Effects of elevated CO_2 on chloroplast pigments of spruce (*Picea abies*) and beech (*Fagus sylvatica*) in model ecosystems as modified by provenance, soil type, and nitrogen supply

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Young beech (*Fagus sylvatica* L.) and spruce (*Picea abies* [L.] Karst.) trees, each from two different provenances, were grown in competition in model ecosystems in open-top chambers for four years. The treatments consisted of elevated concentrations of CO_2 (370 $\mu L\ L^{-1}$ versus 590 $\mu L\ L^{-1}$), increased wet deposition of nitrogen (7 versus 70 kg N ha^{-1} y^{-1}), and two different forest soils (acidic versus calcareous). Chloroplast pigments in dark- and light-adapted leaf material sampled in the last year of the experiment were analysed. Differences in pigment composition between provenances were observed only in beech trees. Soil type significantly affected the pigment composition in both species. Trees grown under calcareous conditions had higher contents of chlorophylls, whereas acidic soil conditions caused significantly enhanced levels of β-carotene and xanthophylls as well as increased values of the xanthophyll de-epoxidation status. For both tree species light-adapted samples had higher carotenoid concentrations and de-epoxidation state values than dark-adapted foliage, whereas neither CO_2 nor N-treatment affected these parameters. Elevated CO_2 application induced decreased concentrations of total chlorophyll contents in both species. Nitrogen deposition had no effects on pigment composition neither for spruce nor for beech trees. Interactions between CO_2 and nitrogen application were not observed for both tree species.

Key words: carbon dioxide, nitrogen deposition, *Picea abies*, *Fagus sylvatica*, soil type, provenance, pigment composition, de-epoxidation status

Introduction

Atmospheric carbon dioxide (CO_2) concentrations have been increasing for the past 150 years from a level of about 270 $\mu L\ L^{-1}$ to the present concentration of nearly 365 $\mu L\ L^{-1}$

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(Drake et al. 1998). Because of their prominent role in the global carbon balance, trees are very important organisms in relation to global changes. Presently much attention is paid to the effects of increasing CO_2 on physiological processes in plants and ecosystems (Ceulemans and Mousseau 1994). Complex interactions of multiple factors highlight the need for studies which examine the effects of elevated CO_2 in concert with multiple environmental conditions (Pritchard et al. 1997). At the physiological level, elevated CO_2 enhances photosynthesis, growth, and belowground allocation of assimilates (Bazzaz 1990). Plants vary widely, however, in the exact nature of their responses to elevated CO_2, within as well as among species. The responses also depend on the availability of other resources, in particular light, water, and nutrients (Idso and Idso 1994).

Changes in photosynthetic pigment composition reflect alterations in plant vitality (Lichtenthaler 1993) which may occur due to various environmental factors in the field, among them nutritional disorder, air pollutants, water parameters or others (Tausz et al. 1998a, b). In plant cells, especially in chloroplasts, stressful environmental conditions (e.g. nutritional disorder, insufficient water supply, high irradiation etc.) promote the formation of harmful reactive oxygen species (ROS) due to an inadequate exploitation of light energy. These ROS can initiate destructive oxidative processes such as protein oxidation, lipid and pigment degradations, etc (Elstner and Oßwald 1994). Since these reactions are driven by light energy, photoprotective energy dissipation in the thylakoids is the first line of biochemical defence (Foyer 1997). The xanthophylls and carotenes play important roles in the protection of the chloroplast from photo-oxidative stress (Demming-Adams and Adams 1994). A higher de-epoxidation status of the xanthophylls cycle indicates a higher need for photoprotection under unfavourable environmental conditions (Demming-Adams and Adams 1994). Pigment loss (reflected in decreased total chlorophyll contents) or shifts of pigment ratios are possible consequences of adverse environmental influences (Tausz et al. 1998a., Wonisch et al. 1999)

The present paper aims at the characterization of the effects of elevated CO_2 and nitrogen deposition on the pigment composition of two provenances of spruce and beech growing under near-natural conditions. Investigations were performed in the frame of the project ICAT (Impact of elevated CO_2 levels and air pollutants on tree physiology), conducted at the Swiss Federal Research Institute WSL in Birmensdorf, Switzerland. The experimental design of the ICAT project is aimed at complex interactions between elevated CO_2 and various environmental conditions and its effects on the photosynthetic and photoprotective pigments.

Materials and Methods

Experimental design

This study was part of a model forest ecosystem experiment (Egli and Körner 1997, Günthardt-Goerg 1997, Egli et al. 1998, Maurer et al. 1999, Egli et al. 2000, Sonnleitner et al. 2001) conducted at the Swiss Federal Research Institute WSL in Birmensdorf, Switzerland described in detail by Landolt et al. (1997). Young beech (*Fagus sylvatica* L.) and spruce trees (*Picea abies* [L.] Karst.) from two different Swiss provenances were grown under near-natural conditions in model-ecosystem chambers (sixteen open-top chambers, each divided into two parts containing either nutrient rich calcareous or nutrient poor acidic soil) during four years. CO_2 and nitrogen treatments applied in the last experimental year (1998)

were four combinations with 4 replications each: control [370 µL L^{-1} CO$_2$/ 7 kg N ha^{-1} y^{-1}], CO$_2$ [590 µL L^{-1} CO$_2$/ 7 kg N ha^{-1} y^{-1}], N [370 µL L^{-1} CO$_2$/ 70 kg N ha^{-1} y^{-1}] and CO$_2$ × N [590 µL L^{-1} CO$_2$/ 70 kg N ha^{-1} y^{-1}]. N treatment was NH$_4$NO$_3$ addition by irrigation.

Plant Material

Light- and dark-adapted foliage (sampled at midday and before dawn, respectively) was sampled from the two different populations (spruce: provenance Maschwanden, 460 m asl. and Bremgarten, 425 m asl., tree age 8 years – beech: provenance Hirschtal and Sihlwald, 600 m asl., tree age 6 and 7 years) at the end of the four-years fumigation period (in July 1998). Needle material of spruce trees were current-year needles, beech samples were taken from leaves of comparable developmental stage (youngest fully developed age).

Sample preparation

Foliage was removed from the branches and frozen in liquid nitrogen, transported under liquid nitrogen to Graz and lyophilised in a Hetosic CD4 lyophilizer (Heto, AllerØd, Denmark). The lyophilised material was powderized under liquid nitrogen in a dismembrator (Braun Mikro-Dismembrator II, Braun, Maria Enzersdorf, Austria). The resulting powder was stored in humidity proof plastic vials at –25 °C until required for extraction.

Chloroplast pigments

Pigments were determined using the high-performance liquid chromatography (HPLC) gradient method described by PFEIFHOFER (1989). This method permits the separation of all important chloroplast pigments in one step: Column Sperisorb S5 ODS 2 250 × 4.6 mm with pre-column S5 ODS2 50 × 4.6 mm. Solvent A: acetonitrile : methanol : water = 100 : 10 : 5 (v/v/v), solvent B: acetone : ethylacetate = 2 : 1 (v/v), linear gradient from 10 % solvent B to 70 % solvent B in 18 min, run time 30 min, flow = 1 ml min^{-1}, photometric detection at 440 nm. Plant material (50 mg lyophilized powder) was extracted two times in acetone (1 ml, 1min. on a Vortex-mixer) and after centrifugation (17500 g at 4 °C) the supernatants were combined and adjusted to a final volume of 3 ml. These acetone extracts were injected (20 µl) using a cooled (4 °C) autosampler.

Statistical analyses

The effects of the factors tree provenance, soil type, sample time (dark-adapted and light-adapted), CO$_2$ and N treatment were evaluated. Each combination of factors contained n=4 trees. Due to the small sample size a non-parametric analysis of variance (an enhanced Kruskal-Wallis test with data-alignment according to Hildebrandt given in BORTZ et al. (1990)) was applied (Tab. 1). $P < 0.01$ was regarded significant.

Results

Effects of provenance, soil type, sampling time, CO$_2$ and N treatment on pigment composition of spruce and beech foliage were investigated. Total chlorophyll contents are expressed on a leaf dry weight basis. Since the protective and light harvesting capacity of carotenoids is important in relation to the amount of absorbed quanta of light, carotenoids are presented per unit chlorophyll.

Results on beech

Elevated CO_2 treatment decreased the total chlorophyll concentrations in beech leaves (Fig. 1, Tab. 1). The provenance significantly affected the total chlorophyll, β-carotene, and xanthophyll cycle concentration of beech trees (Tab. 1). Foliage sampled from trees of the provenance „Hirschtal« had more chlorophylls (Fig. 1) and less β-carotene and xanthophyll cycle pigments (Tab. 1, Tab. 2) than that from the provenance „Sihlwald«. The two provenances also differed significantly in the de-epoxidation status of the xanthophylls cycle under the same illumination conditions (Tab. 1). The soil type had neither significant effects on the total chlorophyll content (Tab. 1, Fig. 1) nor on the de-epoxidation status (Tab. 1, Fig. 2) of beech leaves, but only on some carotenoids (Tab. 1). Significant interaction effects between the factors provenance, soil type, sampling time, CO_2 and N treatment on pigment composition were not found for beech.

Results on spruce

The soil type and the CO_2-treatment had significant effects on pigmentation of spruce needles, whereas the provenance did not affect the measured variables (Tab. 1). The nitrogen treatment had only significant effects on the β-carotene content (Tab. 1). The acidic, nutritionally poor soil type caused significantly decreased contents of chlorophyll (Fig. 1) and

Tab. 1. Non-parametric analysis of variance (enhanced Kruskal-Wallis test) regarding the effects of the factors tree provenance, soil type, sample time (light- and dark-adapted), CO_2 and N treatment on pigment composition of spruce and beech foliage. Each combination of factors contained n = 4 trees. Significant interactive effects were only found in one case (see legend of Fig. 2). ns = not significant, *** = $p < 0.001$. V+A+Z = xanthophyll cycle pool (violaxanthin + antheraxanthin + zeaxanthin).

Fagus sylvatica					
	Provenance	Soil type	Sampling time	CO_2	N
Chlorophyll [mg g⁻¹ dw]	***	ns	ns	***	ns
De-epoxidation status	***	ns	***	ns	ns
β-Carotene [µg mg⁻¹ chl]	***	***	***	ns	ns
α-Carotene [µg mg⁻¹ chl]	ns	***	***	ns	ns
Lutein [µg mg⁻¹ chl]	ns	***	***	ns	ns
V+A+Z [µg mg⁻¹ chl]	***	ns	***	ns	ns
α-/β-Carotene	ns	ns	***	ns	ns
Xanthophylls/carotenes	***	***	***	ns	ns
Chlorophylls/carotenoids	***	***	***	***	ns
Picea abies					
	Provenance	Soil type	Sampling time	CO_2	N
Chlorophyll [mg g⁻¹ dw]	ns	***	ns	***	ns
De-epoxidation status	ns	***	***	***	ns
β-Carotene [µg mg⁻¹ chl]	ns	***	***	ns	***
α-Carotene [µg mg⁻¹ chl]	ns	***	***	ns	ns
Lutein [µg mg⁻¹ chl]	ns	***	***	***	ns
V+A+Z [µg mg⁻¹ chl]	ns	***	ns	ns	ns
α-/β-Carotene	ns	***	ns	ns	ns
Xanthophylls/carotenes	ns	ns	ns	***	***
Chlorophylls/carotenoids	ns	***	***	ns	ns

increased carotenoid concentrations per unit chlorophyll (Tab. 3). Furthermore, the de-epoxidation state of the xanthophylls-cycle was higher in needles of spruce trees grown on the acidic soil type compared to fertile calcareous growth conditions (Fig. 2). On the nutritionally
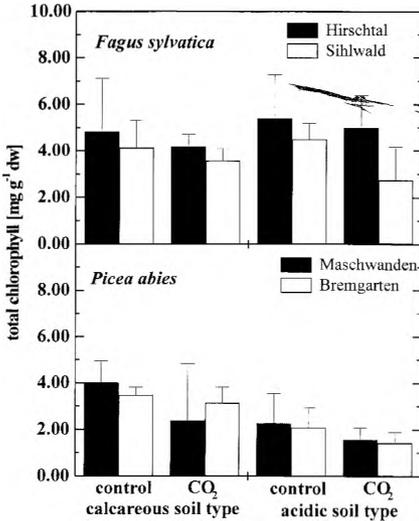

Fig. 1. The effects of elevated CO$_2$, provenances, soil type on total chlorophyll contents of spruce (current year needles) and beech. Data are medians + 80 percentile ranges of 16 individuals (data of the different N treatments and sampling times were pooled).
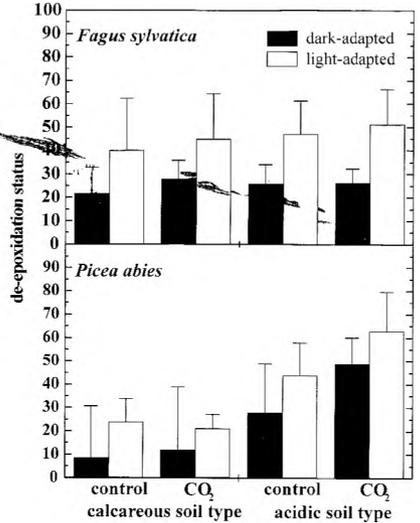
Fig. 2. The effects of elevated CO$_2$ sampling time, and soil type on the de-epoxidation status of the xanthophylls cycle of spruce (current year needles) and beech. Data are medians + 80%ile ranges of 16 individuals (data of different N treatments and provenances were pooled). The interaction CO$_2$ × soil type was significant for spruce trees (p = 0.006).

Tab. 2. Chloroplast pigments of beech leaves from trees of two different provenances growing in micro-ecosystems with spruce in nutrient rich, calcareous (»Rich«) or nutrient poor, acidic soil (»Poor«) treated with elevated CO$_2$. Concentrations in [µg mg^{-1} chlorophyll]. Data are medians (half 20–80 percentile range) of n=16 samples. Data of two N-treatments were pooled because the effect of N-treatment was not significant (Tab. 1) and for the sampling time (morning/midday) which reflects normal diurnal courses no interactions with treatments were found. V+A+Z = xanthophyll cycle pool (violaxanthin + antheraxanthin + zeaxanthin).

	Provenance »Hirschtal«				Provenance »Sihlwald«			
	370 µL L^{-1} CO$_2$		590 µL L^{-1} CO$_2$		370 µL L^{-1} CO$_2$		590 µL L^{-1} CO$_2$	
	Rich	Poor	Rich	Poor	Rich	Poor	Rich	Poor
β-Carotene	39 (9)	46 (8)	42 (11)	48 (3)	48 (11)	51 (11)	50 (19)	53 (8)
α-Carotene	3 (1)	6 (4)	4 (2)	6 (3)	5 (5)	5 (5)	5 (2)	5 (2)
Lutein	81 (8)	76 (12)	91 (8)	71 (7)	88 (13)	74 (14)	90 (12)	82 (13)
V−A−Z	32 (9)	34 (6)	35 (6)	28 (6)	35 (3)	37 (8)	38 (5)	47 (13)

Tab. 3. Chloroplast pigments of spruce needles growing in micro-ecosystems with beech in nutrient rich (»Rich«), calcareous or nutrient poor, acidic soil (»Poor«) treated with additional N and elevated CO_2. Concentrations in [μg mg^{-1} clorophyll]. Data are medians (half 20–80 percentile range) of n=16 samples. Data of two provenances were pooled because the effect of provenances was not significant (Tab. 1) and for the sampling time (light- and dark adapted) which reflects normal diurnal courses and no interactions with treatments were found. V+A+Z=xanthophyll cycle pool (violaxanthin+antheraxanthin+zeaxanthin).

| | No additional N fertiliziation (7 kg N ha^{-1} a^{-1}) | | | | NH$_4$NO$_3$ treatment (70 kg N ha^{-1} a^{-1}) | | | |
| | 370 μL L^{-1} CO$_2$ | | 590 μL L^{-1} CO$_2$ | | 370 μL L^{-1} CO$_2$ | | 590 μL L^{-1} CO$_2$ | |
	Rich	Poor	Rich	Poor	Rich	Poor	Rich	Poor
β-Carotene	35 (4)	45 (9)	34 (8)	51 (5)	34 (6)	42 (7)	32 (12)	48 (7)
α-Carotene	34 (10)	29 (7)	40 (9)	30 (8)	36 (8)	28 (6)	36 (6)	32 (8)
Lutein	78 (19)	91 (10)	88 (19)	102 (15)	79 (6)	85 (15)	86 (12)	99 (12)
V+A+Z	15 (5)	17 (3)	17 (4)	21 (5)	18 (2)	19 (4)	16 (3)	19 (4)

poor soil elevated CO_2 caused a higher de-epoxidation state of the xanthophylls cycle in spruce needles (Fig. 2, significant interaction soil type × CO_2). Comparable to data of beech trees CO_2 treatment caused a reduction of total chlorophyll contents in needles (Tab. 1, Fig. 1). All other interactive effects were not significant.

Discussion

The investigation of the effects of elevated CO_2 and nitrogen deposition on the pigment composition and the de-epoxidation status of two provenances of spruce and beech on two forest soils resulted in different responses of the tree species. While pigmentation of beech leaves was more influenced by provenance, pigment concentrations of spruce needles were affected by soil type (Tab. 1). Pigment patterns and photoprotective xanthophylls of both tree species were not affected by the additional application of nitrogen, but elevated CO_2 decreased the total chlorophyll contents in beech and spruce foliage, respectively. An elevated CO_2-induced decrease in pigment content, mainly in that of chlorophyll, has also been observed in other studies (SURANO et al. 1986, TAUSZ et al. 1996). These decreased contents of total chlorophyll contents could indicate a lower light harvesting capacity, possibly because of the luxury supply of CO_2 as a substrate for photosynthesis. This is supported by data of the carbon assimilation, where increases were found for both spruce and beech trees in the present experiment (GÜNTHARDT-GOERG et al. 1999). While spruce trees allocated the assimilated carbon to the increased production of biomass, beech leaves showed strong starch accumulations in chloroplasts. Spruce trees showed increased values of the de-epoxidation state and increased concentrations of the photoprotective pigment β-carotene when grown on acidic soil type, an effect that was significantly amplified by elevated CO_2. The de-epoxidation of violaxanthin via antheraxanthin to zeaxanthin is an important process in the dissipation of excess energy and protection of the photosynthetic membranes from injury (SIEFERMANN-HARMS 1977). A higher de-epoxidation state indicates less light harvesting capacity and more energy dissipation in the pigment bed (DEMMING-ADAMS and ADAMS 1994). For spruce, the elevated CO_2 may be an additionally environmental stress factor under sub-optimal soil conditions, a fact that is emphasized by

need of spruces for photoprotective mechanisms indicated by the higher de-epoxidation state (Fig. 2). However, because spruce was able to induce these potoprotective mechanism, it finally profited from elevated CO_2 by increased biomass production in contrast to beech.

Soil effects on total chlorophyll contents and the de-epoxidation status were absent for beech trees, while provenances showed pronounced differences between these parameters. Trees from the provenance »Hirschtal« could be characterized by more vitality (more chlorophyll) and less need for photoprotection (e. g. shown by lower contents of β-carotene) compared to trees from »Sihlwald«.

The present results emphasize the complex responses of forest trees to global change factors at the physiological level. The observed differences between species and provenances, and the additional effect of soil and nutrition factors mediate physiological responses and, eventually, can change the competitive behaviour in the ecosystem.

Acknowledgements

References

BAZZAZ, F. A., 1990: The response of natural ecosystems to the rising global CO_2 levels. Annual Rev. Ecol. Syst. 21, 167–196.

BORTZ, J., LIENERT, G. A., BOEHNKE, K., 1990: Verteilungsfreie Methoden in der Biostatistik. Springer Verlag, Berlin.

CEULEMANS, R., MOUSSEAU, M., 1994: Effects of elevated atmospheric CO_2 on woody plants. New Phytol. 127, 425–446.

DEMMING-ADAMS, B., ADAMS III. W. W., 1994: Light stress and photoprotection related to the xanthophylls cycle. In: FOYER, C. H., MULLINEAUX, P. M. (eds.), Causes of photooxidative stress and amelioration of defence systems in plants, 105–126. CRC Press, Boca Raton.

DRAKE, B. G., JACOB, J., GONZÀLEZ-MELER, M. A., 1998: Photosynthesis, respiration and global climate change. In: RAGHAVENDRA, A. S. (ed.), Photosynthesis: A comprehensive treatise, 273–282. Cambridge University Press, Cambridge.

EGLI, P., KÖRNER, CH., 1997: Growth respones to elevated CO_2 and soil quality in beech-spruce model ecosystems. Acta Oecol. 18, 343–349.

EGLI, P., MAURER, S., GÜNTHARDT-GOERG, M. S., KÖRNER, CH., 1998: Effects of elevated CO_2 and soil quality on leaf gas exchange and above-ground growth in beech-spruce model ecosystems. New Phytol. 140, 185–196.

EGLI, P., MAURER, S., SPINNLER, D., LANDOLT, W., GÜNTHARDT-GORG, M. S., KÖRNER CH., 2000: Downward adjustment of carbon fluxes at the biochemical, leaf, and ecosystem scale in beech-spruce model communities exposed to long-term atmospheric CO_2 enrichment. Oikos (in press).

Elstner, E. F., Oßwald, W., 1994: Mechanisms of oxygen activation during plant stress. Proc. Roy. Soc. Edinburgh 102B, 131–154.

Foyer, C., 1997: Oxygen metabolism and electron transport in photosynthesis. In: Scandalios, G. (ed.), Oxidative stress and the molecular biology of antioxidant defence, 587–621. Cold Spring Harbor Laboratory Press, Cold Spring Harbor.

Günthardt-Goerg, M. S., 1997: Leaf and shoot formation of young spruce and beech exposed to elevated CO_2. Acta Oecol. 18, 335–341.

Günthardt-Gorg, M. S., Vollenweider, P., Prati, D., McQuattie, C. J., Mathis, D., 1999: Zellen und Zellinhalt. Proc. of the »Schlussveranstaltung ICAT-Birmensdorf«, Bern, Schweiz, 28–30.

Heagle, A. S., Miller, J. E., Sherrill, D. E., Rawlings, J. O., 1993: Effects of ozone and carbone dioxide mixtures on two clones of white clover. New Phytol. 123, 751–762.

Idso, K. E., Idso, S. B., 1994: Plant responses to atmospheric CO_2 enrichment in the face of environmental constraints: a review of the past 10 years' research. Agric. Forest Meteorol. 69, 153–203.

Landolt, W., Bucher, J. B., Schulin, R., Körner, Ch., Brunold, Ch., 1997: Effects of elevated CO_2 concentration and N deposition on spruce-beech model ecosystem. In: Mohren G. M. J., Kramer K., Sabaté S. (eds.), Impacts of global change on tree physiology and forest ecosystems, 317–324. Kluwer Academic Publishers, Dordrecht.

Lichtenthaler, H. K., 1993: The plant prenyllipids, including carotenoids, chlorophylls, and prenylquinones. In: Moore, T. S. (ed.), Lipid metabolism in plants, 427–470. CRC Press, Boca Raton; Ann Arbor, London, Tokyo.

Maurer, S., Egli, P., Spinnler, D., Körner Ch., 1999: Carbon and water fluxes in Beech-Spruce model ecosystems in response to long-term exposure to atmospheric CO_2 enrichment and increased nitrogen deposition. Funct. Ecol. 13, 748–755.

Pfeifhofer, H., 1989: Evidence of chlorphyll b and lack of lutein in *Neottia nidus-avis* plastids. Biochem. Physiol. Pflanzen 184, 55–61.

Pritchard, S., Peterson, C., Runion, G. B., Prior, S., Rogers, H., 1997: Atmospheric CO_2 concentration, N availability, and water status affect patterns of ergastic substance deposition in longleaf pine (*Pinus palustris* Mill.) foliage. Trees 11, 494–503.

Siefermann-Harms, D., 1977: The xanthophyll cycle in higher plants. In: Tevini, M., Lichtenthaler H. K. (eds.), Lipids and lipid polymers in higher plants, 218–230. Springer Verlag, Berlin, Heidelberg, New York.

Sonnleitner, M. A., Günthardt-Goerg, M. S., Bucher-Wallin I. K., Attinger, W., Reis, S., Schulin, R., 2001: Influence of soil type on the effects of elevated atmospheric CO_2 and N deposition on water balances and growth of mixed spruce and beech forest regeneration. Water Air Soil Pollut. 126, 273–292.

Surano, K., Daley, P., Houpis, J., Shinn, J., Helms, J., Pallassou, R., Costella, M., 1986: Growth and physiological responses of *Pinus ponderosa* Dougl. Ex P. Laws to long-term elevated CO_2 concentrations. Tree Physiol. 2, 243–259.

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, 362–367.

TAUSZ, M., STABENTHEINER, E., WONISCH, A., GRILL, D., 1998a: Classification of biochemical response patterns for the assessment of environmental stress to Norway spruce. Environ. Sci. Pollut. Res. Special Issue 1, 96–100.

TAUSZ, M., JIMÉNEZ, M., S., GRILL, D., 1998b: Antioxidative defence and photoprotection in pine needles under field conditions. A multivariate approach to evaluate patterns of physiological responses at natural sites. Physiol. Plant. 104, 760–764.

WONISCH, A., TAUSZ, M., HAUPOLTER, M., KIKUTA, S., GRILL, D., 1999: Stress-physiological response patterns in spruce needles relate to site factors in a mountain forest. Phyton (Austria) 39, 269–274.