DIFFERENCES IN THE PHOTOSYNTHETIC UV-B RESPONSE BETWEEN EUROPEAN BEECH (*Fagus sylvatica* L.) AND NORWAY SPRUCE (*Picea abies* (L.) K a r s t.) SAPLINGS

MIRKA ŠPRTOVÁ[1], MICHAL V. MAREK[1,3], OTMAR URBAN[1], JIŘÍ KALINA[2], VLADIMÍR ŠPUNDA [2]

[1] Laboratory of Ecological Plant Physiology, Institute of Systems Biology and Ecology Academy of Sciences of the Czech Republic, Poříčí 3b, 603 00 Brno, Czech Republic; e- mail: mirka@usbe.cas.cz
[2] Department of Physics, Faculty of Science, University of Ostrava, 30. Dubna 22, 701 03 Ostrava 1, Czech Republic
[3] Institute of Forest Ecology, Faculty of Forestry and Wood Technology, Mendel Agriculture and Forestry University, Zemědělská 3, 612 00 Brno, 612 00 Czech Republic

Abstract

Šprtová M., Marek V.M., Urban O., Kalina J., Špunda V.: Differences in the photosynthetic UV-B response between European beech (*Fagus sylvatica* L.) and Norway spruce (*Picea abies* (L.) K a r s t.) saplings. Ekológia (Bratislava), Vol. 27, No. 2, p. 130–142, 2008.

Cloned saplings of Norway spruce (*Picea abies* (L) K a r s t.) and beech (*Fagus sylvatica* L.) (7 years old) were exposed to enhanced UV-B irradiation (+25%) continuously over three growing seasons (1999–2001). Selected parameters of variable chlorophyll *a* fluorescence and pigment composition were analysed in the late summer of the third growing season to evaluate the influence of long-term elevated UV-B irradiation on broadleaf and conifer tree species. To obtain information on the xanthophyll cycle, the de-epoxidation state (DEPS) was calculated. These tree species responded differentially to the long-term effects of enhanced UV-B radiation, Norway spruce was more sensitive compared to the European beech. The results show that in Norway spruce a long-term exposure to enhanced UV-B radiation under field conditions caused negative changes at the level of primary photosynthetic reactions. Contrary to the beech, this had higher degree of UV-B protective responses. UV-B radiation is not effective stressor to its primary photosynthetic reaction.

Key words: UV-B radiation, xanthophylls cycle, chlorophyll *a* fluorescence, daily courses

Abbreviations: $DEPS_{act}$ – actual de-epoxidation state; Chl*a*, Chl*b* Chl*a*+*b* – chlorophyll *a,b* and *a*+*b* content; F_V/F_M – potential photochemical efficiency of PSII; F_M, F_o – maximal/ minimal value of chlorophyll *a* fluorescence; Φ_{PSII} – actual yield of chlorophyll *a*; PS II RC – photosystem II reaction centre

Introduction

The biological consequences of the UV-B region irradiance (290–320 nm) may be a potentially harmful factor of the plant environment (Caldwell, 1971; Fiscus, Miller, 1994; Caldwell et al.,

1999; Kakanaki et al., 2003). The increased UV-B irradiance in the plant environment results from a reduction of the thickness amount of the stratospheric ozone layer (Rowland, 1989). However, the significant variability exists in UV-B response among plant species, genotypes and ontogeny stages of plants. On the basis of these facts it is not so easy to formulate some general response plants strategy about the influence of enhanced UV-B (Caldwell et al., 1989; Visser et al., 1997; Flint, Caldwell, 2003a), particularly under the field conditions (Flint, Caldwell, 2003b; Kakanaki et al., 2003). The different responses among the tree species, especially between the coniferous and broad-leaved ones might to be expected.

A number of studies of plant responses to increased UV-B radiation have been published; these differ in their conclusions concerning variations in the magnitude of UV-B effects and among plant species responses (Ziska et al., 1992; Kakanaki et al., 2003). Most of these studies show a potentially damaging effect of enhanced UV-B radiation on plant growth (Mepsted et al., 1996; Wand et al., 1996; Visser et al., 1997; Searles et al., 2001), concerning effects on plant morphology (Murali, Teramura, 1986; Searles et al., 2001), photosynthetic pigments (Teviny et al., 1981) and nucleotide acids, as well as protein synthesis and activity (Teramura, 1980; Caldwell, Flint, 1994). However, a number of studies report no detectable, negligible or positive UV-B effects on either photosynthesis or overall plant productivity (Ziska et al., 1993; Fiscus et al., 1994; Searles et al., 2001; Bassman et al., 2001). Thus, it is possible to assume complicated responses of plant/tree species to the enhanced UV-B radiation. Moreover this response could be related to the plant/foliage ontogeny stage, leaf ecotypes (Krause et al., 2003) or to the interaction among various environmental stresses. For example, coniferous evergreen trees with long-lived foliage have a feasible potential to be sensitive to UV-B damage, because the degree of damage appears to be dependent on a cumulative exposure (Naidu et al., 1993;). Strong on species-specific reaction to UV-B radiation in broad-leaved trees was reported (Keiller, Holmes, 2001; Searles et al., 2001).

This paper reports results obtained from a three-year project in which saplings (7-years old) of spruce (*Picea abies* [L.] K a r s t.) and beeches (*Fagus sylvatica* L.) were grown under an artificially enhanced UV-B irradiance in the mountain region of the Beskydy Mts (NE part of the Czech Republic). The enhanced UV-B radiation was administered at levels that were at any moment at 125% of incident ambient UV-B radiation. The effect of UV-B radiation on the level of chlorophyll *a* fluorescence and pigment composition level was determined. The response to the enhanced UV-B radiation was compared for both investigated tree species with an aim to obtain different mechanism of coniferous and broadleaf trees.

Material and methods

Plant material and experiment design

The long-term influence of enhanced UV-B radiation on young cloned European beech (*Fagus sylvatica* L.) and Norway spruce (*Picea abies* (L.) K a r s t.) saplings (age – 7 years, average height – 0.4 m) was investigated at the Experimental Research Site of Bílý Kříž in the Moravian-Silesian Beskydy Mts (NE Moravia, Czech Republic, 49°30´ N, 18°32´ E, 943 m a.s.l.).

A special illuminating modulated system of similar design as reported by Caldwell et al. (1983) has been constructed to provide a UV-B radiation illumination enhancement under the field conditions of a mountain forest locality. The system consists of four pieces of 1.5x3 m light bank. Each bank contained 12 filtered fluorescent lamps (UV-B-313, Q-Panel OH, USA). Fluorescent lamps modulation was based on the comparison of incident to under-lamp bank UV-B level. The UV-B measurement is based on cosine-corrected SED-240 vacuum photodiodes (Starna, Austria). The SED-240 has a similar spectral response as the UV-B plant action spectrum (DeLucia et al. 1991) and was calibrated annually again a spectrometer (Spectroradiometer 75L, Optronic Lab., USA). The output from the SED-240 was connected to a datalogger (DL-3000, Delta-T, England). The lamp output was then adjusted to a present ambient level using a feedback and amplification circuit to provide total UV-B irradiance of 125% of the incident UV-B. UV-B lamps were filtered with the pre-solarized (8 h) 0.13-mm thick cellulose diacetate (transmit only wave-lengths above 290 nm) to avoid a transmission of UV-C radiation. The cellulose-diacetate film was replaced every 10 days.

Potted saplings (soil volume of the pot cca 0.05 m^3) were located under a metal frame, which held the illuminating system. The pots were irrigated to maintain full field capacity. One treatment was used as control (C-variant), i.e. illuminating lamp-bank was occupied with old non-functioning UV lamps. The second treatment was exposed to enhanced UV-B radiation (E-variant). The modulated lamp system provided a 125% increase of UV-B radiation compared to the ambient UV-B background (Fig. 1). Each variant was located under two lamp-banks. Thus 2x20 of beech and 2x20 of spruce trees were used. The upper part of each spruce and beech sapling was fixed by nylon fibber net to achieve a horizontal level resulting in the perpendicular illumination of leaves/shoots of the upper part of the sapling.

The experiment was started in the spring of 1999. Because of the mountain conditions (snow cover > 1 m), the exposure to enhanced UV-B irradiation was carried out only during the growing season (end of April to November). The results of the third season (i. e. 2001) of the UV-B exposure are presented. The presented results were obtained from the measurements carried out during a sequence of hot sunny days (Fig. 2) in August 2001.

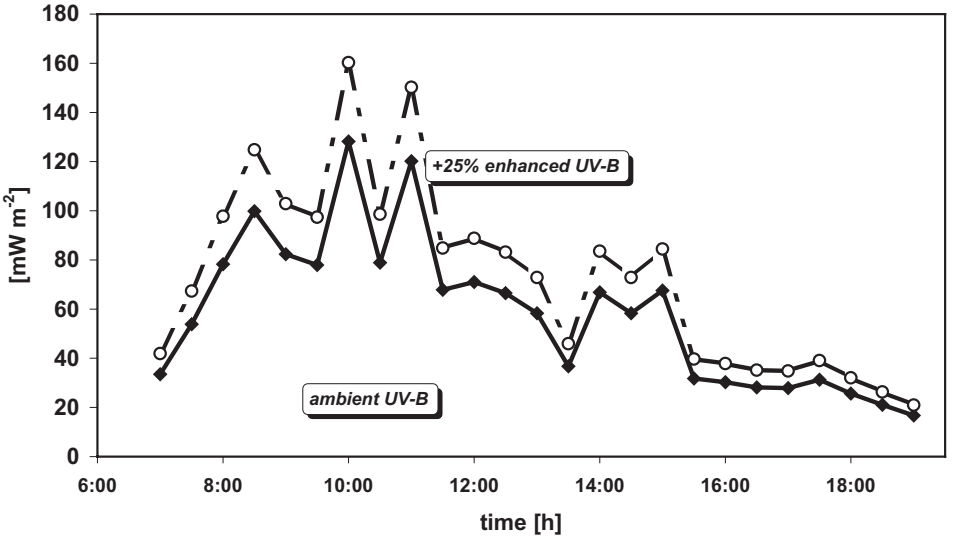

Fig. 1. An example of the daily course of UV-B radiation and its +25% enhancement produced by the constructed modulated lamps system during the example sunny example: 21.06.1999. (from Šprtová et al., 1999).

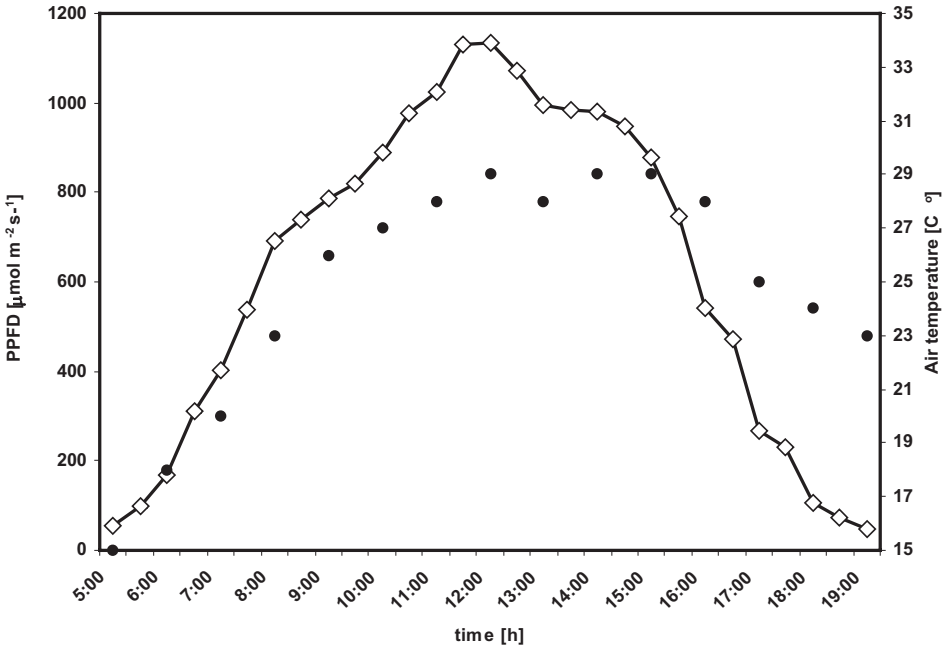

Fig. 2. Average daily course of solar irradiance and air temperature during a period of summer sunny days (18.–23.08.2001), when the investigations of young spruce and beech saplings enhanced UV-B radiation response were carried out. *Open diamonds: photosynthetically photon flux density (PPFD); black circles: air temperature.*

Pigment analysis

Samples for the determination of pigment content of the individual tree species composed of a mixture of adult leaves or current needles. Beech leaves and Norway spruce current year shoots (two from each tree) were excised in the morning (between 7:00 a.m. and 9:00 a.m.). Samples were transported to the laboratory in darkness on a moist cloth and ice. Each mixed sample was divided into three sub-samples. The pigments content was calculated on a dry leaf area basis. Pigments from frozen (in liquid nitrogen) sufficiently dark-adapted foliage segments (approx. 100 mg fresh mass) were extracted with 80% acetone and small amount of $MgCO_3$. The supernatant, obtained after centrifugation of the extract at 4000 rpm for 3 min, was used for spectrophotometric (UV/VIS 550, Unicam, England) estimation of the pigment contents (Chl a, Chl b and total carotenoids) according to Lichtenthaler (1987), and for HPLC quantification of individual carotenoids. In addition to dark-adapted leaf segments, the segments were exposed 10 min either to ambient or high (1870 µmol m^{-2} s^{-1}) irradiance and immediately frozen in liquid nitrogen. These samples were also used for estimation of actual de-epoxidation state ($DEPS_{act}$) – see below.

Isocratic reversed-phase HPLC analysis and conversion factors were used according to Färber and Jahns (1998) with minor modification. The HPLC system consisted of a Rheodyne 5011 valve (Rheodyne Inc., USA), isocratic pump Spectra Series P100, reverse-phase column (5-µm particle size; 25x0.4 cm i.d.; 250/4 RP 18 Lichrocart, Germany) with a guard column (Lichrocart, Germany) and photodiode-array detector UV6000LP (TSP Analytical, USA). The supernatant was filtered through a 0.2 µm syringe filter (PTFE, Whatman, England) and injected using a sample-injection valve (ECOM, The Czech Republic) with a 20x 10^{-6}l sample loop. The pigments were eluted

isocratically for 10 min with solvent system acetonitrile:methanol:Tris (0.1 M) (87:10:3, v/v) followed by an 11 min. isocratic elution with solvent methanol:hexan (4:1,v/v). Total run time was 21 min and the flow rate $2x10^{-3}$ l min^{-1}. Absorbances were detected at 400–500 nm and peak areas were integrated using ChromQuest software for Windows NT (ThermoQuest, Canada). The conversion state of the xanthophyll cycle, i.e. de-epoxidation state (DEPS) was calculated as (Demmig-Adams, Adams 1996; Adams et al. 1999):

$$DEPS = [0.5 \times A+Z]/ [V+A+Z],$$

where Z indicates zeaxanthin, A indicates antheraxanthin and V indicates violaxanthin. The DEPS was estimated from beech leaves and spruce needles samples harvested at 5:00 h; at noon; at 16:00 h; 18:00 h and 20:00 h.

Measurement of modulated chlorophyll a fluorescence

Chlorophyll *a* fluorescence was measured using a portable chlorophyll fluorometer (PAM-2000, Heinz Walz, Effeltrich, Germany). The measurement of chlorophyll *a* fluorescence was carried out on adult beech leaves and current spruce shoots from the upper part of the measured trees. The noon value of potential photochemical efficiency of PSII (F_V/F_M ratio) was measured after a period of 30 minutes of darkness for Norway spruce shoots (Špunda et al. 1998) and 40 minutes of darkness for beech leaves. The F_V/F_M ratio was determined according to Havaux et al. (1991): illumination by the actinic light (10 μmol m^{-2} s^{-1}) was followed by application of a white saturation pulse (1 s duration and PPFD 4000 μmol m^{-2} s^{-1}). This pulse intensity was sufficient to saturate F_M. The parameters F_0 (open PS II RC) and F_M (closed PS II RC) were then estimated. The daily course of the actual yield of chlorophyll *a* fluorescence, (Φ_{PSII}) calculated as: $\Phi_{PSII} = \Delta F/F_{M'}$ (Genty et al., 1989) was estimated each hour from 5:00 to 20:00 h. Measurement of these chlorophyll *a* fluorescence parameters was made using the routine programs of the portable chlorophyll fluorometer (PAM-2000, Heinz Walz, Effeltrich, Germany).

Statistical processing of data

Each treatment (C – control, E – exposed to 25% increase of UV-B) was twice replicated. One replication was represented by 20 trees located under the lamp-bank. Fluorescence measurement and pigment analysis was carried out on six trees per replication. Two leaves/shoots were selected for a measurement on each sampled tree. Three repetitions of the above mentioned chlorophyll fluorescence parameters were realized on each of selected leaf/shoot. Three leaves/shoots were removed for the pigment estimation. The statistical significance of differences between the control and exposed variants was based on the F- and t-test of mean values, respectively. As a null hypothesis the equality of the mean values was accepted. The analysis was carried out using the analytical tool of the Stagraphics program package.

Results

The noon values of potential photochemical efficiency of PSII (F_V/F_M) measured in August 2001 divide the investigated tree species according to F_V/F_M sensitivity to be influenced by enhanced UV-B exposure (Fig. 3). The noon depression of the F_V/F_M ratio values was deeper in the E-variant of Norway spruce (up to 32%) compared to the control and was statistically significant ($p = 0.05$). However, the differences of F_V/F_M ratio for beech leaves of E- and C- variants were not significant. The F_V/F_M ratio of the exposed beech leaves was 1.34 times that of the exposed Norway spruce needles.

When the sample trees were exposed to full sunlight during sunny days, the distinctive daily course of the Φ_{PSII} values with the practically identical shape was observed for both tree species and UV-B treatments (Fig. 4A, B). A conspicuous noon decline in Φ_{PSII} is followed by a recovery upon the return of low light conditions in the late afternoon. The recovery of Φ_{PSII} values could be divided into two phases. The rapid part, which occurred within distinctive time interval (cca 3–4 h) around the noon, was than followed by the much slower recovery phase.

Fig. 3. The noon values of the potential photochemical efficiency of PSII (F_V/F_M) for the enhanced UV-B radiation exposed (Exposed) and control (Control) foliage of the beech (adult leaves) and Norway spruce (current needles) *No. of sample trees: 6; No. of measured leaves/shoots on each sample tree: 2; No. of measurements replication on each leaf/shoot: 3; white: control; grey-exposed variant.*

Towards sunset the Φ_{PSII} values were nearly the same as those determined in the morning before sunrise. Noticeably, the beech and Norway spruce significantly differ in the Φ_{PSII} daily course response to enhanced UV-B. The sensitivity of Φ_{PSII} daily course of the UV-B exposed variant of the Norway spruce was greater and more distinctive.

No significant effects of enhanced UV-B radiation were observed on the pigment composition of UV-B exposed beech leaves (Table 1). Conversely, the UV-B exposed spruce needles showed significantly reduced total chlorophyll *a+b* and chlorophyll *a* content compared to the control plants. Total chlorophyll *a+b* in the control needles was 1.32 times that of the E-variant. The significant differences were found for the chlorophyll *a+b*. Long-term influence of the enhanced UV-B radiation on the chlorophylls *a/b* ratio and total carotenoids content was not significant for either tree species investigated (Table 1).

To be able to obtain the some information on the xanthophyll cycle, the conversion state of the xanthophyll cycle, i.e. de-epoxidation state (DEPS) was calculated (Fig. 5A, B). The daily dynamics of DEPS values was observed for both tree species. The magnitude and direction of differences between control and UV-B exposed foliage differed strongly between Norway spruce and beech leaves. While spruce needles DEPS values were always higher in the control variant, beech leaves DEPS value was always higher in exposed one.

Discussion

Results from the realised long-term experiment are in agreement with the presented results on the variable effects of enhanced UV-B radiation related to the different plant species,

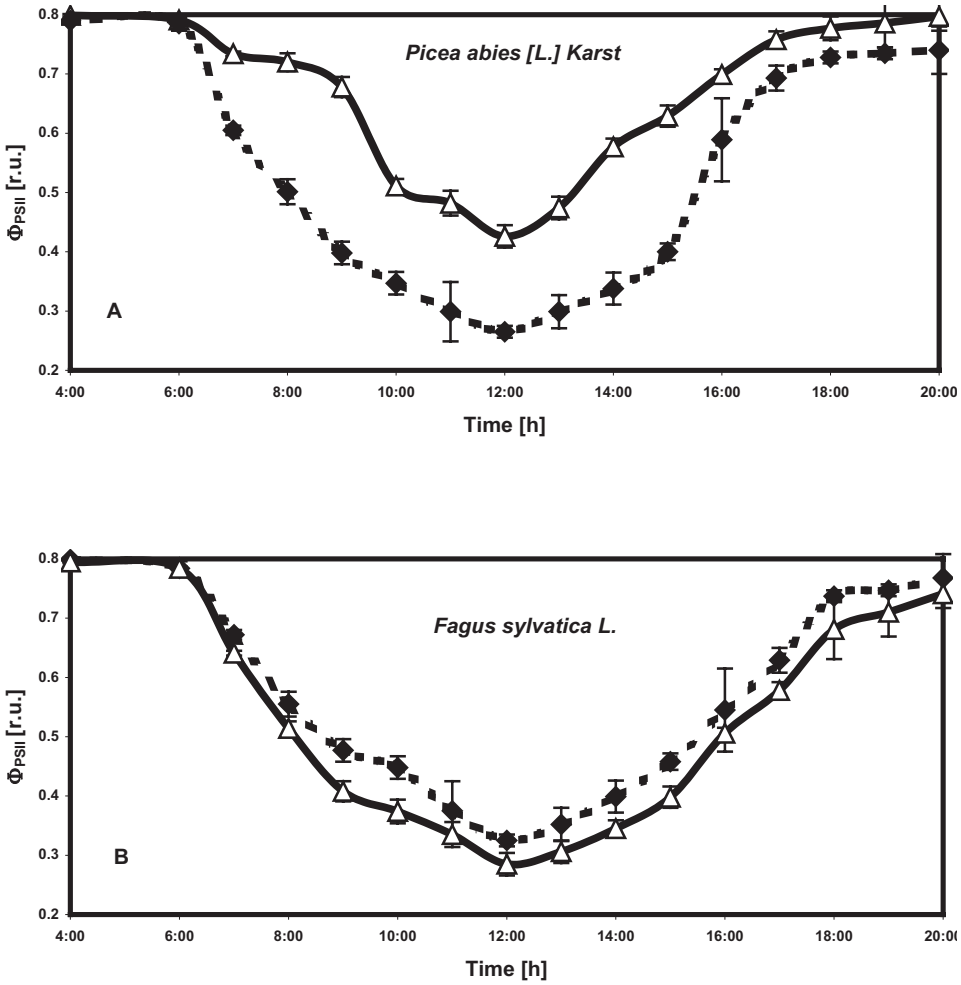

Fig. 4A, B. Daily course of the actual yield of the chlorophyll *a* fluorescence (Φ_{PSII}) for the UV-B exposed (Exposed) and control (Control) **A:** Norway spruce needles (current needles) **B:** beech leaves (adult leaves) *No. of sample trees: 6; No. of measured shoots on each sample tree: 2; No. of measurements replication on each shoot: 3; diamonds: UV-B exposed shoots; triangles: control shoots.*

location of place of long-lasting collecting UV-B doses, altitude (Robakowski, Laitat, 1999). This is supported by the other experiments on the UV-B radiation effects (Wand et al., 1996; Visser et al., 1997; Ziska et al., 1993; Fiscus et al., 1994).

The decline of F_V/F_M was used as a convenient indication of some degree of photoinhibition although Lichtenthaler et al. (1992) showed that ratio F_V/F_o proved to be a better

T a b l e 1. Photosynthetic pigment content of the beech leaves and current Norway spruce shoots from the exposed and control variants. **Chla**; **Chlb**; **Chlab**; **Carx+c**; **Chl a/b –** chlorophyll *a, b, a+b* and total carotenoids content [g m^{-2}] and Chla/*b* ratio. The same letters indicate the statistically significant differences (level 95%) of measured parameters *No. of sample trees: 6; No. of measured leaves/shoots on each sample tree: 3; No. of measurements replication on each leaf/shoot: 3.*

	beech				N. spruce			
	Control	SD	Exposed	SD	Control	SD	Exposed	SD
Chla	**a** 0.124	0.005	**a** 0.120	0.010	**a** 0.185	0.013	**a** 0.138	0.005
Chlb	0.043	0.002	0.042	0.004	**b** 0.071	0.004	**b** 0.056	0.021
Chla+b	0.167	0.007	0.162	0.014	**c** 0.256	0.017	**c** 0.194	0.006
Carx+c	0.041	0.002	0.040	0.003	0.058	0.003	0.053	0.004
Chla/Chlb	2.890	0.057	2.869	0.018	2.61	0.24	2.46	0.36

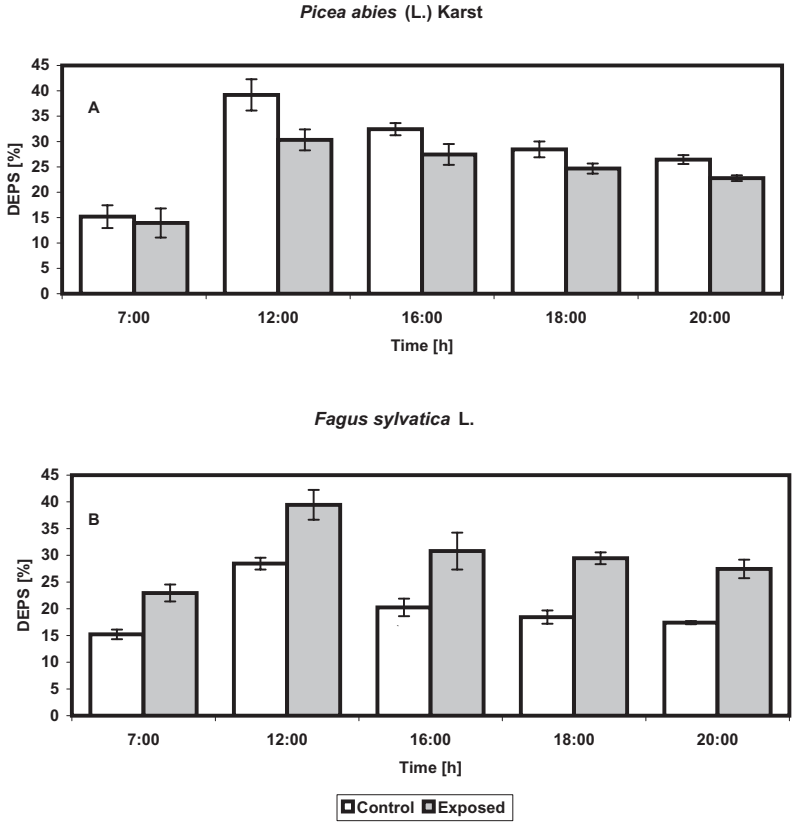

Fig. 5A, B. De-epoxidation state (DEPS) in **A:** current needles of Norway spruce (current needles) **B:** beech leaves (adult leaves)

indicator of photoinhibition with a large amplitude than the ratio F_V/F_M. The relationship between F_V/F_M and ETR in thylakoids isolated from photoinhibited leaves has been shown (Krause et al., 1990; Schnettger et al., 1994; Krause, Winter, 1996). Accepting these conclusions it is possible to draw similar conclusions about the negative effects of enhanced UV-B radiation on Norway spruce electron transport (decline of F_V/F_M up to 32%). Similar results with Norway spruce published Pukacki and Modrzynski, (1998). Conversely, the lack of a UV-B effect in beech leaves F_V/F_M indicates a neglected impact of enhanced UV-B radiation on electron transport. Some increase of photosynthetic characteristic in poplar (*Populus delotides* L.) mature leaves under enhanced UV-B radiation (twice- and triple-ambient) reported Bassman et al., (2001). Contrary to this finding Zeuthen et al. (1997) reported moderate depression of F_V/F_M under the influence of enhanced UV-B radiation (+23%), especially in a combination with increase troposhperic ozone.

A characteristic feature of the investigated trees was the onset of two phase recovery of the Φ_{PSII} values following the mid-day depression (Fig. 4A, B). Two such phases of recovery (i.e. rapid and slow), have previously been reported in laboratory experiments (Leitsch et al., 1994; Krause at al., 1995; Krause, Winter, 1996), were observed and confirmed in this presented field experiment. Thiele et al. (1996) reported that the rapid recovery phase is related to epoxidation of zeaxanthin via the xanthophyll cycle and reflects reversion of a zeaxanthin-dependent state of PSII (Krause, Winter, 1996). This mechanism of dissipation of exceeding irradiance energy has been described in tropical forest tree species by Koniger et al. (1995) and for Norway spruce (Špunda et al., 1998). Zeaxanthin accumulation and a decrease of violaxanthin during the sunny warm day in the maple leaves have been described Demmig-Adams et al. (1996) who reported that the size of the xanthophyll cycle pool is an indicator of the protective ability against photoinhibition. However, some critical comments concerning this performance were reported by Schindler and Lichtenthaler, (1994, 1996). These authors found, that the major part of high-light-induced fluorescence quenching, manifested as photochemical quenching and F'_V/F'_M ratio decline, was apparently not dependent on zeaxanthin.

The rapid phase of Φ_{PSII} recovery was quantified by the initial slope of the linear relation between the lowest noon values of Φ_{PSII} and that obtained at 3 p.m. Changes of these slopes were greater for UV-B exposed variant, i.e. 1.01 times for beech leaves and 1.29 times for Norway spruce needles. Obtained results on the dynamics of the rapid recovery phases of Φ_{PSII} were compared to realised investigation of de-epoxidation state DEPS (Fig. 5A, B). The higher value of the rapid recovery phase slope of Φ_{PSII} in Norway spruce UV-B exposed needles not very well correspond to the daily DEPS dynamics. The rapid recovery phase slope of Φ_{PSII} indicates stronger induction of the inter-conversion between violaxanthin and zeaxanthin (Thiele et al., 1996). However, the xanthophyll cycle pool in spruce needles must be low because the UV-B effects is more pronounced compared to the beech, where this slope induction was lower.

The slow phase of Φ_{PSII} values recovery (Fig. 4A, B), which is observable during late afternoon conditions, thus under decreasing PAR irradiance, probably represents reactivation of PSII by means of D1 protein turnover. Experiments using streptomycin to blocks D1

protein re-synthesis show inhibition of the slow recovery phase (Leitsch et al., 1994). The function of PSII reaction centre depends on continuous synthesis of one of its core proteins (Prášil et al., 1992) and protein synthesis can be expected as UV-B targets (Bornman, 1989, 1991). Hence the capacity for D1 protein synthesis could be limited under the influence of elevated UV-B. The slow phase of recovery was evaluated on the basis of a calculated linear relationship between the values of Φ_{PSII} obtained at the end of the day (i.e. 18:00 to 20:00.). The value of this slope was very different for both investigated tree species. The slope of the Φ_{PSII} slow recovery phase in Norway spruce needles under enhanced UV-B was 0.58 time that under ambient UV-B conditions.

Contrary to this, the slope of the Φ_{PSII} slow recovery phase in beech leaves was higher for UV-B exposed seedlings, i.e. 1.93 times that of under ambient UV-B conditions. These changes in the slow recovery phase obtained under enhanced UV-B radiation Norway spruce needles could been interpreted as an indication of D1 protein turnover impairment due to long-term effects of enhancement UV-B radiation. This D1 impairment was not detected in beech leaves exposed to the enhanced UV-B. One of possible explanation of this could be find in published positive effects of enhanced UV-B radiation of increased leaf thickness (Bornman, 1991; Searles et al., 2001; Šprtová et al., 2003).

Conclusion

The assimilatory apparatus of Norway spruce needles exhibited higher sensitivity to the enhanced UV-B irradiances compared to the beech leaves. This sensitivity is demonstrated by the decrease of the photosynthetic pigment content and by the lower degree of conversion between pigments of the xanthophyll cycle. The observed two-phase recovery of the actual yield of fluorescence probably reflects a protective down-regulation of PSII (rapid phase) and an inactivation of the D1 protein (slow phase). This down-regulation was more pronounced in spruce needles but was connected to the higher inactivation of D1 protein, compared to beech leaves. Thus, the presented results show that in Norway spruce a long-term exposure to enhanced UV-B radiation under field conditions caused negative changes at the level of primary photosynthetic reactions. However, the broad-leaves species, i.e. European beech, shows higher degree of UV-B protection responses and UV-B radiation for this species is not so effective stressor to its primary photosynthetic reactions.

Translated by the authors

Acknowledgements

This work was supported by the grants from the Grant Agency of the Academy of Sciences of the Czech Republic No. S6087005 and by the Research Intention Plan of the Institute of Systems Biology and Ecology No. AVOZ 60870520 and Faculty of Forestry and Wood Technology No. MSM 6215648902 and grant INTERVIRON No. 2B06068.

References

Adams III., W.W., Demmig-Adams, B., Logan, B.A., Baker, D.H., Osmond, C.B., 1999: Rapid changes in xantophyll cycle-dependent energy dissipation and photosystem II efficiency in two vines, *Stephania japonica* and *Smilax australis*, growing in the understory of an open Eucalyptus forest. Plant Cell Environ., *22*: 125–136.

Bassman, J.H., Robberecht, R., Edwards, G.E., 2001: Effects of enhanced UV-B radiation on growth and gas exchange in *Populus deltoides* B a r t r. ex Marsh. International Journal of Plant Sciences, *162*: 103–110.

Bornman, J.F., 1989: Target sites of UV-B radiation in photosynthesis of higher plants. Journal of Photochemistry and Photobiology B: Biology, *4*: 145–158.

Bornman, J.F., 1991: UV radiation as an environmental stress in plants. Journal of Photochemistry and Photobiology B: Biology, *8*: 337–342.

Caldwell, M.M., 1971: Solar UV irradiance and the growth and development of higher plants. In Giese, A.C. (ed.), Photobiology. Academic Press, New York, p. 131–177.

Caldwell, M.M., Gold, W.G., Harris, G., Ashurt, W., 1983: Technical note. A modulated lamp system for solar UV-B (280-320 nm) supplementation studies in the field. Photochem. Photobiology. *37*: 479–485.

Caldwell, M.M., Teramura, A.H., Tevini, M., 1989: The changing solar ultraviolet climate and the ecological climate for higher plants. Trends Ecol. Evol., *4*: 363–367.

Caldwell, M.M., Flint, S.D. 1994: Stratospheric ozone reduction, solar UV-B radiation and terrestrial ecosystems. Climatic Change, *28*: 375–394.

Caldwell, M.M., Searles, P.S., Flint, S.D., Barnes, P.W., 1999: Terrestrial ecosystem response to solar UV-B radiation mediated by vegetation, microbes and abiotic photochemistry. In Press, M.C., Scholes, J.D., Barker M.G. (eds), Physiological plant ecology: the 39th Symposium of the British Ecological Society, held at the University of York, 7–9 September 1998. Blackwell Science, Oxford, p. 214–262.

DeLucia, E.H., Day, T.A., Vogelmann, T.C., 1991: Ultraviolet-B radiation and the Rocky mountain environment: measurement of incident light and penetration into foliage. Current Topics in Plant Biochemistry and Physiology, *10*: 32–48.

Demmig-Adams, B., Adams III., W.W., 1996: Xantophyll cycle and light stress in nature uniform response to excess direct sunlight among higher plant species. Planta, *198*: 460–470.

Demmig-Adams, B., Adams III., W.W., Barker, D.H., Logan, B.A., Verhoeven, A.S., Bowling, D.R., 1996: Using chlorophyll fluorescence to asses the fraction of absorbed light allocated to thermal dissipation of excess excitation. Physiol. Plant., *98*: 253–264.

Farber, A., Jahns, P., 1998: The xantophyll cycle of higher plants: influence of antenna size and membrane organization. BBA, *1363*: 47–58.

Fiscus, E.L., Miller, F.L., Booker, F.L., 1994: Is UV-B a hazard to soybean photosynthesis and yield? Results of an Ozone UV-B Interaction Study and Model Predictions. In Biggs, R.H., Joyner, M.E.B. (eds), Stratospheric Ozone Depletion/ UV-B Radiation in the Biosphere. NATO ASI Series. Springer, Berlin, p. 135–147.

Flint, S.D., Caldwell, M.M., 2003a: The biological spectral weighting function for ozone depletion research with higher plants. Physiol. Plant., *117*: 137–144.

Flint, S.D., Caldwell, M.M., 2003b: Field testing of UV biological spectral weighting functions for higher plants. Physiol. Plant., *117*: 145–153.

Genty, B., Briantais, J.M., Baker, N.R., 1989: The relationship between quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochimica et Biophysica Acta, *990*: 213–224.

Havaux, M., Strasser, R.J., Greppin, H., 1991: A theoretical and experimental analysis of the qP and qN coefficients of chlorophyll fluorescence quenching and their relation to photochemical and nonphotochemical events. Photosynth. Res., *27*: 41–55.

Kakanaki, V.G., Reddy, K.R., Zhao, D., Sailaja, K., 2003: Field crop responses to ultraviolet-B radiation: a review. Agric. For. Meteorol., *120*: 191–218.

Keiller, D.R., Holmes, M.G., 2001: Effects of long-term exposure to elevated UV-B radiation on the photosynthetic performance of five broad-leaved tree species. Photosynthesis Research, *67*: 229–240.

Koniger, M., Harris, G.C., Virgo, A., Winter, K., 1995: Xantophyll cycle pigments and photosynthetic capacity in tropical forest species: a comparative field study on canopy, gap and understorey plants. Oecologia, *104*: 280–290.

Krause, G.H., Somersalo, S., Zambusch, E., Weyers, B., Laasch, H., 1990: On the mechanism of photoinhibition in chloroplasts. Relationship between changes in fluorescence and activity of photosystem II. J. Plant Physiol., *136*: 472–479.

Krause, G.H., Virgo, A., Winter, K., 1995: High susceptibility to photoinhibition of young leaves of tropical forest trees. Planta, *19*: 583–591.

Krause, G.H., Winter, K., 1996: Photoinhibition of photosynthesis in plants growing in natural tropical forest gaps. A chlorophyll fluorescence study. Botanica Acta, *109*: 456–462.

Krause, G.H., Gaile, A., Gademann, R., Winter, K, 2003: Capacity of protection against ultraviolet radiation in sun and shade leaves of tropical forest plants. Functional Plant Biol., *30*: 533–542.

Leitsch, J., Schnettger, B., Critchlev, C., Krause, G.H., 1994: Two mechanisms of recovery from photoinhibition *in vivo:* Reactivation of photosystem II related and unrelated to D 1 protein turnover. Planta, *194*: 15–21.

Lichtenthaler, H.K., 1987: Chlorophylls and carotenoids - pigments of photosynthetic biomembranes. In Colowick, S.P., Kaplan, N.O. (eds), Methods in enzymology, 148. Academic Press, San Diego/New York/Berkeley/Boston/London/Sydney/ Tokyo/Toronto, p. 350–382.

Lichtenthaler, H.K., Burkart, S., Schindler, C., Stober, F., 1992: Changes in photosynthetic pigments and in vivo chlorophyll fluorescence parameters under photoinhibitory growth conditions. Photosynthetica (Prague), *27*: 343–353.

Mepsted, R., Paul, N.D., Stephen, J., Vorlett, J.E., Nogues, S., Baker, N.R., Jones, H.G., Ayres, P.G., 1996: Effects of enhanced UV-B radiation on pea (*Pisum sativum* L.) grown under field conditions in the UK. Global Change Biology, *2*: 325–334.

Murali, N.S., Teramura, A.H., 1986: Effectiveness of UV-B radiation on the growth and physiology of field-grown soybean modified by water stress. Photochem. Photobiol., *44*, p. 215–219.

Naidu, S.L., Sullivan, J.H., Teramura, A.H., DeLucia, E.H., 1993: The effects of ultraviolet-B radiation on photosynthesis of different needle age classes in field-grown loblolly pine. Tree Physiol., *12*: 151–162.

Prášil, O., Adir, N., Ohad, I., 1992: Dynamics of photosystem II: mechanism of photoinhibition and recovery process. In Barber, J. Jr. (ed.), Topics in photosynthesis, 11. Elsevier, Amsterdam, p. 293–348.

Pukacki, P.M., Modrzynski, J., 1998: The influence of ultraviolet-B radiation on the growth, pigment production and chlorophyll fluorescence of Norway spruce seedlings. Acta Physiol. Plant., *20*: 245–250.

Robakowski, P., Laitat, E., 1999: Effects of an enhanced ultraviolet-B irradiation on photosynthetic apparatus of several forest coniferous species from different locations. Acta Physiol. Plant., *21*: 283–296.

Rowland, F.S., 1989: Chlorofluorocarbons and the depletion of stratospheric ozone. Sci. Am., *77*: 36–45.

Schindler, C., Lichtenthaler, H.K., 1994: Is there a correlation between light-induced zeaxanthin accumulation and quenching of variable chlorophyll *a* fluorescence? Plant Physiol. Biochem. (Paris), *32*: 813–823.

Schindler, C., Lichtenthaler, H.K., 1996: Photosynthetic CO_2 assimilation, chlorophyll fluorescence and zeaxanthin accumulation in field grown maple trees in the course of sunny and a cloudy day. J. Plant Physiol., *148*: 399–412.

Schnettger, B., Critchley, C., Sanmtore, U.J., Graf, M., Krause, G.H. 1994: Relationship between photoinhibition of photosynthesis, D 1 protein synthesis inhibitors. Plant Cell Environ., *17*: 55–64.

Searles, P.S., Flint, S.D., Caldwell, M.M. 2001: A meta analysis of plant filed studies simulating stratospheric ozone depletion. Oecologia, *127*: 1–10.

Šprtová, M., Marek, M.V., Nedbal, L., Prášil, O., Kalina, J., 1999: Seasonal changes of photosynthetic assimilation of Norway spruce under the impact of enhanced UV-B radiation. Plant Science, *142*: 37–45.

Šprtová, M., Špunda, V., Kalina, J., Marek, M.V., 2003: Photosynthetic UV-B response of the young forest tree saplings. Photosynthetica, *41*: 533–543.

Špunda, V., Čajánek, M., Kalina, J., Lachetová, I., Šprtová, M., Marek, M.V., 1998: Mechanistic differences in utilization of absorbed excitation energy within photosynthetic apparatus of Norway spruce induced by the vertical distribution of photosynthetically active radiation through the tree crown. Plant Science, *133*: 155–165.

Tegelberg, R., Julkunen-Tiitto, R., Alphalo, P.J., 2001: The effects of long- term elevated UV-B on the growth and phenolics of field-grown silver birch (*Betula pendula*). Global Change Biology, *7*: 839–845.

Teramura, A.H., 1980: Effects of ultraviolet-B irradiance on soybean. Plant Physiol., *65*: 483–488.

Tevini, M., Iwanzik, W., Thioma, U., 1981: Some effects of enhanced UV-B irradiance on the growth and composition of plants. Planta, *153*: 388–394.

Thiele, A.K., Schirwitz, K., Winter, K., Krause, G.H., 1996: Increased xantophyll cycle activity and reduced D 1 protein inactivation related to photoinhibition in two plant systems acclimated to excess light. Plant Science, *115*: 237–250.

Visser, A.J., Tosserams, M., Groen, M.W., Magendarns, G.W.H., Rozema, J., 1997: The combined effects of CO_2 concentration and solar UV-B radiation on faba bean grown in open-top chambers. Plant Cell Environ., *20*: 189–199.

Wand, S.J.E., Midgley, G.F., Musil, C.F., 1996: Physiological and growth responses of two African species, *Acacia karroo* and *Themeda triandra*, to combined increase of CO_2 and UV-B radiation. Physiol. Plant., *98*: 882–890.

Zeuthen, J., Mikelssen, T.N., Paludan Muller, G., Ro Poulsen, H., 1997: Effects of increased UV-B radiation and elevated levels of tropospheric ozone on physiological processes in European beech (*Fagus sylvatica*). Physiol. Plant., *100*: 281–290.

Ziska, L.H., Teramura, A.H., Sullivan, J.H., 1992: Physiological sensitivity of plants along an elevational gradient to UV-B radiation. Am. J. Bot., *79*: 863–871.

Ziska, L.H., Teramura, A.H., Sullivan, J.H., McCoy, A., 1993: Influence of Ultraviolet -B (UV-B) radiation on photosynthetic and growth characteristics in field-grown cassava (*Manihot esculentum* C r a n t z.) Plant Cell Environ., *36*: 73–79.