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Photosynthetic UV-B response of beech (*Fagus sylvatica* L.) saplings

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Abstract

Cloned saplings of beech (7-y-old) were exposed to enhanced UV-B irradiation (+25 %) continuously over three growing seasons (1999–2001). Analysis of CO_2 assimilation, variable chlorophyll (Chl) *a* fluorescence, and pigment composition was performed in late summer of the third growing season to evaluate the influence of long-term elevated UV-B irradiation. This influence was responsible for the stimulation of the net assimilation rate (P_N) over a range of irradiances. The increase in P_N was partially connected to increase of the area leaf mass, and thus to the increased leaf thickness. Even a higher degree of UV-B induced stimulation was observed at the level of photosystem 2 (PS2) photochemistry as judged from the irradiance response of electron transport rate and photochemical quenching of Chl *a*. The remarkably low irradiance-induced non-photochemical quenching of maximum Chl *a* fluorescence (NPQ) in the UV-B plants over the entire range of applied irradiances was attributed both to the reduced demand on non-radiative dissipation processes and to the considerably reduced contribution of the quenching localised in the inactivated PS2 reaction centres. Neither the content of Chls and total carotenoids expressed per leaf area nor the contents of lutein, neoxanthin, and the pool of xanthophyll cycle pigments (VAZ) were affected under the elevated UV-B. However, the contributions of antheraxanthin (A) and zeaxanthin (Z) to the entire VAZ pool in the dark-adapted UV-B treated plants were 1.61 and 2.14 times higher than in control leaves. Surprisingly, the retained A+Z in UV-B treated plants was not accompanied with long-term down-regulation of the PS2 photochemical efficiency, but it facilitated the non-radiative dissipation of excitation energy within light-harvesting complexes (LHC) of PS2. Thus, in the beech leaves the accumulation of A+Z, induced by other factors than excess irradiance itself, supports the resistance of PS2 against combined effects of high irradiance and elevated UV-B.

Additional keywords: carotenoids; chlorophyll *a* fluorescence; photosystem 2; xanthophyll cycle pigments.

Introduction

UV-B irradiance (290–320 nm) is regarded as a potentially harmful factor of plant environment with important biological consequences (Caldwell 1971). The importance of increased UV-B irradiance in the environment results from the reduction in stratospheric ozone layer as a consequence of anthropogenic emission of chlorofluorocarbons and nitrogen oxides (Rowland 1989). Many studies on the potential response of plants to increased UV-B radiation were published. Most of these studies show a potentially damaging effect of enhanced UV-B on plant growth (Wand *et al.* 1996, Visser *et al.* 1997), when considering effects on plant morphology (Murali and Teramura 1986), photosynthetic pigments (Tevini *et al.* 1981), and nucleic acids as well as protein synthesis and activity (Teramura *et al.* 1980, Caldwell and Flint 1994). However, there exist studies showing no UV-B effects on either photosynthesis or overall plant productivity (Ziska *et al.* 1993, Fiscus and Miller 1994). Generally, significant variability in the response to UV-B exists between plant species, genotypes, or stages of ontogeny. Other environmental conditions, particularly irradiance, water status, temperature, and mineral nutrition may also strongly influence the resulting response as combination of these environmental stresses can reveal a synergetic

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action, but in some cases also eliminate the negative response (Ziska *et al.* 1993). Thus different response among the tree species, especially between coniferous and broad leaf species, may be expected. Coniferous evergreen trees with long-lived foliage may be sensitive to UV-B induced damage, because the degree of damage appears to depend on cumulative exposure (Naidu *et al.* 1993, Šprtová *et al*. 1999). The targets of UV-B are multiple. This means, for example, that photosynthesis can be affected by UV-B not only *via* protein turnover but also *via* changes in the membrane composition and enzyme activity. With regard to the photosynthetic processes in thylakoid membranes, both the reaction centre and oxidising side of PS2 were suggested to be primary targets of UV-B radiation, with other sites of electron transport chain being less affected (Bornman 1989). As both the UV-B and excess photosynthetic photon flux density (PPFD) target particularly photosystem 2 (PS2), an interest was focused on the combined effects of enhanced UV-B and high PPFD. Bornman and Vogelmann (1991) reported that enhanced UV-B induced pronounced inactivation of PS2 together with decrease of CO_2 assimilation and reduction of chlorophyll (Chl) content. Similar results were also obtained for the prolonged UV-B exposure of Norway spruce (Šprtová *et al*. 1999). These and other reports (Pfündel *et al.* 1992) suggest that enhanced UV-B increases the sensitivity of photosynthetic apparatus to high irradiance stress. On the contrary, Teramura *et al.* (1980) showed that exposure of soybean to enhanced UV-B with a low PPFD background resulted in a significant decrease in net photosynthetic rate (P_N) in comparison to the combined effect of enhanced UV-B and high PPFD. Moreover, it is possible to expect not only a single response of tree species to enhanced UV-B radiation, since the response could be related to foliage ontogeny or to a combination of environmental stresses (Caldwell *at al.* 1983, DeLucia *et al*. 1991).

In the present paper, we report results of a three-year project in which seedlings of beech (*Fagus sylvatica* L.) were grown under an artificially enhanced UV-B irradiance in the mountain region of NE Czech Republic. The UV-B was administered at levels that were, at any moment, +25 % of the ambient UV-B radiation. The experiments were performed during periods of hot sunny summer days. The UV-B effects on fully developed sun-exposed leaves were monitored at the levels of gas-exchange, Chl *a* fluorescence, and pigment composition. We attempted to confirm or reject the hypothesis that enhanced UV-B increases or reduces the sensitivity of photosynthetic apparatus to photoinhibition.

Materials and methods

Plants and experiment design: The long-term influence of elevated UV-B radiation on young cloned beech (*Fagus sylvatica* L.) saplings (7-y-old, 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 irradiating modulated system was constructed using a similar design as reported by Caldwell *et al*. (1983). The UV-B enhancement was provided with a modulated lamp system operating under the field conditions of the mountain forest stand. The system consisted of a 1.5×3.0 m light bank containing 12 filtered fluorescent lamps (*UV-B-313*, *Q-Panel*, Cleveland, OH, USA). Radiation of UV-B lamps was filtered through a pre-solarised (8 h) 0.13-mm thick cellulose diacetate film (transmits only wavelengths above 290 nm) to avoid UV-C radiation transmission. The cellulose-diacetate film was regularly replaced each 10 d.

Both the ambient incident UV-B level and UV-B level under the lamp-bank were continuously monitored and the lamp output was adjusted using a feedback and amplification circuit to provide +25 % UV-B irradiance of the ambient incident UV-B (Fig. 1). The UV-B detection system was based on cosine-corrected *SED-240* vacuum photodiodes (*Starna*, Hainault, England). The *SED-240* has a similar spectral response as the generalised plant UV-B action spectrum (DeLucia *et al*. 1991) and was calibrated annually against a spectroradiometer *75L* (*Optronic Lab*., Orlando, USA). The output from the *SED-240* was connected to the data logger (*Delta-T*, Cambridge, England).

Potted beech saplings (soil volume of the pot *ca*. 0.05 m^3) were located under a metal frame, which held the irradiating system. To avoid drought stress, the pots were irrigated to maintain full field capacity. Two experimental variants were tested. Each variant contained 40 individuals of beech sapling, respectively. The first variant was exposed to enhanced UV-B radiation (E-variant). The second variant was used as a control (C-variant), *i.e.* irradiating lamp bench was occupied with old non-functioning UV lamps in order to assure the same penetration of sun radiation as for the E-variant.

The experiment was started in the spring of 1999. Due to the mountain conditions (snow cover > 1 m), artificial irradiation was not provided during the winter months. Therefore, the exposure to enhanced UV-B irradiation was carried out only during the growing season (end of April to the beginning of November). The results of the third season (*i.e.* 2001) of the UV-B exposure are presented. The adult leaves used for all the physiological investigations and estimation of pigment contents and composition were located near the top of the canopy, *i.e*. upper layer of leaves. At this canopy layer the UV-B sensors were located. Thus, the measured leaves were distinctly identified as “sunny” leaves.

Gas exchange measurements: The relationship between P_N and PPFD was analysed *in situ* using an open system (*CIRAS-1, PP-Systems*, Hitchin, UK). This relationship was determined in the assimilation chamber at standardised CO_2 concentration [340 μmol(CO_2) mol^{-1}], foliage temperature (20±2 °C), and relative air humidity (55±3 %). The Parkinson conifer chamber was connected to a special laboratory-made artificial light source (two 100 W lamps with two thermal filters and fans) mounted perpendicularly to the top of the assimilation chamber. The light source provided homogenous irradiation (±20 %) of the 6×3 cm plane within the assimilation chamber with maximum PPFD of 1 300 μmol m^{-2} s^{-1}. The P_N-PPFD response curve was measured at steady state under a predetermined set of PPFDs (0, 20, 50, 100, 200, 500, 800, and 1 200 μmol m^{-2} s^{-1}). P_N measurement at each PPFD was recorded within an average 5 min time period. After the gas-exchange and Chl *a* fluorescence measurements, the investigated leaf was removed and the projected leaf area was estimated using a leaf area meter (*LI-3000A*, *LI-COR*, Lincoln, USA).

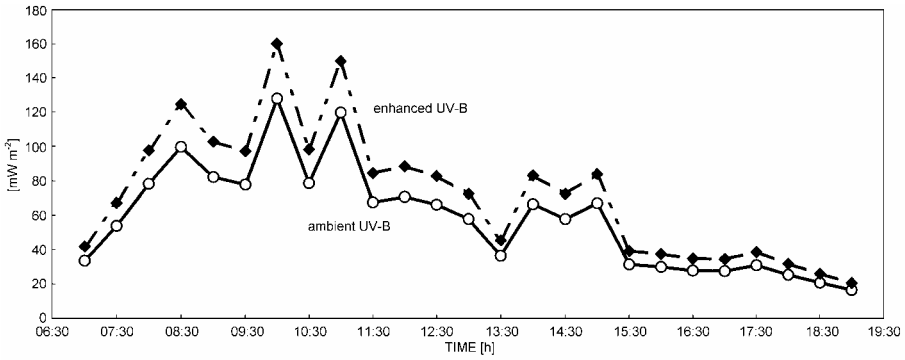

Fig. 1. An example of the daily course of UV-B radiation and its +25 % enhancement produced by the constructed modulated lamp system during a sunny summer day.

Pigment analysis: Samples were prepared from a mixture of adult leaves from the three investigated beech trees. Two leaves from each tree were excised in the morning (between 06:00-07:00). Samples were transported to the laboratory in darkness on a moist cloth and ice at a temperature near to 0 °C. The leaf area was measured as mentioned above. The area leaf mass (ALM) expressed as dry mass [g(DM) m^{-2}] was estimated after 5 h of drying at 105 °C. Aliquots of samples for pigment estimation [*ca*. 100 mg(FM)] were frozen in liquid nitrogen, stored at −18 °C, and analysed within several days. The frozen samples were extracted under dim green radiation with 80 % acetone and a small amount of $MgCO_3$. The supernatant obtained after centrifugation of the extract at 66.7 rps for 3 min was used for spectrophotometric (*UV/VIS 550*, *Unicam*, Cambridge, England) estimation of the pigment contents, *i.e.* Chl *a*, Chl *b*, total carotenoids, and HPLC quantification of individual carotenoids (Lichtenthaler 1987).

To separate pigments, the 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*, Rohnert Park, USA), isocratic pump *Spectra Series P100*, reverse-phase column (5-μm particle size; 25×0.4 cm i.d.; *250/4 RP 18 Lichrocart*, Darmstadt, Germany) with a guard column (*Lichrocart*, Germany), and photodiode-array detector *UV6000LP* (*TSP Analytical*, Arcacle, USA). The supernatant was filtered through an 0.2 μm syringe filter (*PTFE*, *Whatman*, Maidstone, England) and injected using a sample-injection valve (*ECOM*, Praha, Czech Republic) with a 20 mm^3 sample loop. The pigments were eluted isocratically for 10 min with a solvent system consisting of acetonitrile : methanol : Tris (0.1 M) (87 : 10 : 3, v/v), followed by an 11 min isocratic elution with solvent system methanol : hexane (4 : 1, v/v). Total run time was 21 min and the flow rate was 33.3 mm^3 s^{-1}. Absorbance was detected at 400–500 nm and peak areas were integrated using *ChromQuest* software for *Windows NT* (*ThermoQuest*, Miississauga, Canada).

The de-epoxidation state of the xanthophyll cycle pigments (DEPS) was calculated as:

$$DEPS = [A]/2 + [Z]/[V] + [A] + [Z]$$

(Demmig-Adams and Adams 1996, Adams *et al.* 1999), where [V], [A], and [Z] indicate violaxanthin, antheraxanthin, and zeaxanthin concentrations, respectively.

Pigment analysis: Chl *a* fluorescence was measured using a portable Chl fluorometer (*PAM-2000*, *H. Walz*, Effeltrich, Germany). The measurement of Chl *a* fluorescence was performed using the same leaves as for the gas-exchange measurements. Investigated potted trees were transported to the field laboratory located very close to the UV-B lamp system and were pre-darkened for 30 min. Time period of darkness was determined for beech according to recent studies carried out under the same field conditions (Špunda *et al.* 1998). After irradiation by the "analytic light" (less than 1 μmol m^{-2} s^{-1} for estimation

of F_0) a "white" saturation pulse (1 s duration and PPFD 4 000 μmol m^{-2} s^{-1}) was applied. This pulse intensity was sufficient to saturate F'_M. The parameters F_0 (open PS2 reaction centres, RC), F_M (closed PS2 RC), and F'_S (the lowest fluorescence after being irradiated for 5 min by actinic radiation) were then estimated. The value of F'_0 was estimated as the lowest fluorescence level during 10 s of darkness following the irradiation period at each PPFD. To obtain the irradiance responses of ETR, NPQ, and q_P, PPFDs of 60, 90, 130, 220, 330, 460, 630, 950, and 1 300 μmol m^{-2} s^{-1} were provided using the external halogen lamp, which is a part of the *PAM-2000* system. Measurements of fluorescence parameters were carried out at steady state conditions after 15 min of acclimation to each irradiance.

The maximum photochemical efficiency of PS2 (F_V/F_M) was determined according to Havaux *et al.* (1991). The photochemical electron transport rate (ETR) was calculated as: $ETR = \Delta F/F_M \times PAR \times 0.5 \times a$ (Genty *et al.* 1989). The absorption coefficient a = 0.867 was estimated for beech leaves using a spectrophotometer *LI-1800* equipped with an integrating sphere (*LI-COR*, Lincoln, USA). Photochemical quenching coefficient (q_P) was calculated according to Bilger and Schreiber (1986): $q_P = (F'_M - F'_S)/(F'_M - F'_0)$. The non-photochemical quenching of F_M (NPQ) and F_0 (SV_0) were calculated according to the Stern-Volmer formalism (Härtel and Lokstein 1985): $NPQ = F_M/F'_M - 1$; $SV_0 = F_0/F_0' - 1$.

Statistical processing: Three individual trees represented each treatment, *i.e.* control and exposure to enhanced UV-B. Three leaves were measured on each sampled tree. Three replications of measurements of the above mentioned parameters were done on each leaf. The statistical significance of differences between the control and exposed treatments was based on the F- and *t*-tests of mean values, respectively. The zero assumption was the equality of the mean values. The analysis was carried out using the analytical tools of the *STATGRAPHICS* program package.

Results

The data presented were obtained in the third year of the experiment, *i.e.* 2001. The history of the trees at that moment included three full seasons of elevated UV-B exposure. The investigations were carried out during a sequence of hot sunny days of August 2001 (Fig. 2).

Gas exchange: Beech leaves exposed to enhanced level of UV-B (E) showed significantly increased ALM, up to 11 %, in comparison to the control leaves (Table 1). The stimulation of P_N over the whole interval of investigated PPFD was observed in E-leaves compared to the control (C) variant (Fig. 3*A*). The initial slopes of these response curves indicate an increase in apparent quantum yield (α) in E-leaves by 45 %, compared to the C-variant (Table 1). There was a significant increase (up to 35 and 37 %) of the photon saturated photosynthetic rate (P_{Nmax}) and a dark respiration rate (R_D) in beech leaves of the E-variant (Table 1).

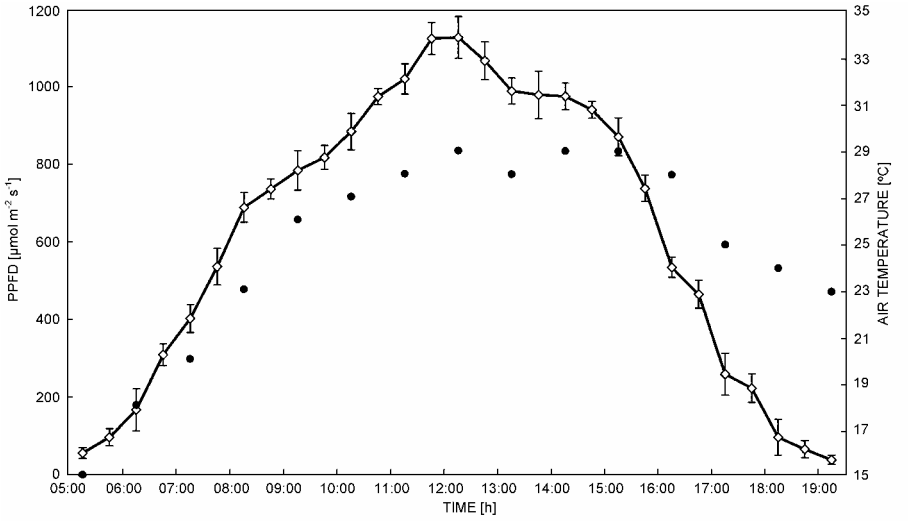

Fig. 2. Solar irradiance and air temperature during a period of summer sunny days, when UV-B response of young beech saplings was studied. *Open diamonds*: photosynthetic photon flux density (PPFD); *black circles*: air temperature (hour means).

Room temperature Chl *a* fluorescence: The noon values of the maximum potential photochemical efficiency of PS2 (F_V/F_M) observed after 30 min of leaf darkening were 0.810±0.012 and 0.780±0.021 for E- and C-variants, respectively. The difference is statistically significant (at $p = 0.05$). However, the occurrence of real PS2 photoin-

hibiton was not observed. The values of electron transport rate between PS2 and PS1 (ETR) showed a positive response to the long-term effects of enhanced UV-B radiation (Fig. 3*B*). The PPFD saturated value of ETR of the E-variant was 1.48 times of that of the control.

The relation between photochemical quenching (q_P) and PPFD (Fig. 3*C*) showed an even more pronounced positive effect of the long-term influence of enhanced UV-B radiation. The PPFD saturated value of q_P in the E-variant was 1.7 times that of the C-variant. The irradiance-induced non-photochemical quenching of F_M fluorescence, NPQ, was remarkably lower in E-variant than in the C-one over the entire range of applied irradiances (Fig. 3*D*). Together with high q_P values this indicates that for the UV-B treatment there was reduced demand on NPQ under higher PPFD. The NPQ reflects the quenching processes localised both within the LHC and the core of PS2 (Gilmore *et al*. 1995, Färber *et al*. 1997) whereas the extent of energy dissipation in the LHC has been related to the quenching of $F_0 - SV_0$ (Adams *et al*. 1999). In agreement with our previous papers (Špunda *et al.* 1998, Marek *et al.* 2001a,b) the presented relations between SV_o and NPQ revealed a linear character for both investigated variants (Fig. 4). The leaves in the E-variant exhibited only a negligibly steeper slope of SV_o-NPQ relation, *i.e*. 1.04, than the leaves in the C-variant. However, the pronounced shift of the relation to higher SV_0 at corresponding NPQ indicated increased contribution of the quenching localised within LHC to the total non-radiative dissipation in the E-variant.

Table 1. Parameters of P_N-PPFD response curve for the beech leaves from the UV-B exposed and control treatments. ALM – area leaf mass [g(DM) m^{-2}]; P_{Nmax} – PPFD saturated P_N [μmol(CO_2) m^{-2} s^{-1}]; α – apparent quantum yield [mol(CO_2) quantum^{-1}]; R_D – dark respiration rate [μmol(CO_2) m^{-2} s^{-1}]. 3 sample trees, 3 measured leaves on each sample tree, 3 replications on each leaf. Means ± S.D. Identical letter in the column: $p = 0.05$.

Treatment	ALM	P_{Nmax}	α	R_D
Control	36.7±2.3 a	9.7±2.2 b	0.022±0.002 c	0.24±0.02 d
Exposed	41.5±1.4 a	13.1±1.6 b	0.032±0.003 c	0.33±0.04 d

Pigment content and composition: No significant effects of enhanced UV-B radiation were observed on the pigment composition of exposed leaves (Table 2). The estimated 1–3 % reduction of the Chl *a*, Chl *b*, and Car *x+c* contents expressed per leaf area in the UV-B exposed leaves is not considered of physiological importance. Thus, essentially the same values of Chl *a/b* ratio and Chl *a+b*/Car *x+c* ratio for the UV-B exposed and control plants indicated that there was no difference in the pigment-protein composition.

Table 2. Content of photosynthetic pigments [g m^{-2}] of beach leaves from the UV-B exposed and control treatments. Chl – chlorophyll; Car x+c – total carotenoids. Means±SD. 3 sample trees, 3 measured leaves on each sample tree, 3 replications on each leaf.

Treatment	Control	Exposed
Chl *a+b*	0.167±0.007	0.162±0.014
Car x+c	0.041±0.002	0.040±0.003
Chl *a/b*	2.890±0.057	2.869±0.018
Chl/Car	4.105±0.183	4.040±0.143

The relative contents of neoxanthin (N), lutein (L), and xanthophyll cycle pool (VAZ), all expressed per Chl *a+b*, were almost identical in both variants (Fig. 5*A*). Only the β-carotene content was reduced by 14 % in UV-B exposed plants, however, this difference was not significant. On the contrary, the composition of the VAZ pool was significantly different (Fig. 5*B*). Whereas in the control leaves violaxanthin (V) accounted for more than 75 % of the VAZ pool, in the UV-B plants it was less than 60 %. Consequently, the contributions of antheraxanthin (A) and especially zeaxanthin (Z) to the entire VAZ pool in UV-B plants were significantly higher ($p = 0.05$), *i.e*. 1.61 and 2.14 times that of the control leaves. Thus, the de-epoxidation state of the xanthophyll cycle (DEPS) was nearly doubled in comparison with control leaves. As the samples for pigment analysis were collected in early morning and darkened for an hour before freezing in liquid nitrogen, the content of de-epoxidised xanthophylls was attributed mainly to the long-term accumulation of A and especially Z rather than to actual conversion of V at the beginning of the day.

Discussion

UV-B induced stimulation of photosynthetic activities: The present long-term experiments with beech growing under elevated UV-B radiation support the view that different responses may occur with different species (Ziska *et al.* 1993, Fiscus and Miller 1994). The observed stimulation of P_N in beech E-leaves is in accordance with results reported by Robakowski and Laitat (1999). However, these results are opposite to the findings of Sullivan and Teramura (1990, 1992). Increased leaf density represented by the ALM values observed in UV-B exposed leaves is consistent with literature data which show UV-B radiation induced changes in leaf morphology (Murali

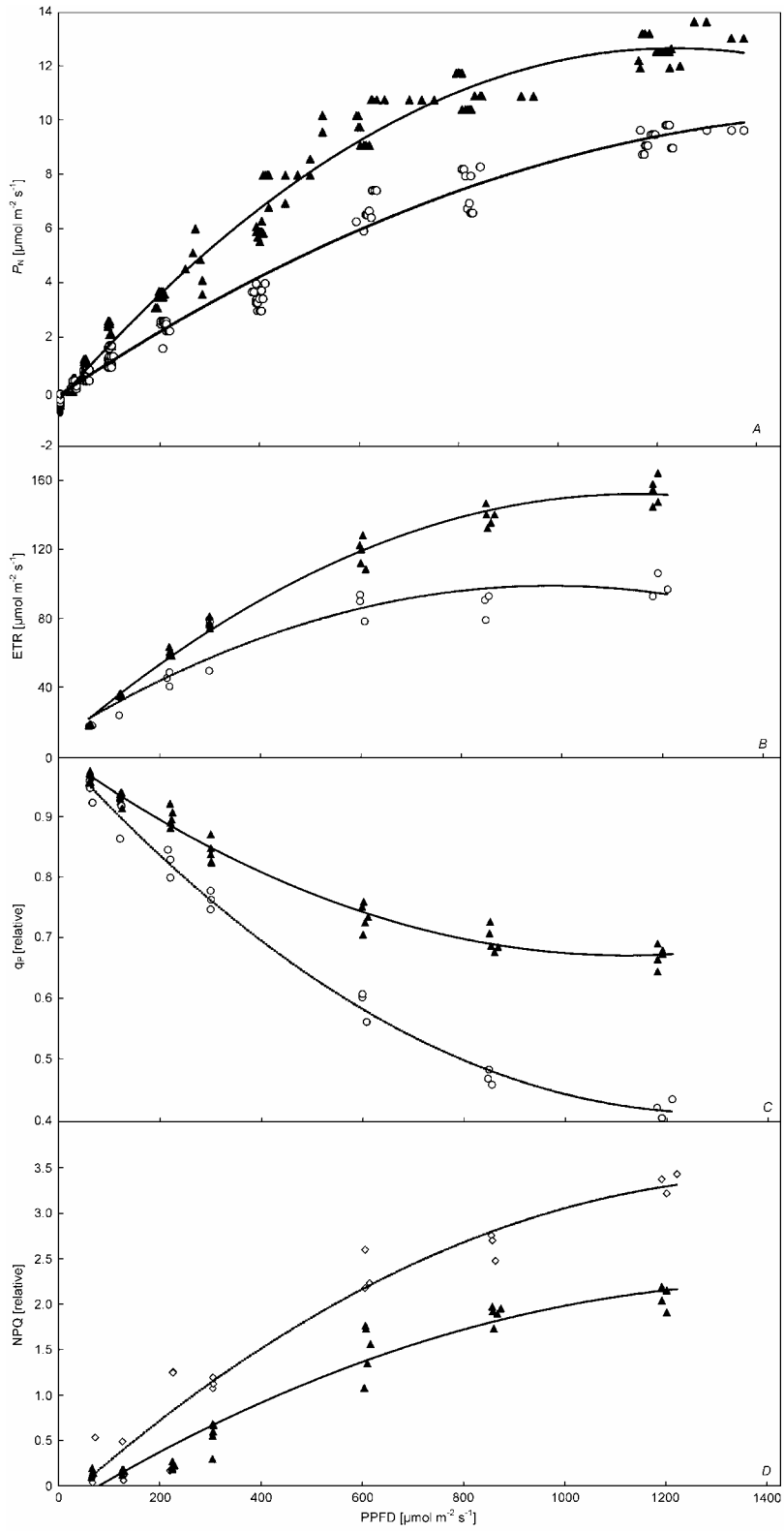

Fig. 3. Relationship between (*A*) net photosynthetic rate of leaves (P_N), (*B*) electron transport rate (ETR), (*C*) photochemical fluorescence quenching (q_P), or (*D*) non-photochemical fluorescence quenching (NPQ) and incident photosynthetic photon flux density (PPFD) for the beech leaves from the UV-B exposed and control treatments. *Black triangles*: UV-B exposed leaves; *open circles*: control leaves. Conditions of measurements: temperature of leaves 20±2 °C; relative air humidity 55+3 %; CO_2 concentration 340±10 μmol mol^{-1}. 3 sample trees, 3 measured leaves on each sample tree, 3 replications on each leaf.

and Teramura 1986, Caldwell *et al.* 1998, Krupa *et al.* 1998). The observed higher P_N in E-leaves may be partly attributed to increased density of these leaves (Fig. 3*A*).
The saturated part of the P_N-PPFD response curve, *i.e.* P_{Nmax}, represents the ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO) limitation of assimilation. The apparent quantum yield (α) can be interpreted as the RuBP regeneration limitation of assimilation (Farquhar *et al.* 1980, Brooks and Farquhar 1985). Significant differences in values of α and P_{Nmax} between C- and E-leaves were observed in our experiment (Table 1). A comparison of the degree of these differences indicates practically the same changes at the level of RuBP regeneration (increase of the α value up to 38 %) and RuBPCO activity limitation (increase of P_{Nmax} value up to 35 %). Contrary to the findings of Sisson and Caldwell (1976) and Vu *et al.* (1984) of the direct negative effects of 25 % UV-B enhancement on RuBPCO content and activity in various C_3 and C_4 plants, a positive effect was found in our experiment.

In accordance to the results published for UV-B exposed loblolly pine under comparable experimental conditions (Naidu *et al.* 1993), we observed a significant effect on dark respiration rate (R_D) (Table 1). It was not clear whether this was because of increased repair processes' actions or if R_D was simply affected by the UV-B irradiance used in our experiment. A similar tendency of increased R_D compared to the control under the short-term influence of UV-B as observed in our experiment was reported for *Rumex* (Sisson and Caldwell 1976). Generally, increased R_D and P_N can be interpreted as an indicator of higher metabolic activity.

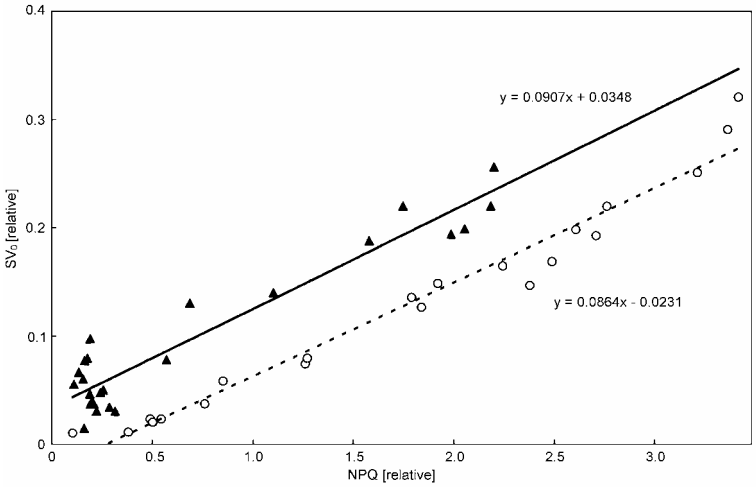

Fig. 4. Relationship between the non-photochemical fluorescence quenching of F_0 (SV_0) and non-photochemical fluorescence quenching of F_M (NPQ) for the beech leaves from the UV–B exposed and control treatments. *Black triangles*: UV-B exposed leaves; *open circles*: control leaves. For conditions of measurements see Fig. 3.

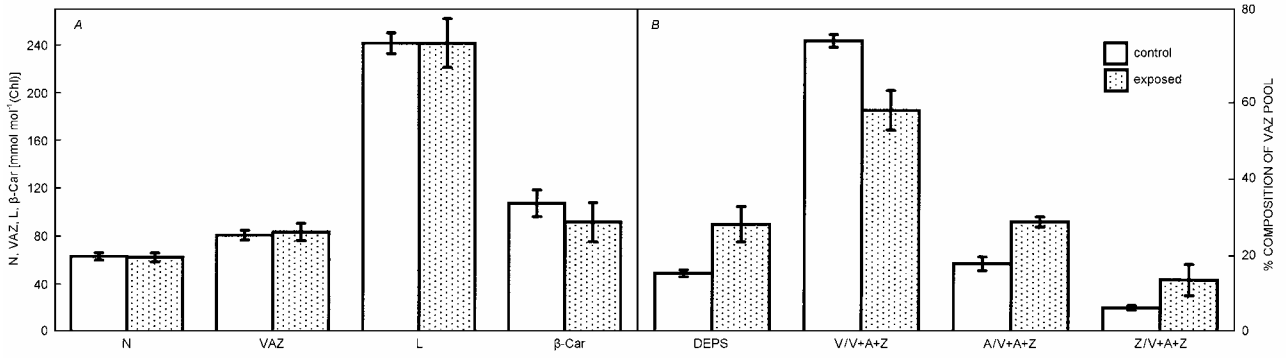

Fig. 5. (*A*) Composition of carotenoids in beech leaves from the UV-B exposed and control treatments. Carotenoid contents expressed on a total chlorophyll basis. β-Car – β-carotene, L – lutein, N – neoxanthin, VAZ – sum of violaxanthin (V) + anteraxanthin (A) + zeaxanthin (Z). (*B*) Per cent expression of the de-epoxidation state of the the xanthophyll cycle pool (DEPS); and of the ratios of the violaxanthin (V/V+A+Z), antheraxanthin (A/V+A+Z), and zeaxanthin (Z/V+A+Z) to the xanthophyll cycle pool in beech leaves from the UV-B exposed and control treatments. HPLC analysis was performed on the dark adapted leaves. 3 sample trees, 3 measured leaves on each sample tree. Means from 9 measurements ± SD.

The stimulation of CO_2 assimilation qualitatively corresponded to an enhanced electron transport rate (ETR, Fig. 3*B*) and increased photochemical quenching of Chl *a* fluorescence (q_P, Fig. 3*C*) over the entire range of applied irradiances. Teramura *et al.* (1980) showed that exposure of soybean to enhanced UV-B at low PPFD resulted in a significant decrease of photosynthetic activity as compared to that at high PPFD. Our results were obtained with sun-exposed leaves of beech saplings that were frequently exposed to PPFD higher than 1 000 μmol m^{-2} s^{-1} (Fig. 2) with a typical spectral composition of direct sunlight, *i.e*. dominating blue-green spectral region (data not shown). Therefore, our results support the idea that high PPFD enriched in photons of 320–520 nm may induce repair mechanisms for UV-B induced damage and allows components of carbon assimilation to attend higher levels than in control plants (Klein 1978, Vu *et al.* 1984).

Effect of enhanced UV-B on PS2 resistance to photoinhibition: UV-B may cause preferential inactivation of PS2, whereas PS1 activity remains mostly unaffected (Naidu *et al.* 1993, Tevini 1994, Greenberg *et al.* 1996). Therefore, the long-term exposure to enhanced UV-B may increase sensitivity of photosynthetic apparatus to high PPFD stress (Bornman 1989, Pfündel *et al.* 1992). Bornman and Vogelmann (1991) reported that enhanced UV-B induced proportional reduction of pigment content and decrease of variable Chl *a* fluorescence, both related to the PS2 lesion and inactivation. Although the present experiments were done during sunny and warm summer days, no signs of severe high irradiance stress were observed in measurements of F_V/F_M in both E- and C-variants, and Chl content of beech leaves was almost identical regardless of the treatment (Table 2). No reduction of pigment content was observed in other samples during the three years of exposure of beech saplings to elevated UV-B. The noon values of F_V/F_M determined after 30 min of darkness were 0.81 and 0.78 for E- and C-variants. This indicated that there was only a small difference in the proportion of inactivated PS2 RCs (small contribution of the slowly relaxing quenching of the variable fluorescence related to the photoinhibited PS2 RCs). Although F_V/F_M was only by 4 % higher in UV-B treated plants in comparison with control, the difference of this magnitude may indicate a slight but significant change of PS2 function. For instance, plants acclimated to a moderate high irradiance stress reveal F_V/F_M typically around 0.80–0.81, that is just 3 % lower than in the plants grown under optimal conditions (Bolhàr-Nordenkampf *et al.* 1991, Kurasová *et al*. 2000). Similar decrease of F_V/F_M reflected the development of acclimation depression of photosynthesis during exposure of Norway spruce to elevated CO_2 (Kalina *et al*. 2000).

The sun exposed leaves of the control beech plants revealed a typical gradual increase of PS2 acceptor side reduction upon increasing irradiance (Fig. 3*C*) as observed earlier for sun exposed leaves of *Picea abies* (Špunda *et al*. 1998) and *Quercus ilex* (Marek *et al*. 2001a). A low q_P around 0.4 indicating a severe degree of PS2 over-reduction (Demmig-Adams 1990, Demmig-Adams and Adams 1996) was reached at irradiances of 800 and 1 200 μmol m^{-2} s^{-1}. On the contrary, for UV-B exposed plants, q_P values around 0.7 were obtained even at the highest applied irradiances, that reflected an extremely high capacity of the E-tratment to maintain PS2 reaction centres in the open state (Fig. 3*C*). Therefore, the slight stimulation of F_V/F_M may be related to the ability of UV-B exposed leaves to avoid PS2 over-reduction even at the highest applied irradiances, indicating that even under the field conditions the probability of PS2 RC photoinactivation was significantly reduced.

Xanthophyll cycle in relation to the photochemical efficiency and non-radiative dissipation in UV-B treated plants: Although the size of xanthophyll cycle pool and content of other xanthophylls was almost the same in control and UV-B treated plants (Fig. 5*A*), the latter exhibited nearly doubled de-epoxidation state of the xanthophyll cycle estimated in night in comparison with control (Fig. 5*B*). Usually, a sustained accumulation of A and Z is related to the slowly reversible suppression of F_V/F_M preventing PS2 photodamage (Adams *et al*. 1995). Recently, we have found a negative linear correlation between de-epoxidation state in dark-adapted leaves of barley grown at different irradiances and F_V/F_M (Kurasová *et al.* 2002). This and many other papers support a suggestion that nocturnally retained Z remains engaged in a state primed for energy dissipation. Here, on the contrary, the increased accumulation of Z+A in UV-B treated plants (Fig. 5*B*) was accompanied with increased quantum efficiency of PS2 photochemistry. Barker *et al.* (2002) found that under summer conditions nearly 80 % of Z+A was accompanied with a slightly sub-optimal F_V/F_M for *Yucca* species growing in the Mojave Desert. Although our results were obtained under much more moderate climate, this is, to our knowledge, the first report of enhanced accumulation of de-epoxidised xanthophylls being inversely related to PS2 efficiency. It supports the idea that the Z+A retention following the night period and the Z+A involvement in the PS2 down-regulation are separate processes that are controlled by separate factors.

Whereas NPQ reflects the quenching processes localised both within the LHC and the core of PS2 (Gilmore *et al*. 1995, Färber *et al.* 1997), the extent of energy dissipation in LHC has been related selectively to the non-radiative quenching of F_0 characterised by the value of SV_0 (Demmig-Adams *et al*. 1996). In our previous papers a slope of SV_0-NPQ relation was correlated to the capacity of non-radiative dissipation within LHC (Špunda *et al.* 1998, Marek *et al.* 2001a,b) and also roughly to the VAZ pool size. In agreement with these reports, the same slope of SV_0-NPQ relation was observed for both E- and

C-variants, corresponding to the identical VAZ size (Fig. 4). However, the consistently higher SV_0 at corresponding NPQ indicated increased contribution of the quenching localised within LHC to the total non-radiative dissipation in the E-variant (Fig. 4). This indicates that accumulated Z+A facilitates the prompt light-induced non-radiative dissipation within LHC, thus preventing long-term photoinhibition of the PS2 RCs. Consequently, the reduced NPQ observed for the E-plants over the entire range of applied irradiances (Fig. 3*D*) may be attributed to considerably reduced contribution of the inactivated RCs to the NPQ. Although usually an acclimation to high irradiance is accompanied with increased quenching of both SV_0 and NPQ (Brugnoli *et al.* 1998, Špunda *et al.* 1998), for other species such as barley reduced NPQ is induced in high-irradiance grown plants in comparison to the low-irradiance ones, in spite of enhanced SV_0 and VAZ pool size (Kurasová *et al.* 2000, 2002). However, in these papers an increased extent of prolonged accumulation of Z+A was caused by excess PPFD as judged from low q_P at the growth irradiance (Kurasová *et al.* 2002). As already mentioned, in UV-B exposed plants a remarkably higher q_P, in comparison to the C-treatment was obtained even at the highest applied irradiances (Fig. 3*C*). This excludes the possibility that retained Z+A was caused by permanent PS2 over-reduction. Thus another factor has to be involved in accumulation of de-epoxidised xanthophylls in UV-B treated beech leaves. Z fulfils also other functions in stress physiology. It is involved in protection of thylakoid lipids against oxidative stress (Tardy and Havaux 1997), serves as a photoreceptor in the blue region of visible spectrum (Srivastava and Zeiger 1995), and is an intermediate in abscisic acid biosynthesis (Marin *et al.* 1996). We speculate that particularly the last function may be a good candidate for UV-B induced reduction of xanthophyll epoxidation as the ABA2 protein may function as a zeaxanthin epoxidase (Marin *et al.* 1996).

Conclusion: The results indicate a positive effect of 25 % enhancement of UV-B radiation on photosynthetic characteristics, as well as on the capacity for utilisation of absorbed photons in PS2 photochemical reactions of beech leaves. Investigations of the gas-exchange parameters suggest that the UV-B stimulation is partially related to increased leaf thickness. The significantly higher retention of the de-epoxidised xanthophylls contributes to the increased resistance of PS2 in UV-B treated plants against photoinhibition. This is to our knowledge the first example that elevated de-epoxidation may be connected to a significant increase of PS2 photochemical efficiency. It suggests that accumulation of A and Z may be driven also by other factors than excess irradiance itself. We suggest that pronounced stimulation of photosynthetic CO_2 assimilation and PS2 photochemical reactions under long-term exposure of beech saplings to enhanced UV-B radiation were related to the fact that other environmental factors were within the range of their physiological optimum. Particularly, the avoidance of drought was achieved due to regular irrigation of the plants.

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