

Ecophysiological Investigation of UV-B Tolerance of Beech Saplings (*Fagus sylvatica* L.)

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Abstract – Our work aimed to reveal UV-B sensitivity of beech both in field, and in outdoor conditions. We examined the role of UV-B radiation in activation of photoprotective mechanisms in photoinhibition and photodamage of photosynthetic apparatus under high light intensity. Our results indicate that under natural irradiances increase of carotenoid content - especially xanthophyll cycle pigments - and xanthophyll cycle activity resulted in not only by visible light but also by UV-B radiation. These pigments have important role in photoprotective processes of photosynthetic apparatus. Amount of these pigments decreased under UV-B exclusion, increased under UV-B exposure, similarly to the UV-B absorbing compounds, which responded susceptibly to changes of UV-B level. These results may suggest that high UV-B tolerance of beech is due to the considerable flavonoid accumulation, which may explain practically unaltered physiological activity of photosynthetic apparatus under UV-B exposition, as these compounds have antioxidant capacity, thus they can reduce lipid peroxidation and damage of photosynthetic apparatus.

enhanced UV-B / UV-B exclusion / photosynthetic pigments / chlorophyll fluorescence / UV-B absorbing compounds

Kivonat – A bükk (*Fagus sylvatica* L.) UV-B érzékenységének ökofiziológiai vizsgálata. Munkánk során az UV-B sugárzás (természetes és emelt szintű) hatásait vizsgáltuk egy hazai erdőalkotó fafaj, a bükk újulatainak ökofiziológiai válaszreakcióira termőhelyi feltételek között és szabadföldi kísérletben. Tanulmányoztuk, hogy a levelekben a fényvédő mechanizmusok aktiválásában, illetve az intenzív napsugárzás alatt fellépő fotoszintézis gátlásban és a fotoszintetikus apparátus károsodásában a látható tartomány mellett milyen mértékben játszik szerepet a természetes UV-B sugárzás. Vizsgáltuk azt is, hogy az emelt szintű UV-B sugárzással szemben milyen a bükk levelekben a fotoszintetikus apparátus védelmét ellátó mechanizmusok hatékonysága.

Eredményeink azt mutatják, hogy természetes fényviszonyok között a fényvédő karotinoidok, ezen belül a xantofill-ciklus pigmentjeinek mennyisége és a ciklus aktivitása nemcsak a látható fény, hanem az UV-B sugárzás hatására is változik. UV-B megvonásnál a levelekben csökkent a mennyiségük, emelt szintű UV-B hatására nőtt, ahogyan az megfigyelhető volt a flavonoid típusú vegyületek esetében is, amelyek a legérzékenyebben reagáltak az UV-B szint megváltozására, és mivel UV-B szűrő képességük mellett antioxidáns tulajdonsággal is rendelkeznek így a fotoszintetizáló szövetek aktivitása viszonylag változatlan maradt.

emelt szintű UV-B sugárzás / UV-B megvonás / fotoszintetikus pigmentek / klorofill fluoreszcencia / UV-B szűrő pigmentek

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1 INTRODUCTION

Owing to the drastic decrease of stratospheric ozone layer in the last two decades studies rightly focused on examination of the potential destructive and inhibiting effects of UV-B radiation (Madronich et al. 1998). In spite of the efforts to restrict the production of ozone depleting substances in the 1990s, thinning of the stratospheric ozone layer and increased penetration of UV-B radiation to the Earth surface will continue. The annually averaged global ozone loss is approximately 3%, but it is not equable over the two hemisphere (McKenzie et al. 2003). Severe declines will occur in years 2010-2019 in the northern hemisphere, that may result up to 50-60% increase in UV-B radiation in springtime (Schindell et al. 1998). Beside ozone depletion UV-B radiation reaching the Earth's surface is influenced by global climate change (clouds, snow-cover), air pollutants, aerosols furthermore slowing ozone recovery due to the warming of troposphere (McKenzie et al. 2003).

UV-B radiation is an important stress factor for plants, which can have direct and indirect effects on the genetic system, the photosynthetic apparatus, and membrane lipids (Björn 1996). UV-B may also play an important ecological role in altering plant growth and competitive ability, with a resultant impact on plant community composition (Sullivan 2005). These indirect effects include changes in the susceptibility of plants to attack by insects and pathogens in both agricultural and natural ecosystems, and changes in nutrient cycling (Caldwell et al. 1998).

Few studies have been carried out on temperate, angiosperm tree species under natural conditions, in spite of that long lived trees may be the most impacted by the changing present-day levels of UV-B radiation owing to the permanent exposure and the accumulation of the effects (Johanson et al. 1995). Plants under field conditions have to cope with several stress factors. Protective mechanisms of well adapted species (to high light intensity, high temperature, water deficit etc.) may be effective against increasing UV-B radiation (Mészáros et al. 2001). However plant species - moreover populations - vary greatly in their response to UV-B: negative, neutral as well as positive effects on plant performance have been reported, which suggest that some plant species may be well adapted to UV-B radiation while others are not. Furthermore the plant response to UV-B seems to depend on experimental set up, treatment regimes and duration (Searles et al. 2001).

Our work aimed to examine possible harmful effects of present and enhanced UV-B radiation on ecophysiological responses of beech (*Fagus sylvatica* L.) both in field, and in outdoor conditions. European beech is one of the major tree species in natural plant associations in Central Europe, and it is characterized by substantial capacity to acclimate to high light intensities. We examined the role of UV-B radiation in activation of photoprotective mechanisms (xanthophyll cycle), furthermore in photoinhibition and photodamage of photosynthetic apparatus under high light intensity. We also studied under enhanced UV-B the efficiency of photoprotective mechanisms in beech leaves, which provide defence for photosynthetic apparatus. In both experimental sites our attention was focused on indicators of UV-B sensitivity and tolerance (Smith et al. 2000), namely changes of photosynthetic pigment composition (chlorophylls and carotenoids), chlorophyll fluorescence parameters of dark and light adapted leaves, and accumulation of UV-B absorbing compounds. We also examined activity of photoprotective xanthophyll cycle, furthermore changes of water content in leaves, mesophyll succulence index and specific leaf mass, which indicate alterations in leaf anatomy.

2 MATERIALS AND METHODS

2.1 UV-B exclusion in the forest site

Field studies were performed in a beech forest site (“Rejtek Research Site”, Bükk Mountains, NE Hungary), where in 1981 a part of the 80 years old forest has been clear-cut for research purposes. In order to examine effects of ambient UV-B radiation in the clear-cut area before budding time beech branches were covered with perforated polyester plastic film, which manipulated the spectral balance of natural irradiance, that is UV-B/PAR ratio considerably decreased. This treatment provided comparable growing conditions for shoots and leaves. Effects of UV-B exclusion (90% \leq) were determined on seedlings growing in the clear-cut area, and in parallel with it comparative measurements were carried out in the southern exposed forest edge and forest interior.

2.2 Outdoor UV-B manipulation in the Botanical Garden

The experiments on UV-B exposure and exclusion have been performed in an outdoor experimental site at the Botanical Garden of Debrecen University. Two year old beech seedlings were planted into 4 l plastic containers and were buried to 30 cm depth in the sandy soil of 1.5 \times 1.5 m plots. A set of the plants were exposed to enhanced UV-B radiation besides the natural radiation using timer-controlled UV-B supplementation system (fluorescent tubes type UV-B 313, Q-Panel, Cleveland, USA). The tubes were wrapped with 0.1mm cellulose acetate filter (Courtaulds, Chemicals, Derby, UK) to eliminate UV-C radiation and it was changed weekly. In the control plot plants received only natural solar radiation. Plants were exposed to UV-B radiation at three intensity levels following daily fluctuation of natural irradiance. Midday maximum of extra UV-B was approximately 40% higher (+80 μ W cm $^{-2}$) than the ambient level. Concerning the experimental conditions in other UV-B studies it can be regarded as a moderately elevated UV-B dose (McLeod 1997).

For UV-B exclusion seedlings were placed under a roof-shaped tent covered with polyester film. The northern part of the frame was uncovered by foil and slits were left in order to provide adequate air circulation. During the experiments (2000-2002) the water supply of the seedlings was equal in all plots. UV-B supplementation and exclusion treatments started before budding stage and it ran to September.

2.3 Chlorophyll fluorescence measurements

In vivo chlorophyll *a* fluorescence was measured with a portable PAM 2000 fluorometer (WALZ Germany) after 20 min dark adaptation period. Fast (F_0 , F_m , F_v/F_m) and slow ($\Delta F/F_m'$, NPQ, RFD) chlorophyll fluorescence induction parameters were calculated by the equations of Schreiber et al. (1994). Slow chlorophyll fluorescence induction was analysed by saturation pulse method after 5 minutes illumination of the leaves with two different actinic light intensities (200 and 1000 μ mol m $^{-2}$ s $^{-1}$).

2.4 Photosynthetic pigments and UV-B absorbing compounds

Photosynthetic pigments were extracted from the leaves with 80% acetone. Absorbances of pigment extract were measured at 470; 646,8; 663,2 nm with Shimadzu UV/VIS 1601 spectrophotometer. Equations of Wellburn (1994) were used to calculate the chlorophyll *a* and *b* concentrations. Carotenoid composition was analyzed by reverse phase HPLC (UV/VIS HPLC, Jasco, Japan; Eluents: ethylacetate, acetonitrile: water 9:1; Column: Nucleosil C18, 5 μ) method (Mészáros et al. 1995) with application of zeaxanthin standard. Chlorophyll content was expressed on dry weight (mg g $^{-1}$), carotenoids and xanthophyll cycle pigments were

expressed on chlorophyll content (mmol mol^{-1} chl a+b). The de-epoxidation state of xanthophyll cycle (Demmig-Adams - Adams 1992) was calculated as

$$\text{DEEPS} = (\text{zeaxanthin} + 0.5 \times \text{antheraxanthin}) / (\text{violaxanthin} + \text{antheraxanthin} + \text{zeaxanthin}).$$

Accumulation of UV-B absorbing compounds in leaves was determined spectrophotometrically from acidified methanol extract (Day 1993). Flavonoid accumulation was expressed as cumulative absorbance of leaf extract at 280-300 nm related to leaf dry weight (g), fresh weight (g) and leaf unit area (cm^2) suggested by Qi et al. (2003).

Actual water content of leaves was determined by thermogravimetric method, after drying at 85°C till weight ceaselessness. Water content of samplings were expressed on fresh leaf weight (WC%). Mesophyll succulence was determined by chlorophyll and water content ($\text{gH}_2\text{O mg}^{-1}$ chl).

2.5 Statistical analysis

Effects of UV-B exclusion and enhanced UV-B were evaluated by one-way ANOVA. Multiple range tests (95% confidence intervals method) were performed to assess differences between the treatments and to identify homogenous groups (LSD, Tukey-b test). Significant differences were determined at $*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.001$. Effects of different light conditions on photosynthesis physiological parameters were analysed using multivariate statistical methods. Classification methods (discriminant analysis) were used for reconnaissance of the similarity using SPSS 11.0 software.

3 RESULTS

3.1 Effects of UV-B exclusion in field and in outdoor experiment

In field it could be generally observed that under UV-B exclusion leaves had lower chlorophyll *a/b* ratio, total carotenoid content, amount of photoprotective xanthophyll cycle pigments, mesophyll succulence and accumulation of UV-B absorbing compounds. Moreover in young leaves activity of the xanthophyll cycle (DEEPS) was also lower than in leaves under natural irradiances. We have shown that significant effects of UV-B exclusion was considerable on young leaves in contrast to the results from the whole growing season, because during senescence differences between leaves under UV-B exclusion and ambient UV-B might be reduced (Láposi et al. 2002). Besides well-known daily changes of some parameters (chlorophyll and water content, DEEPS, F_v/F_m) we also obtained an increase in VAZ/total carotenoid ratio parallel with a decrease in β -carotene/total carotenoid ratio in beech leaves, moreover flavonoid content of leaves was also higher at noon. Daily changes of some parameters were different under UV-B exclusion and under ambient UV-B. In UV-B excluded beech leaves midday decrease of chlorophyll and water content were less expressed, but increase of flavonoid content and decrease of F_v/F_m were larger extent than in leaves under ambient UV-B. UV-B exclusion did not affect daily changes of other examined physiological parameters in beech leaves (*Figure 1*).

From May to September we obtained less extent of decrease in chlorophyll and water content (thus increase in mesophyll succulence) in leaves under UV-B exclusion compared to the leaves under ambient UV-B. Furthermore under UV-B exclusion we have found that midday maximum of DEEPS in beech leaves decreased during the growing season (*Figure 2*).

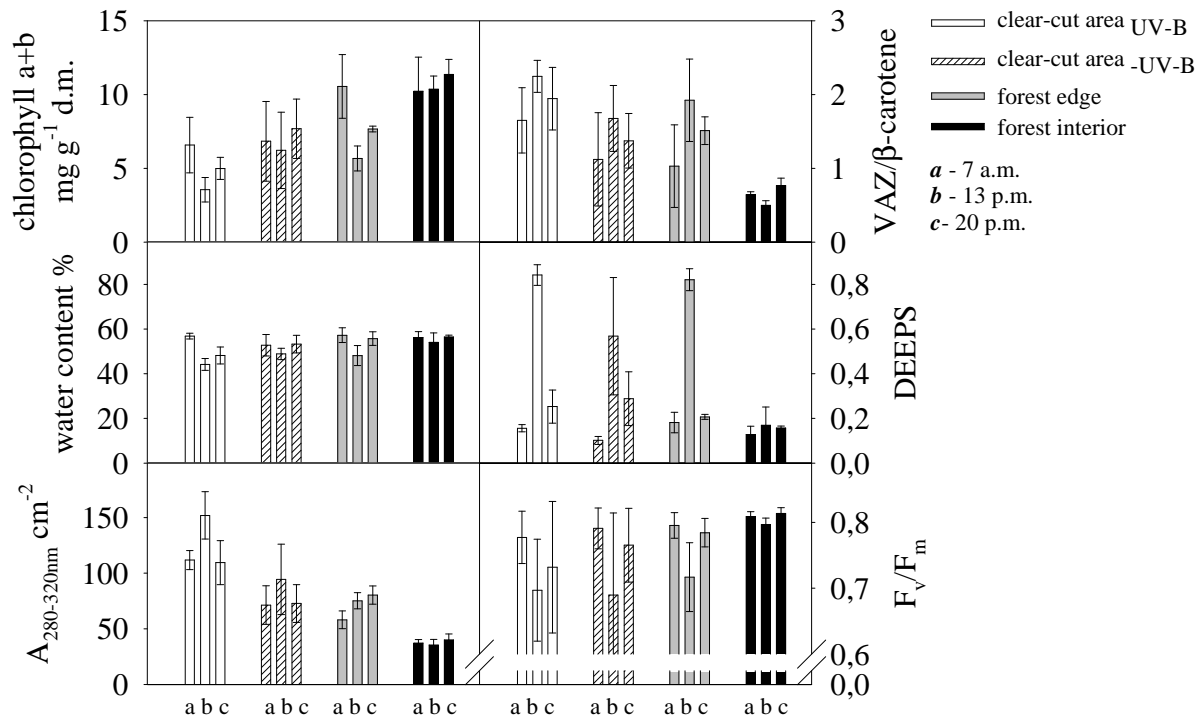


Figure 1. Daily changes of measured parameters of beech leaves in the field experiment (Rejtek Research Site, 08/07/2002) ($n=6\pm SD$).

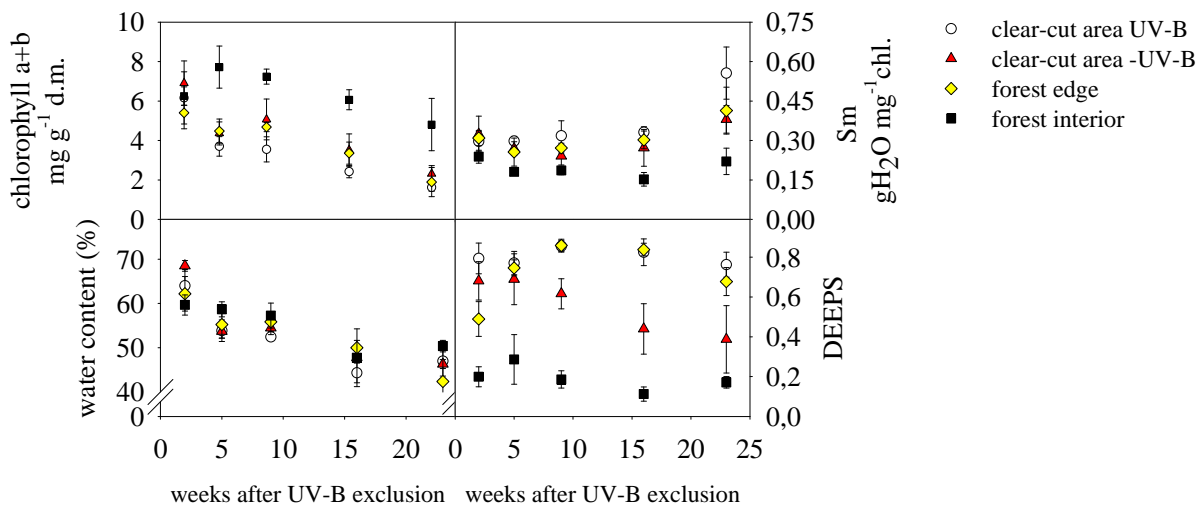


Figure 2. Seasonal changes of midday values of measured parameters in the field experiment (Rejtek Research Site, 2000) ($n=3-6\pm SD$).

In outdoor experiment effects of UV-B exclusion were non significant on the photosynthetic pigment composition and photochemical activity of beech leaves, but at noon the amount of UV-B absorbing compounds was lower than in leaves under ambient UV-B (Láposi et al. 2002). It was very hard to establish obvious significant changes on basis of dark adapted chlorophyll fluorescence parameters. In field beech leaves under UV-B exclusion had higher basal (F_0) and maximal fluorescence (F_m), furthermore higher maximal photochemical efficiency of PSII (F_v/F_m) based on morning values, which probably caused by higher amount

of chlorophyll in leaves. At noon under high light intensity differences decreased between leaves under different light climate (*Figure 1*). In outdoor experiment differences between morning and midday values of F_m and F_v/F_m decreased during growing season especially under UV-B exclusion (*Figure 3*).

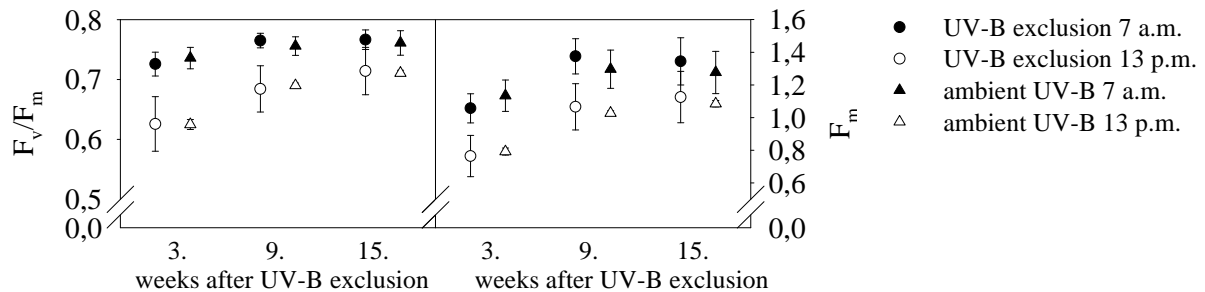


Figure 3. Daily and seasonal changes of maximal chlorophyll fluorescence (F_m) and maximal photochemical efficiency of PSII (F_v/F_m) in beech leaves in the outdoor experiment (Botanical Garden, 2002) ($n=30-78 \pm SE$)

On basis of chlorophyll fluorescence parameters of light adapted leaves we have found that beech leaves under UV-B exclusion had lower actual photochemical efficiency ($\Delta F/F_m'$) and non photochemical fluorescence quenching (NPQ) in both experimental sites. NPQ and $\Delta F/F_m'$ were significantly negatively correlated, demonstrating the competition of the photochemical and non-photochemical processes (*Figure 4*).

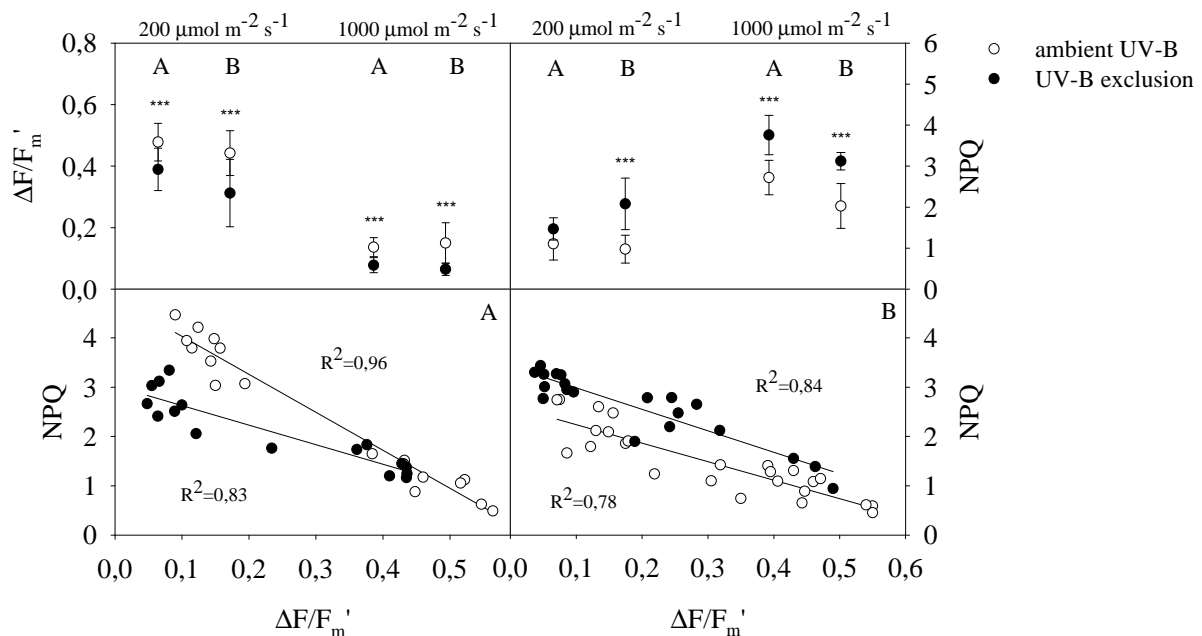


Figure 4. Chlorophyll fluorescence parameters of light adapted leaves after 3 months of treatments. (A-Rejtek Research Site, 2002; B-Botanical Garden, 2002) ($n=7-13 \pm SD$).

3.2 Effects of enhanced UV-B radiation in outdoor experimental conditions

Results of discriminant analysis on basis of photosynthetic pigment composition and parameters of slow chlorophyll fluorescence induction show clear difference between leaves under ambient UV-B and UV-B exposition, while on basis of parameters of fast chlorophyll fluorescence induction leaves under different light climate were widely similar (Láposi et al. 2005). Under UV-B exposition beech leaves had lower chlorophyll and water content, higher chlorophyll *a/b* ratio, higher flavonoid and total carotenoid content (especially *vaz-pool*), and at noon activity of xantofill cycle (DEEPS) was also higher than leaves in the control plot (Láposi et al. 2001).

UV-B treatment did not influence significantly either basal (F_0) and maximal fluorescence (F_m), or maximal photochemical efficiency of PSII (F_v/F_m) (Table 2). However in beech leaves difference between morning and midday values were higher under UV-B exposure, moreover in the end of the growing season midday value of F_v/F_m was lower in the treated leaves than in the control leaves (Láposi et al. 2005). Actual photochemical efficiency ($\Delta F/F_m'$) of UV-B treated beech leaves was lower, non photochemical fluorescence quenching (NPQ) was higher than in the control leaves, while vitality index (RFD) was not affected by enhanced UV-B (Table 1).

Table 1. Effects of enhanced UV-B radiation on the chlorophyll fluorescence parameters of beech leaves in the outdoor experiment. Means \pm SD from the three experiments are given ($n=13-399$). Data were tested by one-way ANOVA (* $P\leq 0.05$, ** $P\leq 0.01$, *** $P\leq 0.001$) (UV-B_{amb}: ambient UV-B; UV-B_{enh}: enhanced UV-B).

		2000		2001		2002			
		UV-B _{enh}	UV-B _{amb}	UV-B _{enh}	UV-B _{amb}	UV-B _{enh}	UV-B _{amb}		
F_0	ns	0,35 \pm 0,04	0,34 \pm 0,04	ns	0,32 \pm 0,02	0,32 \pm 0,03	ns	0,31 \pm 0,03	0,31 \pm 0,03
F_v/F_m	ns	0,77 \pm 0,02	0,77 \pm 0,02	ns	0,76 \pm 0,04	0,76 \pm 0,04	ns	0,72 \pm 0,06	0,72 \pm 0,06
F_m	ns	1,55 \pm 0,21	1,53 \pm 0,21	ns	1,38 \pm 0,21	1,37 \pm 0,24	*	1,16 \pm 0,23	1,11 \pm 0,21
RFD	ns	3,32 \pm 0,8	3,24 \pm 0,76	***	2,79 \pm 0,4	3,32 \pm 0,48	***	2,68 \pm 0,52	3,20 \pm 0,76
$\Delta F/F_m'$	***	0,26 \pm 0,17	0,29 \pm 0,19	ns	0,32 \pm 0,19	0,34 \pm 0,19	ns	0,30 \pm 0,19	0,33 \pm 0,19
NPQ	**	2,25 \pm 1,08	2,08 \pm 1,2	***	2,01 \pm 1,04	1,51 \pm 0,85	ns	1,87 \pm 1,12	1,55 \pm 0,8

Seasonal decrease of values of these parameters was more considerable in the UV-B treated leaves compared to the control leaves (Láposi et al. 2005). Results of discriminant analysis based on measured physiological parameters show that beech leaves under different light climate (ambient UV-B and UV-B exclusion) were more different from each other in field - where other stress factors also occurred - than in outdoor conditions.

In outdoor conditions discriminant analysis also detected a clear difference between UV-B exclusion and UV-B exposition, while leaves under ambient UV-B were more similar to leaves under enhanced UV-B than to reduced UV-B which emphasize the effects of present-day UV-B levels (Table 2).

Table 2. Classification results of discriminant analysis (%) based on the measured ecophysiological parameters of leaves developed under different light conditions (UV-B_{amb}: ambient UV-B; UV-B_{enh}: enhanced UV-B; UV-B_{exc}: UV-B exclusion; FE: forest edge; FI: forest interior).

		Beech forest site (2002)				Outdoor experimental station (2002)			
		clear-cut area		FE	FI	UV-B _{enh}	UV-B _{amb}	UV-B _{exc}	
		UV-B _{amb}	UV-B _{exc}						
Photosynthetic pigments (n=36-46)	UV-B _{amb}	88.9	0	11.1	0	UV-B _{enh}	83.3	16.7	0
	UV-B _{exc}	11.1	88.9	0	0	UV-B _{amb}	22.7	63.6	13.6
	FE	0	0	88.9	11.1	UV-B _{exc}	0	16.7	83.3
	FI	0	0	0	100.0				
UV-B absorbing compounds (n=72-92)	UV-B _{amb}	83.3	5.6	11.1	0	UV-B _{enh}	83.3	16.7	0
	UV-B _{exc}	16.7	44.4	16.7	22.2	UV-B _{amb}	15.9	47.7	36.4
	FE	0	33.3	61.1	5.6	UV-B _{exc}	12.5	12.5	75.0
	FI	0	0	0	100.0				
Fast chlorophyll fluorescence parameters (n=298-311)	UV-B _{amb}	61.0	14.6	17.1	7.3	UV-B _{enh}	35.5	36.8	27.6
	UV-B _{exc}	20.0	25.9	22.4	31.8	UV-B _{amb}	22.2	42.2	35.6
	FE	29.6	14.1	35.2	21.1	UV-B _{exc}	14.9	33.3	51.7
	FI	0	6.8	8.2	84.9				
Slow chlorophyll fluorescence parameters (n=58-69)	UV-B _{amb}	83.3	5.6	11.1	0	UV-B _{enh}	75.0	20.8	4.2
	UV-B _{exc}	18.8	50.0	31.3	0	UV-B _{amb}	29.2	62.5	8.3
	FE	8.3	8.3	83.3	0	UV-B _{exc}	4.8	9.5	85.7
	FI	0	0	0	100.0				

4 DISCUSSION

Some parameters, such as photosynthetic pigment composition, chlorophyll fluorescence, levels of UV-B absorbing compounds and specific leaf mass are known as useful indicators of UV-B tolerance or sensitivity, but display rapid responses to UV-B (and PAR), and often increase or decrease within hours (Smith et al. 2000). In our experiments photosynthetic pigment composition, leaf water content and flavonoid content altered parallel with diurnal light cycle due to not only changes of intensity of photoprotective processes, but also photodamage and resynthesis of some pigments (for example chlorophylls). Due to the higher sensitivity of chlorophyll *b*, than chlorophyll *a* (Tevini et al. 1981) increase of the chlorophyll *a/b* ratio could be observed in UV-B radiation. Activity of the xanthophyll cycle also alter in close positive correlation with diurnal light intensity, as it is an important protective mechanism against photoinhibition and photodamage of PSII (Long et al. 1994). Hader et al. (2004) described that photoinhibition (decrease of F_v/F_m) at noon under high irradiances caused by not only PAR but also UV-B radiation. The total amount of VAZ pigments, as well as the de-epoxidation index, is a good indicator of stress situations in trees as they increase in response to several environmental stress factors (Demmig-Adams - Adams, 1992). Our results show that under enhanced UV-B photoinhibition of PSII, activity of xanthophyll cycle (DEEPS) was higher than in leaves under ambient UV-B. In chloroplasts of higher plants it was found that the de-epoxidation of violaxanthin to zeaxanthin is inhibited upon UV-B exposure (Pfündel et al. 1992), but in field it has smaller importance. In our experiments we observed that enhanced UV-B did not inhibit the activity of xanthophyll cycle in beech leaves, or rather the activity and the pool size increased at noon. In both experimental conditions increases of the VAZ-pool resulted in increases of antheraxanthin and zeaxanthin. Beside the operation of

xanthophyll cycle, protection against photoinhibition can be increased by zeaxanthin synthesis from β -carotene via hydroxylation (Demmig-Adams - Adams, 2002).

The most frequent response to enhanced UV-B radiation is a production of UV-B absorbing compounds (Searles et al. 2001), in particular flavonoids. Flavonoids have been shown to prevent UV-B induced DNA damage (Koostra 1994), and to have free-radical scavenging activity (Barabás et al. 1998), thus offering additional protection to either the photosynthetic apparatus, or membrane lipids. In our experiments these pigments responded the most susceptibly to changes of UV-B level. Amount of these pigments decreased under UV-B exclusion, increased under UV-B exposure in beech leaves.

Our results reveal that beech seedlings are indeed affected by UV-B, even under the present radiation conditions, moreover UV-B exclusion affected to the measured ecophysiological parameters more considerably than enhanced (+40%) UV-B. According to discriminant analysis UV-B affected principally the protective mechanisms, particularly photosynthetic pigment composition and flavonoid content. At the same time photochemical activity of leaves - namely parameters of fast chlorophyll fluorescence induction - were less affected. It indicates that beech may be able to cope with increasing UV-B due to the adaptation ability to the present-day levels of UV-B radiation. Changes in flavonoid accumulation, as well as in carotenoid content (especially xanthophyll cycle pigments) after UV-B exclusion or UV-B exposition show that beech seedlings have effective protective mechanisms to avoid strong damage of the most important metabolism processes, thus photochemical efficiency of photosynthetic apparatus could remain relatively unaffected under changing UV-B levels.

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