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Effects of supplemental UV-B radiation on the photosynthesis — physiological properties and flavonoid content of beech seedlings (*Fagus sylvatica* L.) in outdoor conditions

Réka Láposi, Szilvia Veres, Orsolya Mile, Ilona Mészáros*

Department of Botany, University of Debrecen, Debrecen, Hungary

ABSTRACT Responses of beech seedlings to supplemental UV-B radiation were investigated during three consecutive seasons, in three repeated experiments. Our attention was paid on the alteration of the photosynthetic pigment composition - especially on the xanthophyll cycle pigments - chlorophyll fluorescence parameters, furthermore the accumulation of UV-B absorbing compounds in leaves, the specific leaf mass and leaf water content. The enhanced UV-B radiation generally affected significantly neither photochemical efficiency of PSII, nor photosynthetic pigment composition. UV-B radiation induced some protective mechanisms, thus VAZ-pool increased in beech leaves in every experiment, parallel with the enhancement of non-photochemical quenching. Amount of UV-B absorbing compounds in leaves increased under enhanced UV-B, but no significant changes were observed in the specific leaf mass. Sensitivity of plants to UV-B is largely influenced by other environmental factors and experimental conditions.
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KEY WORDS

UV-B
photosynthetic pigments
xanthophylls cycle
UV-B absorbing compounds
photochemical activity

Thinning of the stratospheric ozone layer and the concomitant increase in UV-B radiation on the Earth's surface are forecasted in the future. UV-B can have direct and indirect effects on the genetic system, the photosynthetic apparatus and membrane lipids (Björn 1996) of plants. The most frequent response to enhanced UV-B radiation is the production of various secondary substances, primarily UV-B absorbing compounds (Barabás et al. 1998; Day et al. 1999), in particular flavonoids. Plant species vary greatly in their response to UV-B. 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. In this study the responses of beech seedlings to the enhanced UV-B were investigated in an outdoor UV-B experimental site during three seasons, in three repeated experiments. Our attention was focused on the accumulation of UV-B absorbing compounds in leaves, and on the alteration of the photosynthetic pigment composition, especially the photoprotective xanthophyll cycle pigments. The maximal (F_v/F_m) and actual ($\Delta F/F_m$') photochemical efficiency of PSII, and other chlorophyll fluorescence parameters (F_0, F_m, RFD, NPQ) were also measured.

Materials and Methods

The UV-B experiments have been performed in an outdoor experimental site at the Botanical Garden of Debrecen University in growing season of 2000, 2001 and 2002. Two years old beech seedlings were planted into slightly acidic soil in 4 l plastic containers. The timer-controlled UV-B supplementation system consisted of racks equipped with six fluorescent tubes (type UV-B 313, Q-Panel, Cleveland, USA), which were wrapped with 0.1mm cellulose acetate filter (Courtaulds, Chemicals, Derby, UK) to eliminate the UV-C radiation. Supplemental UV-B of 80 μW cm^{-2} (approximately 40% of the ambient summer maximum UV-B. In the control plot plants recieved only natural solar radiation. During the experiments the water supply of the seedlings was equal in all plots. UV-B exposure started before bud break and continued till leaf senescence.

Chlorophyll fluorescence parameters were measured with a portable PAM 2000 fluorometer (WALZ Germany) after 20 min dark adaptation period, and they were calculated by the equations of Schreiber et al. (1994). Actual photochemical efficiency of PSII ($\Delta F/F_m' = (F_m' - F_s)/F_m'$), non-photochemical chlorophyll fluorescence quenching ($NPQ = (F_m - F_m')/F_m'$) and relative fluorescence decrease ($RFD = (F_m - F_s)/F_s$) were determined during the slow fluorescence induction after 5 minutes illumination of the leaves with two different actinic light intensities (200 and 1000 μmol m^{-2} s^{-1}). Chlorophyll content was measured in 80% acetone extract with spectrophotometric method (Wellburn 1994). Carotenoid composition was analysed by reversed phase HPLC method. Accumulation of UV-B absorbing compounds in leaves was determined spectrophotometrically, using the method described by Day (1993). Flavonoid accumulation was expressed as cummulative absorbance of leaf extract at 280-300 nm related to leaf

*Corresponding author. E-mail: immeszaros@tigris.klte.hu

Table 1. Effects of enhanced UV-B radiation on the photosynthesis physiological parameters of beech. Means ± SD from the three experiment are given (n=13-399). Data were tested by one-way ANOVA (* P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001).

		2000			2001			2002	
		+UV-B	ambient		+UV-B	ambient		+UV-B	ambient
total carotenoid	ns	445,3±55	414,2±30	ns	289,1±41	308,2±33	**	384,6±38	339,3±39
neoxantin	ns	36,3±6	35,9±4	ns	27,9±4	28,2±3	ns	29,.2±2	28,6±3
lutein	ns	216,7±24	211,3±18	ns	138,4±18	148,6±16	*	165,1±13	151,8±13
β-carotene	ns	57,2±10	59,8±10	ns	55,5±15	57,0±9	ns	78,9±15	75,8±13
β-carotene / tot. car.	ns	0,13±0,02	0,14±0,03	ns	0,19±0,04	0,19±0,03	ns	0,21±0,03	0,22±0,03
VAZ-pool	**	135,2±23	107,0±19	ns	67,3±14	74,3±14	***	111,2±19	82,9±23
VAZ-pool / tot. car.	**	0,30±0,03	0,26±0,04	ns	0,23±0,04	0,24±0,03	**	0,29±0,03	0,24±0,05
DEEPS	ns	0,68±0,15	0,65±0,14	ns	0,35±0,27	0,34±0,27	ns	0,46±0,31	0,37±0,26
VAZ/ β-carotene	*	2,42±0,53	1,86±0,58	ns	1,28±0,39	1,33±0,34	*	1,44±0,3	1,11±0,3
chlorophyll a	ns	1,54±0,26	1,73±0,22	ns	2,22±0,63	1,95±0,54	*	1,10±0,21	1,38±0,36
chlorophyll b	*	0,52±0,11	0,61±0,08	ns	0,75±0,25	0,66±0,2	*	0,34±0,07	0,45±0,14
chlorophyll a+b	*	2,05±0,36	2,34±0,29	ns	2,98±0,87	2,61±0,74	*	1,44±0,28	1,83±0,5
chlorophyll a/b	**	3,01±0,21	2,81±0,13	ns	3,01±0,2	2,98±0,13	*	3,22±0,11	3,08±0,16
water content (%)	ns	52,0±6,4	52,0±5	*	49,1±3,8	51,1±4,1	ns	45,1±4,2	47,8±4,5
Sm (gH_2O chl^{-1})	*	0,24±0,05	0,21±0,04	ns	0,20±0,05	0,21±0,05	ns	0,25±0,05	0,22±0,05
SLM (g dm^{-2})	ns	0,44±0,08	0,41±0,04	***	0,57±0,07	0,41±0,06	ns	0,48±0,1	0,45±0,07
$A_{280\text{-}300nm}$ d.w.$^{-2}$	*	73,2±9	64,9±11	***	126,1±29	78,8±15	*	70,2±9	55,0±14
$A_{280\text{-}300nm}$ fr.w.$^{-2}$	*	16930±4055	15149±2937	***	21740±6646	15956±3653	***	16755±4334	14407±3356
$A_{280\text{-}300nm}$ cm^{-2}	*	8003±1379	7381±988	***	10974±3,63	7715±1675	***	9625±1817	7620±1257
F_0	ns	0,35±0,04	0,34±0,04	ns	0,32±0,02	0,32±0,03	ns	0,31±0,03	0,31±0,03
F_v/F_m	ns	0,77±0,02	0,77±0,02	ns	0,76±0,04	0,76±0,04	ns	0,72±0,06	0,72±0,06
F_m	ns	1,55±0,21	1,53±0,21	ns	1,38±0,21	1,37±0,24	*	1,16±0,23	1,11±0,21
RFD	ns	3,32±0,8	3,24±0,76	***	2,79±0,4	3,32±0,48	***	2,68±0,52	3,20±0,76
ΔF/Fm'	***	0,26±0,17	0,29±0,19	ns	0,32±0,19	0,34±0,19	ns	0,30±0,19	0,33±0,19
NPQ	**	2,25±1,08	2,08±1,2	***	2,01±1,04	1,51±0,85	ns	1,87±1,12	1,55±0,8

unit area (cm^2). For statistical analyisis of data SPSS 11.0 software was used. The results were evaluated by one-way ANOVA, and discriminant analysis.

Results and Discussion

Significant differences (Table 1) in some photosynthetic parameters was found between the seedlings exposed to enhanced and ambient UV-B radiation. In the first and third experiment leaves under enhanced UV-B had higher amount of photoprotective VAZ pigments (related to the total carotenoid content, too) and lower chlorophyll content. Increase of the chlorophyll a/b ratio was observed under enhanced UV-B. Reduction in chlorophyll content might be due to the inhibition of biosynthesis or degradation of pigments under UV-B exposure (Strid and Porra 1992). In the third experiment treated leaves had higher amount of total carotenoid content and lutein than the control plants. UV-B treatment did not affect the β-carotene content and the xanthophyll cycle activity (DEEPS) in beech leaves, although the VAZ-pool size increased. Pfünder et al. (1992) reported that in chloroplasts of higher plants the de-epoxidation of violaxanthin to zeaxanthin is inhibited upon UV-B exposure, but in field it may have smaller importance. UV-B radiation caused higher flavonoid accumulation in leaves under UV-B exposure in every experiment, and it probably caused that enhanced UV-B did not affect significantly the fast chlorophyll fluorescence parameters (F_0, F_m, F_v/F_m, F_m/F_0). Veit et al. (1996) reported that the synthesis of UV-B absorbing compounds prevents damage to PSII. In contrast to results by Wand (1995) specific leaf mass (SLM) did not change under enhanced UV-B. We observed decrease of actual photochemical efficiency of PSII in the first experiment parallel with the increase of NPQ. In the second experiment NPQ also increased under enhanced UV-B. RFD did not decrease below 2, but in the second and third experiment treated plants had lower RFD, than control plants. Discriminant analysis (Table 2) also pointed to that enhanced UV-B had the largest effects on flavonoid content and photosynthetic pigments, as the similarity of treated and control leaves is the least, while in case of the slow chlorophyll fluorescence induction parameters effects of the UV-B is less, so the similarity of the leaves is higher. Separation

Table 2. Classification results of discriminant analysis (% of similarity) based on data from 2000-2002.

		2000		2001		2002	
		+UVB	ambient	+UVB	ambient	+UVB	ambient
Photosynthetic pigments *(n=108)*	+UVB	92,3	7,7	84,6	15,4	100	0
	ambient	20	80	15	85	5,9	94,1
UV-B absorbing compounds *(n=198)*	+UVB	70,6	29,4	85,5	14,5	79,2	20,8
	ambient	38,9	61,1	7,5	92,5	18,2	81,8
Fast chlorophyll fluorescence induction parameters *(n=1587)*	+UVB	49,6	50,4	55,1	44,9	45,1	54,9
	ambient	41,3	58,7	51,2	48,8	36,5	63,5
Slow chlorophyll fluorescence induction parameters *(n=349)*	+UVB	82,1	17,9	73,5	26,5	64,0	36,0
	ambient	43,3	56,7	30	70	31,5	68,5

of plants on the basis of the fast chlorophyll fluorescence induction parameters is minimal (Table 2). We concluded that enhanced UV-B did not affect considerably either photochemical efficiency of PSII, or leaf photosynthetic pigment composition of beech under the experimental conditions. However, UV-B radiation induced some protective mechanisms, thus the content of UV-B absorbing compounds and vaz-pool/total carotenoid content of beech leaves increased in every experimental season.

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