Deciduous and evergreen tree responses to enhanced UV-B treatment during three years

Odziv listopadne in vednozelene drevesne vrste v času 3-letne izpostavljenosti povečanemu sevanju UV-B

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Abstract: This paper reports a study of the strategies in Norway spruce (*Picea abies* (L.) Karst.) and European beech (*Fagus sylvatica* L.) for coping with enhanced UV-B radiation. Trees, as plants in general, possess diverse systems which respond to UV-B radiation. Changes in physiology, biochemistry and morphology have been observed in trees under enhanced UV-B radiation. The efficiency of trees' UV-B protective systems depends on plant characteristics and state of development as well as can be correlated with the UV-B dose and the environmental conditions. The two tree species were exposed outdoors to enhanced UV-B simulating 17% ozone depletion for three years during which time, selected parameters were monitored. Selected physiological parameters were monitored three times a year on beech leaves and three needle age classes of spruce. Spruce and beech exhibited great variability in the amounts of chlorophyll, methanol-soluble UV-B and UV-A absorbing compounds, and optimum quantum yield of photosystem II. The effects of UV-B radiation also varied with needle and leaf development stage and interaction with environmental conditions. Enhanced UV-B radiation triggered responses in both trees and a reduced negative effect of UV-B radiation on spruce photochemical efficiency was observed during prolonged drought. The results show high UV-B tolerance of both tree species and indicate the complexity of plant response to UV-B, involving multilevel interactions with environmental factors and thus emphasizes the necessity of long-term investigations on trees in a natural ecosystem.

Keywords: *Picea abies, Fagus sylvatica*, UV-B radiation, long-term field experiment

Izvleček: V raziskavi smo preučevali strategije spoprijemanja s povečanim sevanje UV-B pri smreki (*Picea abies* (L.) Karst .) in bukvi (*Fagus sylvatica* L.). Sadike obeh drevesnih vrst so bile posajene na prostem in za obdobje treh let izpostavljene povečanemu sevanju UV-B. Izbrane parametre smo spremljali trikrat letno na listih oziroma treh starostnih razredih iglic. Tako pri smreki kot pri bukvi smo izmerili veliko variabilnost v vsebnostih UV-B absorbirajočih snovi, fotosinteznih barvil in fotokemični učinkovitosti. Učinek povečanega sevanja UV-B je bil odvisen od razvojne faze iglice oziroma lista ter od okoljskih razmer. Povečano sevanje UV-B je sprožilo posamezne odzive pri obeh drevesnih vrstah. Zmanjšan negativni učinek sevanja UV-B

na fotokemično učinkovitost smreke smo opazili v tretji poskusni sezoni in ga razlagali kot omilitveni učinek suše. Pri letošnjih iglicah, ne pa tudi listih ali starejših iglicah, je bila prisotna neznačilna tendenca povečane sinteze UV-B absorbirajočih snovi pod povečanim sevanjem UV-B. Rezultati so pokazali veliko strpnost obeh drevesnih vrst do povečanega sevanja UV-B, obenem pa potrdili kompleksen odziv na povečano sevanje UV-B, ki je odvisno tudi od razvojne faze rastline in okoljskih razmer.

Ključne besede: *Picea abies, Fagus sylvatica*, sevanje UV-B, večletni poskus

Introduction

Depletion of the stratospheric ozone over the past several decades has resulted in enhanced levels of UV-B radiation reaching the biosphere (Madronich et al. 1998, Ajavon et al. 2006). A United Nations report states that it is estimated that full recovery of stratospheric ozone on a global scale will not occur before 2050–2100 and will be depend upon continued compliance with the Montreal Protocol and addressing the interactions between ozone recovery and atmospheric changes, such as climate change. The enhanced UV-B radiation remains an issue which can affect biocenosis significantly.

Most of the studies on the effects of enhanced UV-B radiation on plants have involved agricultural species, with much fewer studies on trees, even though the importance of trees in both ecosystems and ecosystems and in economics is considerable. The knowledge of UV-B radiation effects on trees is mainly based on short-term experiments and/or controlled growth conditions. Detrimental effect of UV-B observed in those studies occurs rarely in field-grown trees, where natural light conditions and other environmental factors contribute to diverse responses of trees (Mirecki and Teramura 1984, Laakso and Huttunen 1998, Laakso et al. 2000, Sullivan at al. 2005). Long-term field UV-B effects have been studied scarcely, and various responses of trees to enhanced UV-B radiation were reported. Three-year studies on conifers reported reductions in growth (Sullivan and Teramura 1992) and a reduction of UV-B absorbing compounds Kinnunen et al. 2001) on pine trees under UV-B exposure. An increase of UV-B absorbing compounds was observed in Douglas fir and Ponderosa pine during two year UV-B irradiation (Warren et al. 2002). No reduction of growth and photosynthesis/secondary compounds was detected at Douglas fir, Norway spruce and Scots pine after two or three years of UV-B exposure (Bassman et al. 2002, Turtola et al. 2006). A five-year UV-B irradiation of Norway spruce led to a decrease of some growth parameters, but not of photosynthesis or UV-B absorbing compounds (Trošt Sedej and Gaberščik 2008). A five-year exposure to increased UV-B of deciduous trees (ash, *Fraxinus excelsior*; silver birch, *Betula pendula*; lime, *Tilia cordata*; English oak, *Quercus robur*; and sycamore maple, *Acer pseudoplatanus*) resulted in decreased photosynthesis, transpiration and stomatal density (Keiller and Holmes 2001). In a three-year study on red maple (*Acer rubrum*), tulip poplar (*Liriodendron tulipifera*) and sweetgum (*Liquidambar styraciflua*), photosynthesis generally did not decline and poplar exhibited an increase of UV-B absorbing compounds (Sullivan et al. 1994), while European beech (*Fagus sylvatica*) showed increased photosynthesis after three years of UV-B exposure (Šprtová at al. 2003). Reduced photosynthetic activity led to reduced leaf elongation, plant growth and biomass production in some cases (Warren et al. 2002, Bassman et al. 2003, Kirchgessner et al. 2003, Lavola et al. 2003, Lenk and Buschmann 2006, Trošt Sedej and Gaberščik 2008). Leaf size in deciduous trees has been variously reported to have been decreased by enhanced UV-B radiation (Newsham et al. 1999, Keiller and Holmes 2001, Sullivan at al. 2003), increased (Sullivan at al. 2003, Šprtová at al. 2003) or unchanged (Kostina et al. 2001). Increase in leaf thickness under enhanced UV-B radiation was observed in some deciduous trees (Sullivan et al. 1994, Antonelli et al. 1998, Newsham et al. 1999), where leaf thickening was due to an increase in either the thickness of the spongy parenchyma (Kostina et al. 2001) or of the palisade parenchyma (Nagel et al. 1998) .

Trees possess diverse biochemical, physiological and morphological mechanisms which respond to UV-B radiation, so that the ambient UV-B might be viewed both as a stressor and a photomorphogenic signal (Prado et al. 2012). Trees' resistance to enhanced UV-B is partially based on a high epidermal screening capacity due mainly to phenolics (Fischbach et al. 1999, Hoque and Remus 1999, Trošt Sedej and Gaberščik 2008, Rozema et al. 2002, Turtola et al. 2006). Important components of the defence systems against UV and a number of stress factors are also other secondary compounds such as terpenes, (Turtola et al. 2006, Prado et al. 2012), reflectance of UV (Hoque and Remus 1999, Láposi et al. 2009), special anatomical arrangement and increased cell wall thickness of epidermal cells (Sullivan at al. 1994, Antonelli at al. 1998, Newsham at al. 1999, Hoque and Remus 1999, Chalker-Scott and Scott 2004) and small, thick leaves (P´yankov and Kondrachuk 1998). The proportion of UV-B radiation reaching the leaf mesophyll is generally higher in deciduous broadleaf trees than in evergreen conifer trees (Day 1993), that indicate greater sensitivity of deciduous trees to enhanced UV-B radiation but lower maintenance costs. UV-B sensitivity is closely related to the development state of leaves, where the epidermis of fully grown leaves filters UV-B more efficiently than that of young leaves (Day et al. 1992, DeLucia at al.1992, Day et al. 1996, Ruhland and Day 1996, Laakso et al. 2000, Trošt and Gaberščik 2001, Neitzke and Therburg 2003, Trošt Sedej and Gaberščik 2008).

The degree of UV-B shielding in trees depends on environmental conditions (Neitzke and Therburg 2003, Julkunen-Tiitto et al. 2005, Lenk and Buschmann 2006, Trošt Sedej and Gaberščik 2008). The higher sensitivity to UV-B was due to low temperatures in spruce (Bavcon et al. 1996) and increased ozone at beech (Zeuthen et al. 1997). Drought exposure in pine and spruce (Petropoulou et al. 1995, Manetas et al. 1997, Trošt Sedej and Gaberščik 2008) and nutrient deficiency at birch (Keski-Saari et al. 2005) alleviated UV-B effect. Experiments testing elevated CO_2 and enhanced UV-B radiation indicated that increased CO_2 either amelioratied or had no effect on photosynthesis or biomass allocation (Sullivan, 1997, Caldwell at al. 1998, and Lavola at al. 2000). Some studies (Laakso et al. 2000, Sullivan 2005) proved species/

population specific responses to enhanced UV-B radiation, with woody species varying widely in their responses under changing environmental conditions and also responding slowly, that is why further long-term outdoor research is necessary.

Norway spruce (*Picea abies* (L.) Karst.) and European beech (*Fagus sylvatica* L.) are the most common tree species of natural forests in Central Europe. Plants were exposed to ambient and enhanced UV-B levels at the outdoor experimental plot. We have examined physiological and growth responses to UV-B radiation (Sullivan and Teramura 1992, Bassman et al. 2002, Šprtová et al, 2003 Turtola et al. 2006, Trošt Sedej and Gaberščik 2008) taking into account the effect of concomitant environmental conditions (Petropoulou et al. 1995, Manetas et al. 1997, Neitzke and Therburg 2003, Keski-Saari et al. 2005, Trošt Sedej and Gaberščik 2008) and considering the variation of tree response according to needle/leaf development stage (Naidu et al. 1993, Latola et al. 2001). This paper offers an insight into complex response of Norway spruce and European beech to UV-B exposure during a three year study under realistic environmental conditions, thus it provides an additional view to a deciduous and an evergreen tree strategy of coping with enhanced UV-B radiation.

Materials and methods

Outdoor experimental plot

Norway spruce (*Picea abies* (L.) Karst.) and European beech (*Fagus sylvatica* L.) one-year seedlings were planted in an outdoor research plot (Botanical Garden, University of Ljubljana: 320m a.s.l., 46°35´N, 14°55´E). Fifty seedlings for each of the two treatments were planted in 5 clay pots (62x21x19 cm) in a mixture of compost and peat (1:1). Plants were transplanted to a new soil mixture every February. The pots were buried at ground level to minimise soil temperature variation and desiccation (Sullivan and Teramura 1992).

Two different treatments were applied in the experiment. A UV-B supplement system was designed, as described by Björn and Teramura (1993) and exposure resulting from 17% ozone depletion, corresponding to a 35–55% increase of ambient

UV-B$_{BE}$ (UV-B+), was simulated using Q-Panel UV-B 313 lamps (Cleveland, OH, USA), which emit from 275 nm to 400 nm with peak emission at 313 nm, filtered with cellulose diacetate filters. Ambient radiation (UV-B) was simulated using the same lamps filtered with Mylar foil. The doses were calculated and adjusted weekly (Björn and Murphy 1985) using the generalised plant action spectrum of Caldwell (1968). The system was timer controlled. Ambient UV-B (Fig. 1a). UV-A and PAR radiation were monitored at the site by a three-channel dosimeter (Häder et al. 1999). The experiment was conducted over three growing seasons from May till October.

Cumulative water balance data (Fig. 1b) calculated as the difference between total monthly precipitation and total monthly potential eva-potranspiration according to Penman's equation, were obtained from Slovenian Environment Agency. Seasonal cumulative water balance (CWB) represents three seasonal periods of beech growth and development: March to June,

March to August and March to October. Principal Component Analysis showed a high correlation between seasonal cumulative water balance and environmental factors air and soil temperature, insolation, precipitation and potential evaporation and other plant parameters at the outdoor experimental plot. Seasonal cumulative water balance, as a water disposability indicator, was chosen as the most characteristic environmental factor, and was used in the correlation analyses.

Physiological and biochemical measurements

Measurements were carried out three times per growth season, in May, August and October, the key phases for tree growth and development (young leaves, growth phase peak, end of growth) as determined in the preliminary year (Trošt and Gaberščik 2001). Leaves and needles, the latest of three needle-age classes: current (c), current+1 (c+1) and current+2 (c+2), were sampled from five (for biochemical measurements) and ten (for
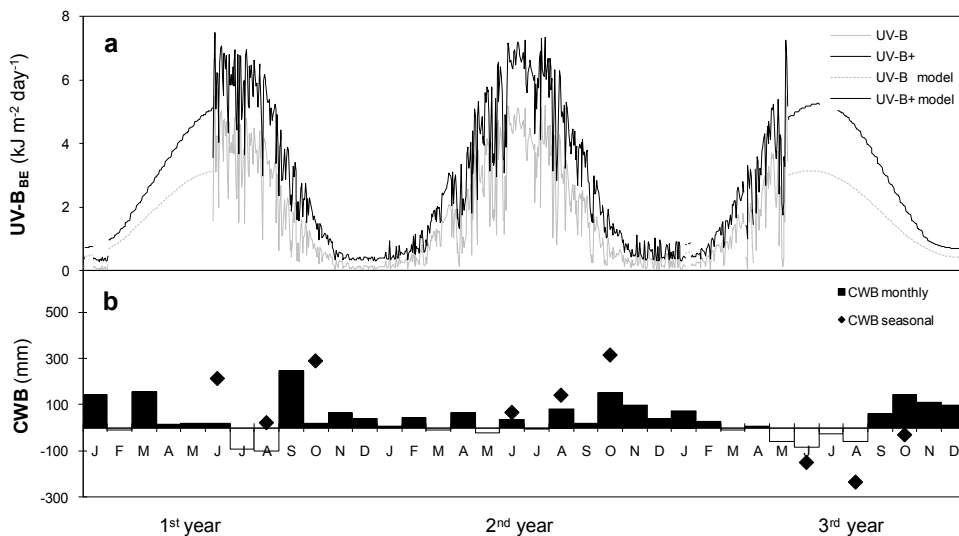
Figure 1a: Monitored ambient (UV-B) and enhanced (UV-B+) daily dose of biologically active UV-B (UV-B$_{BE}$) radiation, calculated ambient (UV-B model) and enhanced (UV-B+ model) daily dose of biologically active UV-B (UV-B$_{BE}$) radiation (Björn and Murphy, 1993) in the two treatments established at the experimental site in Ljubljana. 1b. Monthly and seasonal cumulative water balance (CWB) in Ljubljana.

Slika 1a: Izmerjeni naravni (UV-B) in povečani (UV-B+) dnevni odmerki biološko aktivnega sevanja UV-B (UV-B$_{BE}$), izračunani naravni (UV-B model) in povečani (UV-B+ model) dnevni odmerki biološko aktivnega sevanja UV-B (UV-B$_{BE}$) po modelu Björn and Murphy (1993) na poskusni ploskvi Botaničnega vrta v Ljubljani. 1b. Mesečna in sezonska kumulativna vodna bilanca (CWB) v Ljubljani.

physiological measurements) of the upper horizontal branches of randomly selected trees *per* plot.

Photochemical efficiency was estimated by measuring the chlorophyll *a* fluorescence of photosystem II using a modulated fluorometer (OS-500, Opti-Sciences, USA). Measurements were carried out *in vivo* at noon on clear days. Optimal quantum yield (F_v/F_m), defined as ($F_m - F_o)/F_m$, where F_m represents maximal and F_o minimal fluorescence of a dark adapted sample, was determined.

Total chlorophyll content (Chl $a+b$) was determined as described by Lichtenthaler (1987). The Chl $a+b$ content was calculated per sample DM, from extinction coefficients at 644 and 662 nm in acetone [100% (v/v)] (UV/VIS spectrophotometer Lambda 12, Perkin-Elmer, Norwalk, CT, USA).

The total content of methanol-soluble UV-B ($A_{280–320}$) and UV-A ($A_{320–400}$) absorbing compounds was estimated according to Caldwell (1968). The extinction coefficients of the samples were measured in the UV-B and UV-A spectral range 280–400 nm (UV/VIS spectrometer), calculated per sample DM and integrated to estimate the total content of UV-B and UV-A absorbing compounds.

Morphometric measurements

At the end of growth season, the leaf length, width and thickness as well as needle total length and diameter (optical microscope Zeiss KF2, Carl Zeiss, Germany) were measured. The parameters were determined in ten randomly selected needles of three age-classes and leaves *per* plot sampled at the upper horizontal branches.

Statistical analysis

The independent-samples t-test was used to compare means of measured parameters at the two UV-B treatments. Spearman's coefficient was used to investigate bivariate correlations between the environmental factors and measured parameters. Samples in all the tests were randomly chosen without replication. Statistically significant differences were marked as: non-significant ($p < 0.05$), * ($p \leq 0.05$), ** ($p \leq 0.01$) and *** ($p \leq 0.001$). Analyses were accomplished by SPSS for Windows 13.0.0.

Results

Physiological and biochemical responses

The responses of Norway spruce and European beech varied from neutral to negative or positive during three years exposure to UV-B, and were varying with plant developmental state, growth season and environmental conditions (Fig. 2., Table 1).

In the autumn of the first season Norway spruce manifested reduced F_v/F_m under enhanced UV-B in c+1 needles and in spring of the second season in c and c+1 needles. In the third season, higher F_v/F_m under enhanced UV-B was observed in c and c+1 needles in spring, while in summer of this season F_v/F_m increased in all three needle age classes. In all three spruce needle age classes, F_v/F_m exhibited a tendency to reduced values in spring and optimal values later in the season. The F_v/F_m values of the European beech were significantly reduced under enhanced UV-B in the spring of the second season. F_v/F_m values were low during the second and third season under both treatments (Fig. 2).

In the Norway spruce, the Chl $a+b$ content responded to enhanced UV-B in all three needle age classes. The young needles manifested increased Chl $a+b$ content twice under enhanced UV-B, while Chl $a+b$ content in c+1 and c+2 needles was decreased three times and increased once. In the European beech Chl $a+b$ content significantly decreased under enhanced UV-B in autumn of the second season and increased in autumn of the third season (Table 1).

The $A_{280–320}$ content was high in both tree species under both UV-B treatments during all growth seasons. Needles/leaves were poorly responsive to enhanced UV-B radiation. Under UV-B+ exposure, spruce current needles showed tendency to higher $A_{280–320}$ content, while beech leaves manifested tendency to lower $A_{280–320}$ content under UV-B+ exposure. Compared with UV-B treatment, the $A_{280–320}$ content under UV-B+ radiation decreased significantly once, in summer of the dry third season. $A_{320–400}$ content was high in both tree species and responded rarely to enhanced UV-B (Table 1).
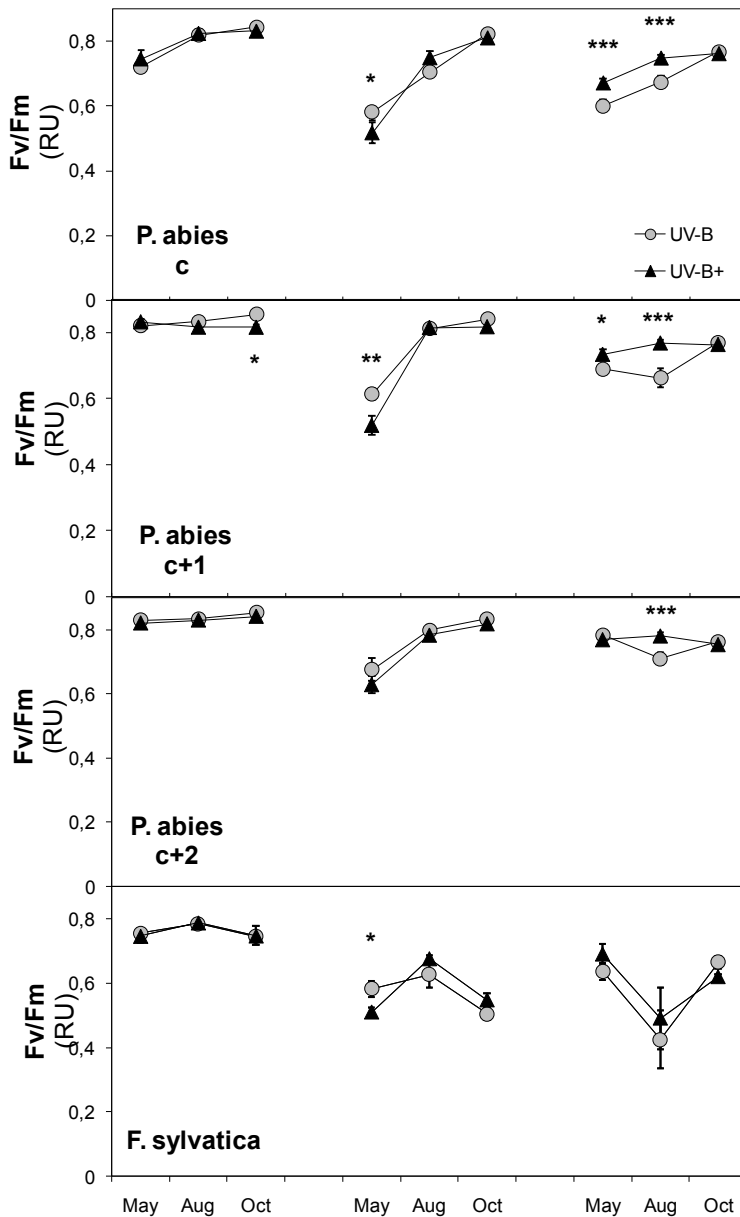
Figure 2: Optimal quantum yield (F_v/F_m) for three needle age classes of Norway spruce (c, c+1, c+2) and leaves of European beech exposed to ambient (UV-B) and enhanced (UV-B+) UV-B radiation during three growth seasons. Data are means ± SE, n = 10, significant differences (Independent-samples t-test) are marked with: * (p ≤ 0.05), ** (p ≤ 0.01), *** (p ≤ 0.001).

Slika 2: Potencialna fotokemična učinkovitost (F_v/F_m) treh starostnih razredov iglic smreke (c, c+1, c+2) in listov bukve, izpostavljenih naravnemu (UV-B) in povečanemu (UV-B+) sevanju UV-B tekom treh let. Podatki so srednje vrednosti ± SE, n = 10, značilna razlika (neodvisni t-test) je označena z: * (p ≤ 0.05), ** (p ≤ 0.01), *** (p ≤ 0.001).

Table 1: Biochemical parameters: chlorophyll content (Chl $a+b$ [mg g⁻¹ DM]), content of UV-A absorbing compounds ($A_{320-400}$ [relative units]) and content of UV-B absorbing compounds ($A_{280-320}$ [relative units]) for three needle age classes (c, c+1, c+2) of Norway spruce and leaves of European beech exposed to ambient (UV-B) and enhanced (UV-B+) UV-B radiation during three growth seasons. Data are means ± SE, n = 5, significant difference (Independent-samples t-test) is marked with: * ($p \leq 0.05$), ** ($p \leq 0.01$), *** ($p \leq 0.001$).

Tabela 1: Biokemijski parametri: vsebnost klorofila (Chl $a+b$ [mg g⁻¹ DM]), vsebnost UV-B absorbirajočih snovi ($A_{280-320}$ [relativna enota]) in vsebnost UV-A absorbirajočih snovi ($A_{320-400}$ [relativna enota]) v treh starostnih razredih iglic (c, c+1, c+2) smreke in v listih bukve, izpostavljenih naravnemu (UV-B) in povečanemu (UV-B+) sevanju UV-B tekom treh let. Podatki so srednje vrednosti ± SE, n = 5, značilna razlika (neodvisni t-test) je označena z: * ($p \leq 0.05$), ** ($p \leq 0.01$), *** ($p \leq 0.001$).

| | | | 1st year | | | | | | 2nd year | | | | | | 3rd year | | | | | |
| | | | May | | Aug | | Oct | | May | | Aug | | Oct | | May | | Aug | | Oct | |
			UV-B	UV-B+	UV-B	UV-B+	UV-B	UV-B+	UV-B	UV-B+	UV-B	UV-B+	UV-B	UV-B+	UV-B	UV-B+	UV-B	UV-B+	UV-B	UV-B+
P. abies Chl a+b	c	Mean	3,15	3,10	2,96	3,12	7,66	4,82	6,94	14,36	7,42	5,60	6,64	5,84	6,89	9,23	2,62	2,78	1,87	2,70
		± SE	0,76	0,35	0,11	0,34	1,07	0,31	0,81	0,59	0,98	0,76	0,70	0,53	1,16	1,56	0,15	0,34	0,12	0,27
		p		ns		ns		ns		***		ns		ns		ns		ns		*
	c+1	Mean	2,91	1,90	3,65	3,95	7,93	8,91	6,19	10,76	3,61	3,46	4,85	5,58	1,31	1,29	1,68	2,18	1,19	1,96
		± SE	0,32	0,22	0,37	0,38	0,58	1,00	0,77	1,83	0,27	0,28	0,41	0,83	0,14	0,35	0,14	0,27	0,11	0,21
		p		ns		ns		ns		*		ns		ns		ns		ns		*
	c+2	Mean	5,55	2,49	4,16	2,50	4,37	5,19	5,41	6,45	3,42	4,01	4,47	3,91	0,41	0,34	1,60	1,88	1,07	1,36
		± SE	0,42	0,51	0,45	0,39	0,43	0,40	0,73	0,54	0,50	0,14	0,48	0,27	0,27	0,06	0,14	0,17	0,16	0,27
		p		ns		ns		ns		***		*		ns		ns		ns		ns
F. sylvatica Chl a+b		Mean	1,31	2,86	3,52	4,05	2,71	2,75	2,43	3,71	3,35	2,96	3,62	2,87	5,71	6,51	4,07	3,31	3,21	3,92
		± SE	0,60	0,28	0,61	0,57	0,14	0,12	0,35	0,54	0,42	0,13	0,23	0,20	0,36	0,31	0,45	0,28	0,11	0,19
		p		ns		ns		ns		ns		ns		*		ns		ns		*
P. abies A₂₈₀₋₃₂₀	c	Mean	1820	2052	2239	2365	2395	2702	2689	2961	2410	2886	3073	3877	3790	3518	1603	1720	1683	1845
		± SE	91,8	106,6	26,7	235,7	81,4	64,3	318,6	350,9	304,2	457,4	298,7	373,2	715,1	1016,5	322,3	303,4	214,4	261,5
		p		ns		ns		ns		ns		ns		ns		ns		ns		ns
	c+1	Mean	2724	2237	2690	2576	3088	2937	2612	2585	2147	1898	3250	2959	1994	1356	2250	1657	2060	2887
		± SE	190,7	85,1	44,1	84,6	125,8	263,8	47,6	249,3	473,7	439,2	190,9	309,4	491,8	310,5	276,3	165,1	99,1	898,1
		p		ns		*		ns		ns		ns		ns		ns		ns		ns
	c+2	Mean	2535	2011	2984	2893	2900	2888	2473	2432	2894	2236	3172	3294	1178	1043	1884	1681	1915	1831
		± SE	361,0	81,9	168,3	86,4	108,8	75,7	182,4	169,9	550,3	262,9	214,4	193,6	308,2	120,9	235,2	171,7	173,9	129,1
		p		ns		ns		ns		ns		ns		ns		ns		ns		ns
F. sylvatica A₂₈₀₋₃₂₀		Mean	2506	2223	1879	1898	1724	1796	2062	1927	2545	2403	1821	1705	1550	1470	1700	1467	1937	1586
		± SE	205,0	199,9	106,6	65,0	94,9	263,0	179,6	104,3	326,7	239,3	72,7	67,0	95,0	134,8	71,1	42,3	189,5	181,9
		p		ns		ns		ns		ns		ns		ns		ns		ns		ns
P. abies A₃₂₀₋₄₀₀	c	Mean	2543	2596	1774	2118	1286	1467	2961	3060	1900	2399	2083	3015	2897	2998	978	1173	854	1251
		± SE	173,7	52,8	125,6	241,2	126,2	139,1	441,2	296,0	236,8	382,0	197,6	215,5	551,7	1041,1	218,2	180,7	110,9	174,1
		p		ns		ns		ns		ns		ns		*		ns		ns		ns
	c+1	Mean	2319	1705	1858	1784	1869	1767	1818	1685	1512	1422	2154	2096	1014	798	1204	962	1001	1267
		± SE	161,1	72,3	55,5	102,6	212,3	156,7	77,2	134,5	293,8	263,7	126,7	260,7	233,8	175,4	183,8	95,8	47,2	242,8
		p		*		ns		ns		ns		ns		ns		ns		ns		ns
	c+2	Mean	1947	1518	2057	2181	1717	1747	1664	1584	2125	1614	2142	2471	653	655	1041	1096	937	1055
		± SE	197,5	122,7	98,3	97,9	76,9	58,0	72,9	152,2	436,7	186,5	243,7	171,9	114,7	60,0	126,1	139,5	83,2	99,1
		p		ns		ns		ns		ns		ns		ns		ns		ns		ns
F. sylvatica A₃₂₀₋₄₀₀		Mean	3053	2946	2287	2307	1945	1833	1943	2045	2710	2732	1655	1556	1480	1475	1745	1571	1914	1588
		± SE	366,9	290,8	249,8	68,6	180,9	275,4	219,0	137,0	386,9	354,7	119,7	86,0	103,4	265,8	83,2	43,7	213,5	254,4
		p		ns		ns		ns		ns		ns		ns		ns		*		ns

Table 2: Morphometric needle/leaf parameters of Norway spruce and European beech exposed to ambient (UV-B) and enhanced (UV-B+) UV-B radiation determined at the end of growth seasons. Parameters: needle total length (m), average diameter (mm), leaf length (mm), width (mm), thickness (μm). Data are means ± SE, n = 50, significant difference (Independent-samples t-test) is marked with: * (p ≤ 0.05), ** (p ≤ 0.01), *** (p ≤ 0.001).

Tabela 2: Morfometrični parametri iglic smreke in listov bukve izpostavljenih naravnemu (UV-B) in povečanemu (UV-B+) sevanju UV-B tekom treh let. Parametri: skupna dolžina iglic (m), premer iglice (mm), dolžina lista (mm), širina lista (mm), debelina lista (μm). Podatki so srednje vrednosti ± SE, n = 10, značilna razlika (neodvisni t-test) je označena z: * (p ≤ 0.05), ** (p ≤ 0.01), *** (p ≤ 0.001).

| | | 1st year | | 2nd year | | 3rd year | |
		UV-B	UV-B+	UV-B	UV-B+	UV-B	UV-B+
P. abies							
Needle length	Mean	77.82	81.19	99.03	100.2	65.86	58.73
	± SE	*11.16*	*12.23*	*30.07*	*30.67*	*8.98*	*3.49*
	p		ns		ns		ns
Needle diameter	Mean	0.59	0.56	0.41	0.39	0.53	0.43
	± SE	*0.018*	*0.016*	*0.009*	*0.012*	*0.026*	*0.014*
	p		ns		ns		ns
F. sylvatica							
Leaf length	Mean	–	–	–	–	44.0	40.8
	± SE					2,3	4,1
	p						ns
Leaf width	Mean	–	–	–	–	26.2	25.4
	± SE					1.6	2.1
	p						ns
Leaf thickness	Mean	–	–	–	–	97.5	89.9
	± SE					8.3	2.2
	p						ns

Morphometric modifications

Enhanced UV-B radiation did not affect needle/leaf morphology of neither of the trees after the three year observation (Table 2).

Interactions of UV-B radiation with environmental conditions and plant development

Correlations between the measured tree parameters and tree age, seasonal tree development, seasonal cumulative water balance and enhanced UV-B radiation are shown in Table 3. In Norway spruce and European beech tree age was generally correlated with decreasing parameter values, with the exception of UV-B and UV-A absorbing compounds in spruce young needles, which failed to correlate with tree age and beech chlorophyll content which increased with tree age. Seasonal tree development correlated scarcely with measured parameters, but there was a strong positive correlation with seasonal cumulative water balance. The content of UV-B absorbing compounds in spruce c needles failed to correlate with tree characteristics or environmental conditions and none of the tree responses could be correlated with the UV-B radiation dose (Table 3).

Discussion

Diverse physiological responses to enhance UV-B varying with environmental condition

The responses of spruce and beech to enhanced UV-B radiation varied according to the needle/leaf development stage, growth season and environmental conditions. In both species, most of monitored parameters did not vary with enhanced UV-B solely (Fig. 2, Table 1).

F_v/F_m was the parameter most sensitive to enhanced UV-B radiation. The F_v/F_m decrease

Table 3: Correlations between tree parameters and tree age, seasonal tree development, seasonal cumulative water balance (CWB) and UV-B treatment (UV-B+) in Norway spruce (different needle age classes: c, c+1, c+2) and European beech. Tree parameters: optimal quantum yield (F_v/F_m), total chlorophyll content (Chl $a+b$), content of UV-B ($A_{280-320}$) and UV-A ($A_{320-400}$) absorbing compounds. Bivariate correlation (Spearman´s coefficient ρ) is marked with: ns ($p > 0.05$), * ($p \leq 0.05$), ** ($p \leq 0.01$), *** ($p \leq 0.001$).

Tabela 3: Korelacije med merjenimi parametric dreves in starostjo bdrevesa, sezonskim razvojem, sezonsko kumulativno vodno bilanco (CWB) ter povečanim (UV-B+) sevanjem UV-B treh starostnih razredov iglic smreke (c, c+1, c+2) in listov bukve. Merjeni parametric drevesa: potencialna fotokemična učinkovitost (F_v/F_m), vsebnost klorofila (Chl $a+b$), vsebnost UV-B absorbirajočih snovi ($A_{280-320}$) in vsebnost UV-A absorbirajočih snovi ($A_{320-400}$). Bivariatna korelacija (Spearmanov koeficient ρ) je označen z: ns ($p > 0.05$), * ($p \leq 0.05$), ** ($p \leq 0.01$), *** ($p \leq 0.001$).

		Tree age		Seasonal development		CWB		UV-B+	
		ρ	p	ρ	p	ρ	p	ρ	p
P. abies									
Fv/Fm	c	−0.389	*	0.740	**	0.477	**		ns
	c+1		ns	0.471	**	0.525	**		ns
	c+2	−0.734	***		ns	0.597	**		ns
F. sylvatica									
Fv/Fm		−0.602	**		ns	0.444	**		ns
P. abies									
Chl *a+b*	c	−0.452	*		ns		ns		ns
	c+1	−0.767	***		ns	0.601	***		ns
	c+2	−0.856	***		ns		ns		ns
F. sylvatica									
Chl *a+b*		0.491	**		ns	−0.469	**		ns
P. abies									
A $_{280-320}$	c		ns		ns		ns		ns
	c+1	−0.452	*	0.396	*	0.725	***		ns
	c+2	−0.769	***		ns	0.495	*		ns
F. sylvatica									
A $_{280-320}$		−0.406	**		ns	0.291	**		ns
P. abies									
A $_{320-400}$	c		ns		ns	0.423	*		ns
	c+1	−0.811	***		ns	0.726	***		ns
	c+2	−0.856	***		ns		ns		ns
F. sylvatica									
A $_{320-400}$		−0.514	**	−0.228	*	0.310	**		ns

under enhanced UV-B was measured in spring of the second season in the young needles of spruce and beech leaves as well. It has been demonstrated that UV-B radiation alters the structure and function of chloroplasts, so that the potential photochemical efficiency might decrease (Björkman and Demmig-Adams 1994, Musil 1996, Adams and Barker 1998, Wu et al. 2011). Some studies on trees indeed showed a decrease of photochemical efficiency (Naidu et al. 1993,

Bavcon et al. 1996), but in others no decrease was observed (Petropoulou et al. 1995, Manetas et al. 1997, Chalker-Scott and Scott 2004). A lowered F_v/F_m may be considered also as a down-regulation mechanism whose aim is lowering of the electron supply in the Calvin cycle as a result of the UV-B induced stress, which was apparently due to UV-B penetration into the mesophyll of recently emerged needles/leaves. It has been shown that in some trees the epidermis of fully grown needles

and leaves filters UV-B more efficiently than the epidermis in young ones (Neitzke and Therburg 2003, Day et al. 1992, DeLucia at al.1992, Day et al. 1996, Ruhland and Day 1996, Laakso et al. 2000, Trošt Sedej and Gaberščik 2008). The effect of UV-B on older leaves was diminished due to higher UV-B absorbing compounds content, self-shading and increased xeromorphic characteristics of needles/leaves. In the third season, the period of the most negative cumulative water balance, the photochemical efficiency of both UV-B exposed and control trees was low, but in Norway spruce the values were significantly higher in irradiated plants (Fig. 2) indicating the alleviating effect of UV-B radiation on drought. Such effect has been observed in Mediterranean conifers (Petropoulou et al. 1995, Manetas et al. 1997). Since UV-B radiation is unable to penetrate into the meso-phyll of fully grown spruce needles (Fischbach et al. 1999), it can be concluded that the effect of UV-B on photochemical efficiency is indirect. UV-B radiation could benefit the water relations of plants through stomata closure (Petropoulou et al. 1995, Manetas et al. 1997), through promoted wax synthesis (Björn et al. 1997) and through cross-resistance of plants which are exposed to any oxidative stress (Turtola et al. 2006). In our study reductions of photochemical efficiency were more common in the recently emerged needles/leaves than in the later development stages. This reflects a remarkable capability for recovery, in which the disturbances of young organs are not expressed in the later stages. Similar findings have been reported in loblolly pine by Naidu and co-workers (1993).

Chlorophyll levels showed no consistent response to enhanced UV-B in any of the trees studied, the results suggesting dependence on development phase and environmental conditions. Other studies reported negative, neutral and rarely, positive effects of enhanced UV-B radiation on chlorophyll content (Šprtová et al. 1999, Bassman et al. 2003, Kirchgessner et al. 2003, Lavola et al. 2003, Trošt Sedej and Gaberščik 2008, Láposi et al. 2009). It has been shown that UV-B radiation can not only inhibit chlorophyll synthesis or cause its photo-oxidation (Bornman 1989, Middleton and Teramura 1993), but also increase the biosynthesis of photosynthetic pigments under favourable irradiation conditions (Middleton and Teramura 1993, Jordan 1996).

High tolerance to UV-B radiation

Norway spruce and European beech appear to possess an effective filter consisting of UV-B and UV-A absorbing compounds already present in young needles/leaves in May, soon after emergence from buds. The content did not change with seasonal development or enhanced UV-B radiation but it was affected by drought (Tab. 3). The total amount of methanol-soluble UV-B absorbing compounds in Norway spruce and European beech was three and two times higher, respectively (Tab. 1) than in some herbaceous species, where the same analytical method was used (Gaberščik et al. 2001, Gaberščik et al. 2002a, Gaberščik et al. 2002b, Breznik et al. 2005).

The low variability of UV-A and UV-B absorbing compounds content is probably part of a protective strategy of long-lived woody plants. Studies indicate that most conifers contain large amounts of UV-B absorbing compounds in the epidermis (Sullivan et al. 1996, Fischbach et al. 1999, Hoque and Remus 1999, Turtola et al. 2006) and the epidermis of fully grown leaves effectively filters UV-B (Day et al. 1992, Day et al. 1996, Ruhland and Day 1996, Fischbach et al. 1999, Hoque and Remus 1999). In the young needles of *Abies lasiocarpa* and *Picea engelmannii* less than 1% of UV-B radiation penetrates into the mesophyll (DeLucia et al. 1992), in European beech the UV-B penetration into young leaves was greater than in developed leaves (Neitzke and Therburg 2003). The production of UV-B absorbing compounds does not always depend on the UV-B dose (Rau and Hofmann 1996, Turtola et al. 2006), and consequently some higher plants from tropical, high-altitude and aquatic environments contain saturating amounts of UV absorbing compounds (Teramura and Sullivan 1994, Germ et al. 2002). It was hypothesized that the receptors triggering the biosynthesis of UV-B absorbing compounds are saturated in plants growing in open environments, therefore they provoke maximal synthesis over a wide range of irradiance (Sullivan et al. 1996), thus, UV absorbing compounds seem to be mainly constitutive. Meta-analysis which generalised an overall response of woody plants under two supplemental UV-B levels proved that woody plants show no significant changes in most variables under the low supplemental UV-B level (Li at al. 2010).

In the dry third season, the content of UV-B and UV-A absorbing compounds was low under both UV-B treatments. Production of UV-B absorbing compounds is an energy demanding process (Gaberščik et al. 2002a), which is why low levels of UV absorbing compounds coheres to low Chl and F_v/F_m values. The subtle changes observed in these parameters may have additional causes, such as changes in leaf histology and biochemistry (Hoque and Remus, 1999), both of which vary with the environmental conditions. The results pronounce the complex influences of UV-B and environmental condition on plants.

Tolerance to elevated UV-B in trees is, in addition to the content of the high methanol-soluble UV-B absorbing compounds, also increased by the presence of cell wall bound UV-B absorbing compounds in conifers (Fischbach et al. 1999, Hoque and Remus 1999, Rozema et al. 2002, Turtola et al. 2006). Other factors supporting this tolerance include reflectance of UV light (Hoque and Remus 1999), special anatomical arrangements and increased epidermal cell wall thickness of epidermis (Hoque and Remus 1999, Chalker-Scott and Scott 2004), small, thick leaves (P´yankov and Kondrachuk 1998), and large amounts of other secondary compounds, which are part of plants' defence systems against many stress factors (Turtola et al. 2006).

Our results show that both Norway spruce and European beech possess effective protection against the UV-B radiation. This protection depends not only on UV-B dose but generally on the state of development and environmental conditions that influence the efficiency of the UV-B protective systems.

Needle/leaf morfology

In the three year study period, it was found by comparing samples from ambient and enhanced UV-B treatment at the outdoor experimental plot (Tab. 2) that elevated UV-B radiation exerts no significant influence on the needle/leaf morphology of Norway spruce and European beech.

Earlier studies found that leaf size of deciduous trees decreases upon exposure to enhanced UV-B radiation (Antonelli at al. 1998, Newsham at al. 1999, Keiller and Holmes, 2001 and Sullivan at al.2003), other studies found that it increases

(Sullivan at al.2003) or is not affected (Kostina at al. 2001). In the UV-B exposed conifers, reduced needle area (Laakso et al. 1996, Bassman et al. 2003, Zu et al. 2010) and shorter needles (Naidu et al. 1993, Sullivan et al. 1996) were reported. Compared to our study those experiments used 2–3 times higher suppplemental UV-B doses, which might be the reason for reduced leaf area in some cases. Other studies reported no effect on growth in UV-B exposed conifers (Petropoulou et al. 1995, Lavola et al. 2003). The response of deciduous trees to UV-B radiation is thickening of the leaves, which decreases the penetration of UV-B radiation to the leaf mesophyll (Sullivan et al. 1994, Antonelli et al. 1998, Newsham et al. 1999, Šprtová et al. 2003). The increase in leaf thickness is due to anatomic changes of spongy parenchyma (Kostina et al. 2001) or palisade parenchyma (Nagel at al. 1998) and corresponds to xeromorphic characteristics of plants from harsh environments, adapted to high irradiation, water stress and nutrient-poor soil (Turunen and Latola 2005). There are several studies on cross-tolerance of plants which are exposed to UV-B and other oxidative stresses (Turtola et al. 2006), uch as drought (Petropoulou et al. 1995, Manetas et al. 1997), drought and high light (Poulson et al. 2005), and cold (Mendez et al. 1999, Chalker-Scott 1999). On the other hand, there are again cases which show a decrease in cuticle thickness with increasing altitude (Turunen and Latola 2005). Diversity of results in different studies, including the current study, indicates the complexity of plant response to UV-B as a function of the whole spectrum of environmental factors and their multilevel interactions and emphasizes the necessity of long-term investigations on trees in natural ecosystems.

Conclusions

The responses of Norway spruce and European beech to enhanced UV-B radiation varied moderately according to the needle/leaf development stage, growth season and above all, environmental conditions. Most of monitored parameters did not respond only to enhanced UV-B solely in any of the studied tree species. Photochemical efficiency was the parameter most responsive

to enhanced UV-B radiation and reductions of photochemical efficiency were observed in the recently emerged needles/leaves, but not in the later development stages, suggesting a recovery capability. Chlorophyll levels showed no consistent response to enhanced UV-B radiation in any of the studied trees, results suggesting dependence on development phase and environmental conditions. Norway spruce as well as European beech manifested an effective filter consisting of UV-B and UV-A absorbing compounds already present in young needles/leaves in May, soon after emergence from buds. The content of the compounds did not change neither with seasonal development nor with enhanced UV-B radiation but was most sensitive to drought. The total amount of methanol-soluble UV-B absorbing compounds in Norway spruce and European beech was as much as three and two times higher, respectively than in tested herbaceous species. During three years' observation, elevated UV-B radiation exerted no significant influence on the needle/leaf morphology of Norway spruce and European beech. Our results prove that the evergreen Norway spruce and the deciduous European beech possess effective protection against the UV-B radiation, which depends specifically on UV-B dose levels but generally on the developmental state and environmental conditions that influence the efficiency of the UV-B protective systems. This study also indicates the complexity of plant response to UV-B, involving multilevel interactions with environmental factors and thus emphasizes the necessity of long-term investigations on trees in a natural ecosystem.

Povzetek

Drevesa se na povečano sevanje UV-B odzivajo preko različnih struktur in mehanizmov na biokemijskem, fiziološkem in morfološkem nivoju. Učinkovitost zaščite pred povečanim sevanjem UV-B je odvisna od značilnosti rastlinske vrste, razvojne faze rastline, okoljskih razmer ter odmerka UV-B sevanja. V raziskavi smo preučevali odzive na povečano sevanje UV-B pri smreki (*Picea abies* (L.) Karst.) in bukvi (*Fagus sylvatica* L.). Sadike obeh drevesnih vrst smo posadili na prosto za obdobje treh let ter jih izpostavili naravnemu in povečanemu sevanju UV-B. Izbrane parametre, optimalno fotokemično učinkovitost, vsebnost klorofilov, vsebnost UV-A in UV-B absorbirajočih snovi, smo spremljali trikrat letno na listih oziroma treh starostnih razredih iglic. Morfološke analize smo izvedli po treh letih obsevanja. Tako pri smreki kot pri bukvi smo izmerili veliko variabilnost v vsebnostih UV-B absorbirajočih snovi, fotosinteznih barvil in fotokemični učinkovitosti. Povečano sevanje UV-B je sprožilo posamezne odzive pri obeh drevesnih vrstah. Učinek povečanega sevanja UV-B se je spreminjal z razvojno fazo iglice in lista ter z okoljskimi razmerami. Zmanjšan negativni učinek sevanja UV-B na fotokemično učinkovitost smreke smo opazili v tretji poskusni sezoni in ga razlagamo kot omilitveni učinek suše. Pri letošnjih iglicah, ne pa tudi pri listih ali starejših iglicah, je bila prisotna tendenca povečane sinteze UV-B absorbirajočih snovi pod povečanim sevanjem UV-B. Rezultati so pokazali veliko strpnost obeh drevesnih vrst do povečanega sevanja UV-B in tudi kompleksen odziv na povečano sevanje UV-B, ki se spreminja tako z razvojno fazo rastline kot z okoljskimi razmerami. Dolgotrajne raziskave v naravnem okolju so zato, za dolgožive vrste kot so drevesa, nujne.

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