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UV-B Transmittance of Greenhouse Covering Materials Affects Growth and Flavonoid Content of Lettuce Seedlings

H. Behn[1,2], S. Tittmann[2], A. Walter[2], U. Schurr[2], G. Noga[1] and A. Ulbrich[1,2,3]
([1]Institute of Crop Sciences and Resource Conservation, Horticultural Sciences, University of Bonn, Bonn, Germany, [2]Institute for Chemistry and Dynamic of the Geosphere (ICG-3 Phytosphere), Forschungszentrum Jülich GmbH, Jülich, Germany, and [3]Faculty of Agricultural Science and Landscape Architecture, University of Applied Science Osnabrück, Osnabrück, Germany)

Summary

In Europe, lettuce (*Lactuca sativa* L., *Asteraceae*) is commonly raised in greenhouses and transplanted to the field at the age of two to four weeks in order to prolong the growing season. The sudden exposure to outdoor conditions including altered temperature, radiation levels and rainfall events is extremely stressful for non-acclimated seedlings. Particularly the increase in ultraviolet-B radiation is considered a serious threat. A new approach to pre-acclimate seedlings to ambient ultraviolet-B radiation is the use of ultraviolet-B transparent covering materials. In order to estimate the benefit of UV-B pre-acclimation, lettuce plants were raised in greenhouses covered with three different materials varying in ultraviolet-B transmittance and transplanted to the field at the age of three weeks. Ultraviolet-B exposure during the greenhouse period led to a reduction in growth (leaf length, leaf area and leaf number) and an increase in flavonoid content. Transplantation to the field induced a strong enhancement in flavonoid content and a severe growth reduction overriding differences between UV-B treatment groups within a few days. At the time of harvest plant fresh weight was therefore independent from previous ultraviolet-B treatment. Effects of UV-B acclimation on plant performance immediately after transplantation require more detailed examination.

Key words. *Lactuca sativa* – biomass – flavonoids – leaf area – leaf length – lettuce – UV-B radiation

Introduction

In order to extend the growing season in Europe, sensitive horticultural crops such as lettuce (*Lactuca sativa* L., *Asteraceae*) are commonly raised in protected cultivation and are transplanted to the field at the age of two to four weeks. Transplantation causes severe stress to the seedlings due to sudden changes in the biotic and abiotic environment coupled with possible mechanical injuries (South and Zwolinski 1997; Korkmaz and Dufault 2001; Mittler 2006). Among abiotic stress factors increased UV-B (280–315 nm) radiation may be particularly harmful to non-acclimated seedlings (Rozema et al. 1997). UV-B radiation is a minor component of sunlight but may cause severe damage due to generation of reactive oxygen species (ROS) and absorption by biologically active molecules such as nucleic acids, proteins (aromatic amino acids) and lipids (Schmitz-Hoerner and Weissenbock 2003; Ulm and Nagy 2005). Plant UV-B responses generally aim at protecting sensitive tissues from UV-B penetration and repairing UV-B induced damage (Jansen et al. 1998). UV-B protection is mainly achieved by epidermal accumulation of UV-absorbing flavonoids and hydroxycinnamic acids (Caldwell et al. 1983; Lois 1994). Repair of UV-B induced damage includes induction of enzymatic and non-enzymatic scavengers of ROS and activation of DNA repair mechanisms (Jansen et al. 1998; Britt 1999). Plants have developed a number of repair mechanisms such as photo-reactivation and excision repair (dark repair) to reduce the UV-B induced damages. The presented study will focus only on growth behavior and accumulation patterns of secondary compounds in the specific system *Lactuca sativa* var. *crispa* during pre-acclimation phase in greenhouses and under field conditions. The diverse molecular responses of plants to UV-B radiation are well reviewed by Britt (1999), Mackerness (2000) and Jordan (2002).

In horticulture, diverse strategies have been developed to pre-acclimate greenhouse grown seedlings to ambient or above-ambient UV-B levels and thereby increase the plant's stress tolerance and facilitate coping with outdoor conditions (Del Corso and Lercari 1997; Hoffmann 1999; Teklemariam and Blake 2003; Chalker-Scott and Scott 2004). A new approach is the use of recently developed covering materials. These innovative foils and glasses allow for pre-acclimation due to an increased UV-B transmittance (Kuhlmann and Müller 2009a). Recent studies have addressed the effects of UV-B transparent covering materials on growth and metabolism of lettuce. Biomass was reduced while flavonoid content was enhanced with increasing UV-B level (Krizek et al. 1998; Garcia-Macias et al. 2007; Tsormpatsidis et al. 2008). Yet, performance

of lettuce seedlings after transplantation to the field has not been examined so far.

Habitus and flavonoid content of lettuce plants are not only critical for the plant`s stress resistance at transplantation but also for product quality at harvest (Ryder 2001). Both affect optical appearance and durability of harvested lettuce (Couture et al. 1993). Flavonoids, with red oak leaf lettuce as an important source, are supposed to exert health-promoting effects since the intake of flavonoid-rich fruit and vegetables was found to be negatively correlated with the occurrence of cardiovascular disease and certain forms of cancer (Hertog et al. 1993; Crozier et al. 1997, Ferreres et al. 1997, Hollman 2001; Llorach et al. 2008).

The present study was therefore based on the hypothesis that UV-B exposure during greenhouse cultivation enhances flavonoid content and reduces either leaf growth or leaf number of lettuce seedlings in a dose-dependent manner. Particular interest was also focused on effects of UV-B acclimation on plant performance after transplantation to the field. The experiment was divided into two consecutive phases: a greenhouse and a field period. Lettuce plants were first raised from seed in greenhouses covered with three materials differing in UV-B transmittance. At the age of three weeks, half of the lettuce seedlings were transplanted to the field while half of them remained in the greenhouses. During the greenhouse period leaf length and area, leaf number, plant fresh weight, and flavonoid content of the seedlings were continuously determined, while during the field period plant fresh weight and flavonoid content were assessed. The experiment was performed three times in planting month I (April), planting month II (May) and planting month III (June 2007), which allowed for an estimation of the planting monthly impact, as well.

Materials and Methods

Plant material and cultivation conditions

Experiments were performed in newly developed greenhouses providing ideal features for examination of plant responses to solar UV-B radiation (design: Gerhard Reisinger, University of Bonn, construction: Siedenburger Gewächshausbau, Rahden, Germany). The experimental greenhouses, installed at Marhof Experimental Station in Wesseling (Germany), were characterized by a light-weight construction in order to minimize shading and a small ground area of 4.2 × 3 m. Six greenhouses were covered with three different materials (two greenhouses each) substantially varying in UV-B transmission: ETFE film ("high UV-B" treatment, ethylene-tetrafluoroethylene, 100 µm, Asahi Glass Green-Tech, USA, China, South Korea, Japan) and MM glass ("intermediate UV-B" treatment, microstructured low iron glass, CENTROSOL MM; Centrosolar Glas, Fürth, Germany) exhibit a UV-B transparency of about 86 and 30 %, respectively, whereas the conventional Float glass ("low UV-B" treatment, Siedenburger Gewächshausbau, Radhen, Germany) almost excludes radiation in the UV-B range (Table 1) (see also Kuhlmann and Müller 2009a). Transmission spectra of Float glass, MM glass and ETFE film, determined in the range between 280 and 750 nm by means of a UV/Vis spectrometer (LAMBDA, Perkin Elmer, Massachusetts, USA) are given in Fig. 1.

Table 1. Proportion of UV-B radiation transmitted by Float glass (low), MM glass (intermediate) and ETFE film (high UV-B). (PAR= photosynthetically active radiation).

Treatment	Covering material	Transmission (%)	
		UV-B	PAR
Low UV-B	Float glass	0.7	89.1
Intermediate UV-B	MM glass	30.1	91.3
High UV-B	ETFE film	86.2	93.1

Red oak leaf lettuce (*L. sativa*, L. cv. 'Bughatti') (Hild Samen GmbH, Marbach, Germany) was grown from seed in trays with 100 small press pots placed on tables inside the greenhouses. Manual irrigation with well water was done every morning. On day twenty-four, twenty-one and twenty after sowing (in planting month I-III, respectively) 200 seedlings from each greenhouse were transplanted to four field plots (50 plants to each plot), while 200 seedlings were kept in the greenhouses.

Solar radiation was monitored inside the greenhouses and in the field with an $X1_2$ Optometer (Gigahertz Optik, Puchheim, Germany). Triple radiation sensors [detecting UV-B, UV-A and photosynthetically active radiation (PAR) separately] were positioned at plant height in the centre of the greenhouses. Temperature and humidity were measured using dataloggers (ELV Elektronik AG, Leer, Germany); values were comparable under the different covering materials.

Growth monitoring

Determination of leaf area and length as well as the rate of leaf formation was restricted to the greenhouse period while assessment of plant fresh weight was continued during field cultivation.

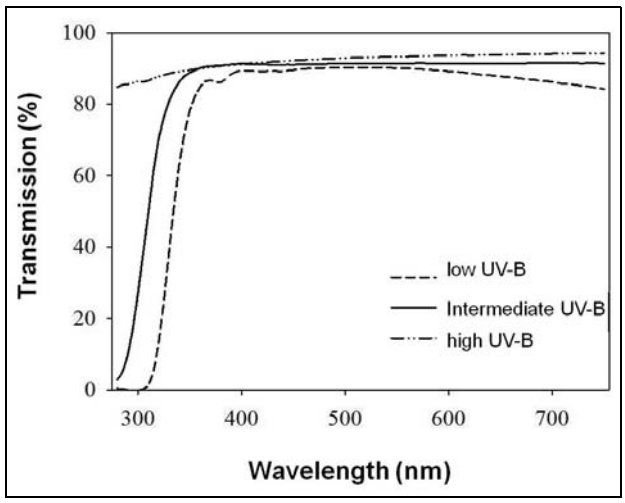

Fig. 1. Transmission spectra of Float glass (low), MM glass (intermediate) and ETFE film (high UV-B transmittance) from 280 to 750 nm, measured with a UV/Vis spectrophotometer.

For growth analysis, three to four trays per greenhouse were screened photographically every second day and total leaf area per plant was calculated. Images of entire germination trays were acquired using a digital camera (Panasonic DMC-FZ7), installed in a fixed position on a tripod. In order to avoid any influence of the circadian rhythm pictures were always taken at the same daytime. Picture analysis was based on the method of "ColorSegmentation"– an analysis tool developed at Forschungszentrum Jülich, ICG-3. The amount of green pixels characterizing the leaf area was transformed into units of leaf area by the use of an internal standard where 2285 pixel correspond to a leaf area of one cm^2. Color segmentation between green leaf area and brown/black background was performed on the basis of hue, saturation and value (HSV)-formatted images. They were transformed from RGB (red, green, blue) images provided by the camera. For more details, see WALTER et al. (2007). Due to leaf curling and overlapping, photographic examination of leaf area was restricted to early stages of plant development (stage 13 according to BBCH code, FELLER et al. 1995).

Leaf lengths of 20-day old plants were measured starting with the oldest (referred to as leaf number 1) and proceeding to the youngest leaf (number 6 or 7). Eighty replicates were taken of each treatment group. The number of leaves per plant was counted between day 14 and 28 after sowing.

Determination of plant fresh weight was started during the greenhouse period and continued throughout the field period until harvest. Fresh weight of above-ground biomass was assessed by cutting the entire plant just above the roots. Six replicates per treatment were taken during the greenhouse period and eight replicates were taken during field cultivation. The calculation of relative growth rate is based on following equation:

$$RGR = \frac{((LN)FW_{t2}) - (LN - FW_{t1})}{(t_2 - t_1)}. \quad (1)$$

(FW = fresh weight, t = timepoint)

Determination of flavonoid content

Samples for flavonoid analysis were collected three times during greenhouse cultivation and twice after transplantation between day 15 and 30 after sowing. Mixed samples of three to twelve whole plants ($n_{day\ 14} = 12$, $n_{day\ 17} = 6$, $n_{day\ 20} = 6$, $n_{day\ 26} = 3$, $n_{day\ 33} = 3$) were frozen in liquid nitrogen and stored at −25 °C. Frozen plant tissue was lyophilized and subsequently ground in a swing mill (MM 2000, Retsch, Haan, Germany) to fine powder. The powder (0.25 g) was extracted with 3 and 2 ml 62.5% aqueous methanol (AppliChem, Darmstadt, Germany) and centrifuged at 4000 rpm for 10 min. The combined supernatants were washed with 2 × 4 ml petrolether (AppliChem, Darmstadt, Germany). Acidic hydrolysis of flavonoid glycosides was performed by addition of 3 ml of 18.5 % HCl (2 M in total) and incubation at 70 °C for 2 h (see HERTOG et al. 1993). Extracts were brought up to a volume of 10 ml with methanol. Before HPLC analysis, samples were filtered through syringe filters (polypropylene membrane, 0.2 µm, VWR International GmbH, Darmstadt, Germany) and stored at −25 °C.

Samples were analysed using an Agilent (Santa Clara, California, USA) 1100 series automated liquid chromatography equipped with a MWD. A C_{18} column (LiChrosorb RP-18, 125 × 3 mm, 5 µm, Chromatographie Service GmbH, Langerwehe, Germany) served for reversed phase separation. The mobile phase performed a 42 min. gradient, (15–100 %) of 0.1 % formic acid (solvent A) and acetonitile (solvent B, both purchased at AppliChem, Darmstadt, Germany) at a flow rate of 0.8 ml min^{-1}. Compounds were identified by comparison of retention times and absorption maxima with standard substances. Utilized standard substances were cyanidin chloride and quercetin (purchased at Carl Roth GmbH & Co. KG, Karlsruhe, Germany and Fluka AG, Buchs, Switzerland, respectively).

Statistical analysis

Significant differences in the growth parameters between plants of the three treatment groups were tested and compared by means of a two-way ANOVA after a root square or log transformation, respectively. This mathematical function (square root transformation) is useful to reduce the imbalance of the dataset and build a normality distribution. Biomass at harvest and leaf length data were analysed via one way ANOVA using Tukey as PostHoc test (SigmaPlot 11.0 and SPSS 11.5, SPSS Inc., Chicago, USA). The number of leaves was analysed with a nonparametric test (two related samples, Wilcoxon signed ranks test using SPSS 11.5). An overview of the statistical dataset is given in the Table 4–6.

Results

Radiation measurements

Radition levels in the UV-B range under the three different covering materials were continuously recorded. During each of the three experiments the degree of UV-B

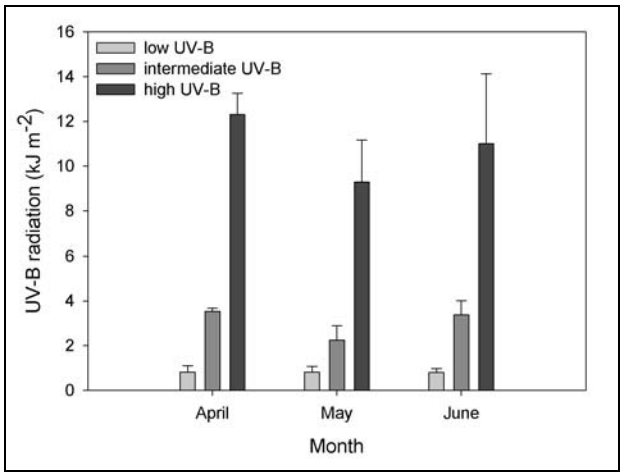

Fig. 2. Mean UV-B radiation level (kJ m^{-2}) transmitted by different UV-B transmittance materials: Float glass (low), MM glass (intermediate) and ETFE film (high UV-B) in planting month I–III (April, May and June). Radiation was recorded by means of a triple sensor (UV-B, UV-A and photosynthetically active radiation – PAR) for five days at a frequency of one minute.

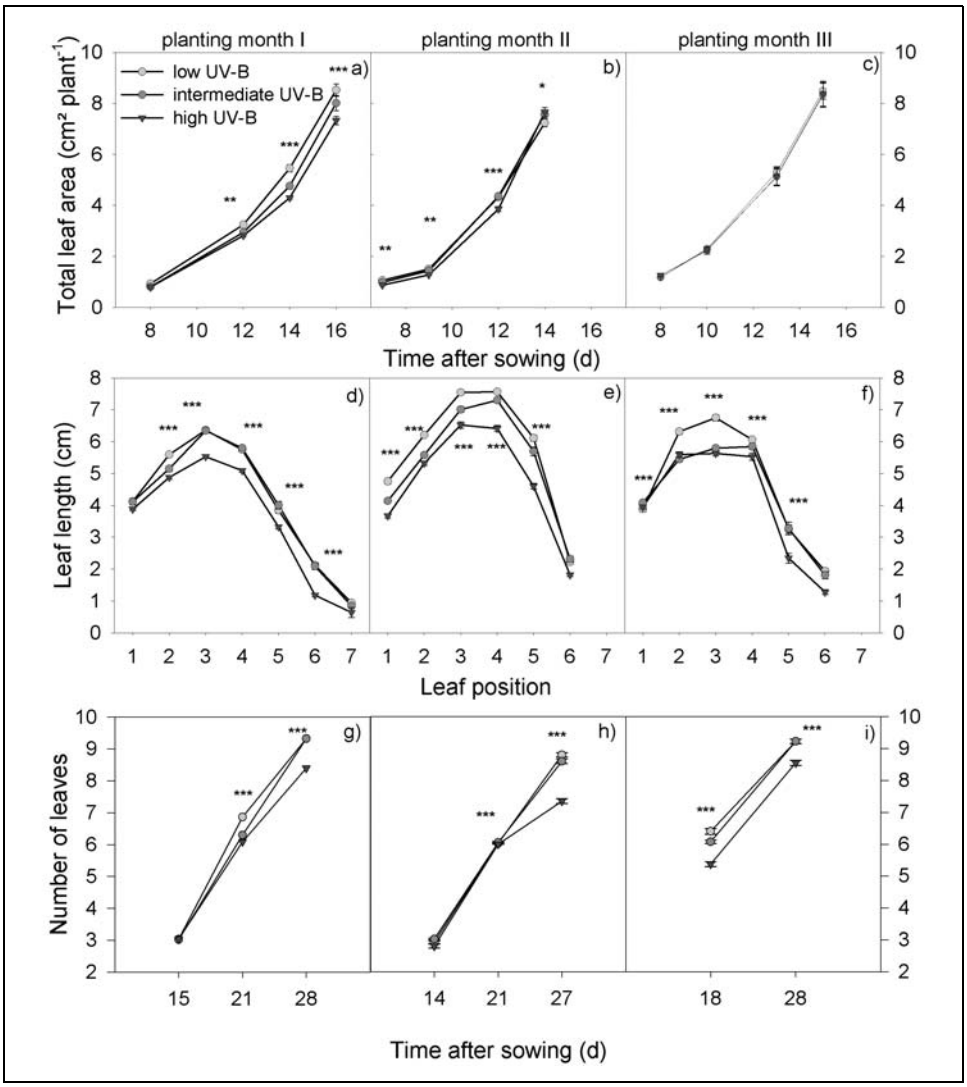

Fig. 3. Morphological parameters. a–c) Total leaf area per plant (cm^2) between day 7 and 16 of seedling development in a) planting month (pm) I (April 2007), b) pm II (May 2007) and c) pm III (June 2007), (mean ± SE, n=104); d–f) Leaf lengths (cm) of leaf positions from 1 (oldest) to 6/7 (youngest) of 20-day old lettuce plants in d) pm I, e) pm II and f) pm III, (mean ± SE, n=40); g–i) Total leaf number between day 14 and 28 after sowing at high, intermediate and low UV-B conditions in g) pm I, h) pm II and i) pm III, (mean ± SE, pm I: n=80, pm II and III: n=40). Statistical analyses were performed by Two way ANOVA, followed by a Tukey PostHoc test, tested the differences in dependence of time after sowing or leaf position, respectively; stars denote significant differences between low and high UV-B treatment, *: $p \leq 0.05$, **: $p \leq 0.01$, ***: $p \leq 0.001$.

transmission showed highest values under ETFE film (9–12 kJ m^{-2}), intermediate under MM glass (2.8–3.5 kJ m^{-2}) and lowest under Float glass (0.7–0.8 kJ m^{-2}, Fig. 2). Minor differences were detected in the transmission of ultraviolet-A (UV-A) and PAR (data not shown). The proportion of UV-B radiation transmitted by the three covering materials was comparable in all experiments although variability was higher in planting month II and III as compared to planting month I (Fig. 2).

Growth analysis

Total leaf area per plant was gradually reduced with increasing UV-B exposure at all four dates (day 8, 12, 14 and 16) in planting month I (April). The reduction in leaf area accounted for 6 and 14 % when grown at 30 and 86 % UV-B transmission, respectively, compared to the control group kept in a UV-B free environment (day 16, Fig. 3a–c, Table 2). This difference in total leaf area was found in planting month I but not in planting month II and III (May and June).

Leaf lengths were significantly lowered by UV-B exposure in all three experiments. In almost every leaf position, a clear reduction in leaf length was found at high UV-B treatment compared to UV-B exclusion. Plants exposed to the intermediate UV-B level ranged between the extreme treatments (Fig. 3d–f). In general, leaf area and length increased at a higher rate in planting month II and III than in planting month I. In planting month II and III the projected leaf area per plant on day 14 accounted for 7.5 cm^2, whereas it was only about 4.8 cm^2 in planting month I (Fig. 3a–c).

Leaf number of lettuce seedlings was significantly lower at high compared to intermediate and low UV-B conditions on day 27/28 in all three experiments (Fig. 3g–j). The previous measurements (day 14/15 and day 18/21) showed the same results except for two measurements, which did not show any differences.

Plant fresh weight did not differ significantly between UV-B treatments (Fig. 4, Table 2) although the relative growth rate calculated from fresh weight data between day 20, the day of transplantation, and 25 indicates a slight growth reduction under +UV-B conditions (Table 3). After transplantation to field conditions, the plant fresh weight of all treatment groups was strongly reduced compared to plants kept under controlled conditions. Plant fresh weight of field grown plants was 25 % lower compared to control plants in the greenhouse after

Table 2. The effect of different UV-B radiation levels on leaf area, plant fresh weight, cyanidin and quercetin content. The values within the table are means with grouping due the significance level of $p < 0.05$, a, b and c after values indicating significantly different means by varying letters.

Treatment/ Parameter		Planting month I			Planting month II			Planting month III					
		Greenhouse			Greenhouse			Greenhouse			Field		
		Low	Inter.	High	Low	Inter.	High	Low	Inter.	High	Low	Inter.	High
Total leaf area (cm^2)	d 7/ 8	0.93	0.81	0.795	1.06 a	0.98	0.87 b	1.17	1.18	1.26			
	d 9/10				1.50 a	1.44 a	1.28 b	2.30	2.26	2.23			
	d 12/13	3.24 a	2.94	2.82 b	4.33 a	4.35 a	3.86 b	5.30	5.12	5.14			
	d 14	5.45 a	4.76 b	4.30 c	7.24 a	7.57	7.66 b						
	d 15/16	8.52 a	8.01 a	7.33 b				8.50	8.34	8.36			
Leaf length (cm)	leaf 1	4.12 a	4.11a	3.88 b	4.76 a	4.14 b	3.67 c	2.89	4.09	3.95			
	leaf 2	5.60 a	5.15 b	4.88 c	6.21 a	5.56 b	5.32 c	6.32 a	5.45 b	5.59 a			
	leaf 3	6.36 a	6.35 a	5.53 b	7.54 a	7.01 b	6.52 c	6.75 a	5.80 b	5.63 a			
	leaf 4	5.75 a	5.80 a	5.09 b	7.57 a	7.30 a	6.42 b	6.06 a	5.85 a	5.53 b			
	leaf 5	3.85 a	4.00 a	3.33 b	6.11 a	5.70 b	4.60 c	3.26 a	3.28 a	2.35 b			
	leaf 6	2.12 a	2.09 a	1.18 b	2.23 a	2.32 a	1.82 b	1.94 a	1.81 a	1.28 b			
	leaf 7	0.95	0.85	0.64									
Number of leaves	date 1	3.03	3.02	3.04	2.93 a	3.03 b	2.80 a						
	date 2	6.86 a	6.30 b	6.09 c	6.03	6.05	6.00	6.40 a	6.08 b	5.38 c			
	date 3	9.33 a	9.33 a	8.40 b	8.80 a	8.60 b	8.35 c	9.23 a	9.23 a	8.56 b			
Plant fresh weight (g)	d 17							2.50	2.63	2.28			
	d 20							4.73	4.92	4.82			
	d 25							20.27	19.50	16.83	12.69	13.76	13.53
	d 29							29.62	29.27	28.05	20.18	19.36	20.38
Quercetin content ($\mu mol\ g^{-1}dm$)	d 14							9.72 a	11.51 a	11.87 b			
	d 17							18.11 a	20.75 b	24.74 c			
	d 20							14.31 a	16.98 b	20.79 c			
	d 26										33.67	35.25	33.84
	d 33										25.66	26.00	24.02
Cyanidin content ($\mu mol\ g^{-1}dm$)	d 14							8.62 a	13.46 a	14.60 b			
	d 17							19.59 a	23.68 ab	33.32 b			
	d 20							14.67 a	21.69 ab	31.40 b			
	d 26										47.36	49.55	47.25
	d 33										47.79	49.10	42.87

Abbreviations: Low, Inter. = intermediate and High UV-B level; dm = dry matter; d = day.

four days of outdoor exposure (day 25, Table 3). At the stage of harvest (age: 61 d) plant fresh weight did not vary between UV-B treatment groups except for experiment in planting month III where fresh weight of plants under intermediate UV-B radiation differed significantly from high and low UV-B treated plants ($p = 0.017$, $p = 0.021$), respectively (see Table 4, Fig. 6).

Flavonoid content

The main flavonoid aglycones detected in extracts of red oak leaf lettuce were quercetin and cyanidin. During greenhouse cultivation, cyanidin and quercetin content were gradually elevated with increasing UV-B level in the order low > intermediate > high UV-B transmission (Fig. 5, data obtained in planting month II (June), Table 2). On day 20, plants exposed to intermediate and high UV-B showed an increase in quercetin content by 19 and 45 % and in cyanidin content by 23 and 78 %, respectively, compared to plants kept at low UV-B. The established differences of plants under low and high UV-B conditions were significant for quercetin ($p < 0.001$ for all three days) and cyanidin from day 14 to 20 after sowing ($p = 0.014$, $p = 0.01$ and $p = 0.006$). The changes

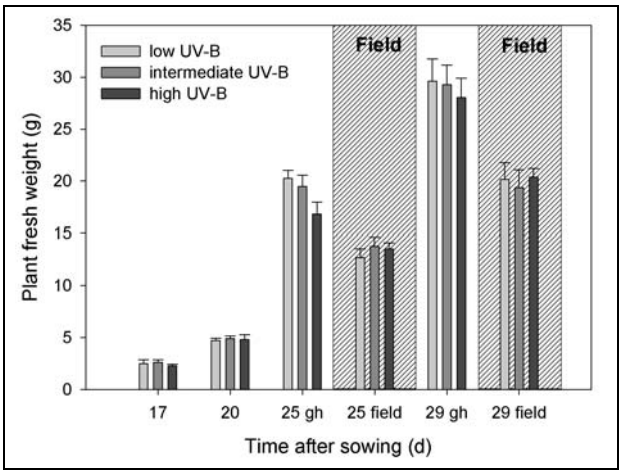

Fig. 4. Plant fresh weight (g) of different UV-B treatment groups on day 17, 20, 25 and 29 in the greenhouse (gh) and on day 25 and 29 in the field (indicated by grey boxes) during June experiment (Mean ± SE, n = 6–8). Data were statistically analysed by Two-way ANOVA and Tukey test; no significant differences were found.

in cyanidin content from day 17 to 20 are not time dependent (p = 0.975) but UV-B dependent. Six days after transfer to the field, quercetin content was increased by 97 % and cyanidin content by 104 % (average of the three treatment groups), respectively. The enhancement did not differ significantly between UV-B conditions. This strong enhancement was coupled to an equalization of differences between treatment groups. Between day 26 and 33 quercetin and cyanidin content declined by 26 and 3 %, respectively. These observations were comparable in all experiments, independent of season.

Table 3. Relative growth rate (% d^{-1}), based on fresh weight data, between day 20 and 25 and between day 20 and 29 under greenhouse and field conditions.

	Day 25		Day 29	
	Green-house	Field	Green-house	Field
Low UV-B	29	18	20	15
Intermediate UV-B	27	21	19	15
High UV-B	25	22	19	18

Discussion

Greenhouse period

During greenhouse cultivation flavonoid content as well as leaf growth and leaf number of lettuce seedlings were clearly affected by the specific proportions of solar UV-B radiation transmitted by Float glass (low), MM glass (intermediate) and ETFE film (high UV-B treatment). The induction of flavonoid accumulation observed in the intermediate and high UV-B treatment group (Fig. 5a, b) is a common response to UV-B radiation, found in numerous plant species including lettuce (Caldwell 1981; Lois 1994; Krizek et al. 1998; Garcia-Macias et al. 2007). Our results are consistent with the assumption that flavonoid induction is a dose-dependent response (Tsormpatsidis et al. 2008) as flavonoid contents seemed to be gradually

Table 4. The influence of different UV-B radiation on leaf area (during three planting months), leaf number, plant fresh weight and cyanidin and quercetin content in dependency of the developmental stage (Days after sowing – DAS) of lettuce plants. (The mean difference is significant at the 0.05 level (bold numbers indicate a significance level, Two way ANOVA, Tukey test).

		Leaf area, p-value				Plant fresh weight, p-value		Cyanidin content, p-value	Quercetin content, p-value
Factor	df	Pm I	Pm II	Pm III	df		df		
DAS	3	<0.001	<0.001	<0.001	5	<0.001	4	<0.001	<0.001
UV-B	2	<0.001	<0.004	0.875	2	0.477	2	<0.001	<0.001
DAS × UV-B	6	**0.032**	**<0.001**	0.995	10	0.703	8	<**0.021**	**<0.001**

Pm = Planting month.

Table 5. The influence of different UV-B levels for biomass at harvest and leaf length (in dependence on leaf position) were tested with a one way ANOVA and Tukey-test. Bold numbers indicate a significance level.

Leaf length/UV-B	Pm I, Leaf position 1-7, p-value	Pm II, Leaf position 1-6, p-value	Pm III, Leaf position 1-6; p-value
Low – intermediate	0.99/**0.00**/0.98/0.85/0.49/0.96/0.29	**0.00**/**0.00**/**0.00**/0.51/**0.02**/0.69	0.11/**0.00**/**0.00**/0.25/0.99/0.64
Intermediate – high	**0.037**/**0.019**/**0.00**/**0.00**/**0.00**/**0.00**/0.31	**0.00**/**0.01**/**0.00**/**0.00**/**0.00**/**0.00**	0.345/0.41/0.31/0.052/**0.00/0.01**
Low – high	**0.028**/**0.00**/**0.00**/**0.00**/**0.00**/**0.00**/0.68	**0.00**/**0.00**/**0.00**/**0.00**/**0.00**/**0.001**	0.081/**0.00**/**0.00**/**0.00**/**0.0**/**0.001**

Pm = Planting month.

Table 6. Influence of different UV-B radiation on biomass at harvest [The mean difference is significant at the 0.05 level (one way ANOVA, Tukey test)] and on number of leaves (Wilcoxon signed rank test). Bold numbers indicate a significance level.

	Biomass at harvest			Number of leaves		
UV-B Treatment – Interaction	Pm I, p-value	Pm II, p- value	Pm III, p-value	Pm I, p-value 15/21/28 DAS	Pm II, p-value, 14/21/27 DAS	Pm III, p-value, 18/28 DAS
Low – intermediate	0.481	**0.018**	**0.045**	0.414/**0.00**/0.73	**0.046**/0.564/**0.046**	**0.002/**1.00
Intermediate – high	0.495	**0.022**	0.993	0.257/**0.01/0.00**	**0.003/**0.157/**0.00**	**0.00/0.00**
Low – high	0.999	0.997	**0.034**	0.739/**0.00/0.00**	0.132/0.317/**0.00**	**0.00/0.00**

Pm = Planting month: DAS = Days after sowing.

elevated with increasing UV-B level (Fig. 5). The most abundant flavonoid aglycones found in extracts of red oak leaf lettuce are quercetin and cyanidin, which in vivo are mainly represented by quercetin-3-O-(6"-O-malonyl)-glucoside and cyanidin-3-O-(6"-O-malonyl)-glucoside (Garcia-Macias et al. 2007; Llorach et al. 2008). Flavonols such as quercetin provide UV-B protection as epidermally deposited UV shields (Caldwell et al. 1983; Lois 1994), whereas anthocyanins such as cyanidin are supposed to contribute relatively little to total UV absorbance (Woodall and Stewart 1998). Both, quercetin and cyanidin possess strong antioxidant activity in vitro (Rice-Evans et al. 1996). Yet, their contribution to the mitigation of UV-B induced oxidative stress in vivo is difficult to estimate since they are for the most part localized in epidermal vacuoles and thereby isolated from ROS generation in the chloroplasts of the palisade mesophyll (Gould and Lister 2006).

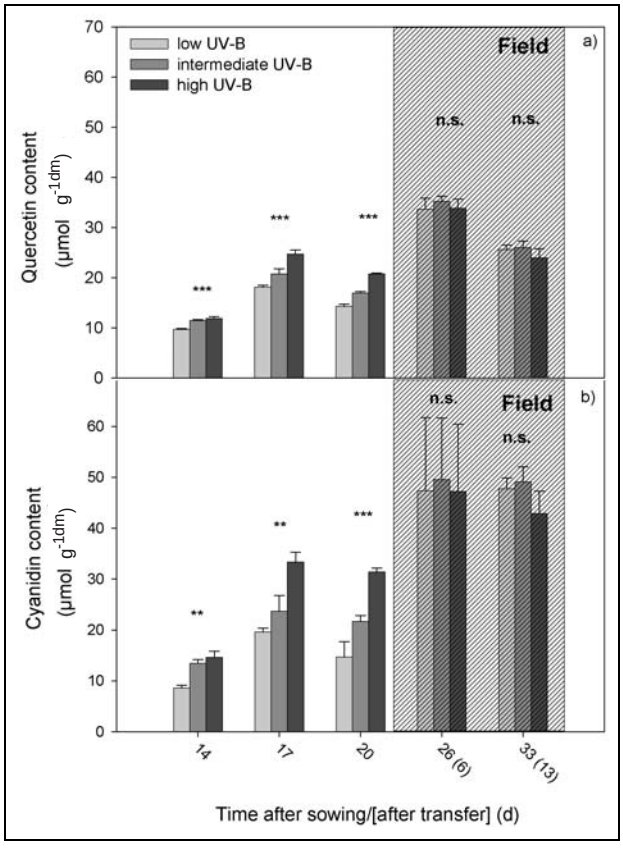

Fig. 5. a) Cyanidin and b) Quercetin content (µmol g^{-1}dm) of lettuce seedlings from different UV-B treatment groups between day 14 and 33 after sowing at greenhouse and field conditions (indicated by grey boxes). (Mean ± SE, $n_{day\ 14}$ = 12, $n_{day\ 17/20}$ = 6, $n_{day\ 26/33}$ =3). (Statistical information of quercetin and cyanidin content is presented in Table 4–6).

The reduction in total leaf area per plant found at intermediate and high UV-B conditions is obviously due to two processes: a decrease in leaf number and a decline in leaf expansion as indicated by lower leaf lengths (Fig. 3a-i). Both is consistent with previous studies reporting UV-B treated lettuce plants to show a reduction in leaf number and leaf area which is often coupled with an increased leaf thickness (Krizek et al. 1998; Rousseaux et al. 2004). Tsormpatsidis et al. (2010) found an induction in leaf number after transferring lettuce plants from UV-blocking to UV-transparent houses. UV-B induced changes in leaf morphology are supposed to diminish UV-B exposure of sensitive tissues (Jansen et al. 1998). The decline in leaf length was observed in most leaf positions indicating that this response is independent of the leaf developmental stage in 20-day-old plants. Plant fresh weight was expected to be reduced by UV-B exposure as described by several other authors (Krizek et al. 1998; Garcia-Macias et al. 2007; Tsormpatsidis et al. 2008), but the differences we found were not significant (Fig. 4).

Planting month was also found to affect growth and growth responses to UV-B. The finding that leaf length was reduced by UV-B exposure in all three months whereas leaf area was only affected in planting month I (Fig. 3a-c) might be due to changes in leaf morphology (e.g. stronger curling) and methodological limitations. For the analysis of the projected leaf area a nearly planar leaf surface is necessary to avoid underestimation of the real leaf area. With increasing curling of leaves, the accuracy of the method decreases. In general, relative growth rate (based on fresh weight) was higher in planting month II and planting month III than in planting month I, indicating a higher photosynthetic productivity in early summer (Fig. 3, Table 3). This effect may be due to elevated temperatures (Medlyn et al. 2002; Walter et al. 2009). This is in contrast with results obtained by Tsormpatsidis et al. (2008) who found no interaction between vegetative growth and planting month in experiments conducted in a more northern region.

At the time of transplantation lettuce seedlings grown at intermediate and high UV-B showed an increase in quercetin content by 18.7 and 45.3 %, an elevation in

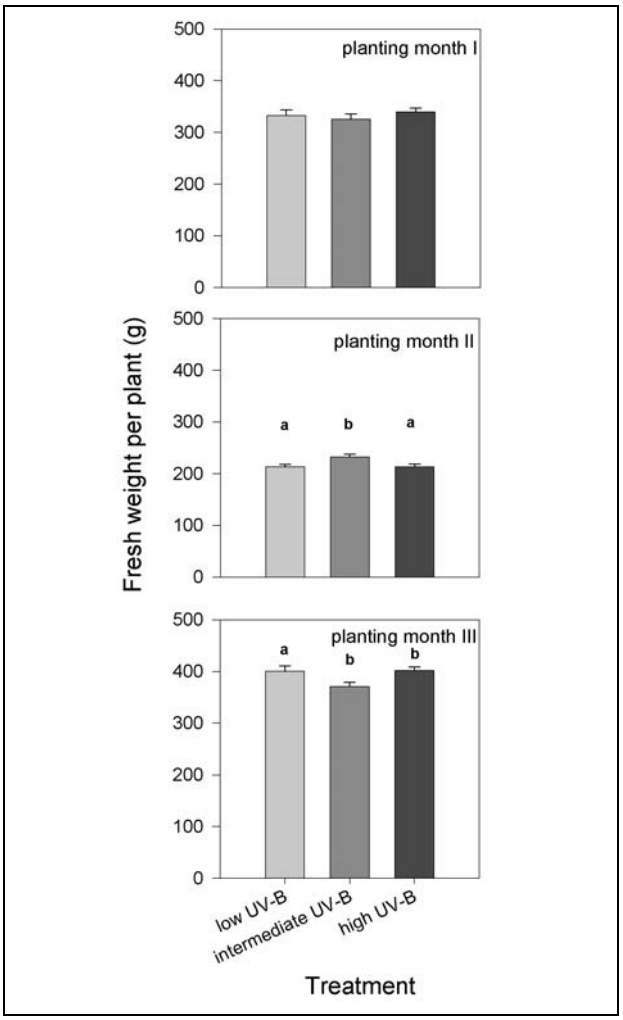

Fig. 6. Fresh weight (g) of plants of different UV-B treatment groups at harvest in a) planting month I (April); b) planting month II (May) and c) planting month III (June) experiment. Mean ±SE, n=70. (Letters a, b and c indicating a different significance level of p = 0.05 after One-way ANOVA and Tukey test).

cyanidin content by 23.2 and 78.4 % (day 20, planting month III), respectively, and a decrease in total leaf area per plant by 6 and 14 % (day 16, planting month II), respectively, compared to plants grown in the absence of UV-B radiation (Fig. 5 and 3f). The increase in epidermally deposited flavonols, such as quercetin, and the decline in leaf area reduce the penetration of harmful UV-B radiation into metabolically active tissues (Burchard et al. 2000). Elevated flavonoid contents contribute to the elimination of stress-induced ROS (Rice-Evans et al. 1996). Therefore, these compositional and structural changes might possibly enhance the plant's stress tolerance under outdoor conditions.

Field period

Transplantation to field conditions induced rapid and strong compositional and morphological responses in propagation plants. The increase in flavonoid content by 97 (quercetin) and 104 % (cyanidin), respectively, and the reduction in fresh weight accumulation by 25 % compared to greenhouse cultivation within four to five days indicate that sudden exposure to outdoor conditions is quite challenging for plants raised in protected cultivation (Fig. 4 and 5). Reduced biomass and elevated flavonoid content are well defined responses of field grown compared to greenhouse grown lettuce plants as previously described by Romani et al. (2002). These non-specific stress responses are supposed to be induced by a broad array of biotic and abiotic stress factors including pathogen attack, increased UV-B level, altered temperature, humidity, availability of water and nutrients, wind and mechanical injury (Rabino and Mancinelli 1986; Rozema et al. 1997; Mittler 2006; Treutter 2006).

The coincidence of flavonoid induction and growth attenuation in lettuce has been reported in former studies (Krizek et al. 1998; Garcia-Macias et al. 2007; Tsormpatsidis et al. 2008) and is generally explained by the 'growth-differentiation balance hypothesis' postulating a trade-off from growth to defense due to the induction of protecting metabolites (Herms and Mattson 1992). This resource allocation mechanism is particularly found in young plants with limited stock reserves (Kuhlmann and Müller 2009b).

UV-B acclimation during the greenhouse period had no long-term effect on growth and flavonoid content under field conditions (Fig. 4 and 5). In several studies addressing pre-adaptation, UV-B treatment during seedling development had proven beneficial, e.g. in terms of an enhanced stress tolerance (Del Corso and Lercari 1997; Teklemariam and Blake 2003, Hoffmann 1999). In the study of Tsormpatsidis and coworkers (2010) it was shown, that cultivation of lettuce seedlings under UV blocking conditions followed by transfer to UV transparent conditions six days before harvest, increased the flavonoid content and did not reduce crop yield. Subsequent cultivation under these two materials differing in UV-transparency is beneficial for producers and consumers.

The present results confirm the hypothesis that UV-B exposure during greenhouse cultivation leads to a reduction in leaf growth and leaf number as well as to an increase in flavonoid content of lettuce seedlings in a mostly dose-dependent manner. Previous UV-B treatment had no long-term effect on the plant's response to field conditions after transplantation. While former studies on performance of lettuce at different UV-B levels are restricted to greenhouse cultivation, our work includes subsequent transplantation to the field and therefore represents a first approach to estimate the effects of UV-B pre-acclimation on performance of lettuce seedlings under outdoor conditions. In future studies, the response of a variety of cultivars with respect to their reaction to a transient exposure to UV-B and subsequent field cultivation will be addressed.

A more positive effect of UV-B pre-adaptation on plant performance under field conditions is conceivable under generally more stressful conditions. If lettuce plants would have been exposed to more adverse environmental conditions after transfer to the field, which often occurs in practice, increased flavonoid content and reduced biomass might have been beneficial for plant performance. Assessment of specifically stress-related parameters, as well as a higher temporal resolution of the data obtained immediately after transplantation might have shown clearer differences between UV-B treatment groups. In future studies, the benefit of pre-acclimation to near-ambient

solar UV-B radiation will be investigated in more detail along the lines of the experimental procedures described here, taking a closer look at stress tolerance and product quality.

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Addresses of authors: Helen Behn (corresponding author 1), Susanne Tittmann (corresponding author 2) [both authors contributed equally to this work], Achim Walter, Ulrich Schurr and Andreas Ulbrich, Institute for Chemistry and Dynamic of the Geosphere (ICG-,3 Phytosphere), Forschungszentrum Jülich GmbH, D-52425 Jülich; Helen Behn, Georg Noga and Andreas Ulbrich, Institute of Crop Sciences and Resource Conservation, Horticultural Sciences, University of Bonn, Auf dem Hügel 6, D-53121 Bonn; Andreas Ulbrich, Faculty of Agricultural Science and Landscape Architecture, University of Applied Science Osnabrück, Oldenburger Landstraße 24, D-49090 Osnabrück, and Susanne Tittmann (current address), Research Center Geisenheim, Institute of Viticulture, Von-Lade-Str. 1, D-65366 Geisenheim, e-mail: h.behn@fz-juelich.de (corresponding author 1) and susanne.tittmann@fa-gm.de (corresponding author 2).