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# Development-dependent UV-B Responses in Red Oak Leaf Lettuce (*Lactuca sativa* L.): Physiological Mechanisms and Significance for Hardening

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**Summary**

Plants protect themselves against potentially harmful UV-B radiation mainly by epidermal accumulation of UV-B shielding phenolic compounds and repair of cellular damage. The capacity and necessity for these protection mechanisms alter with the stage of plant and leaf development, which might be crucial for horticultural hardening techniques aiming at a UV-B pre-acclimation of greenhouse grown seedlings before transplantation to the field. In order to examine the development-dependence of UV-B responses and to estimate the efficiency of different hardening strategies, lettuce (*Lactuca sativa* L., var. *crispa* L. 'Bughatti') was raised in greenhouses covered with a UV-B transmitting and a UV-B excluding covering material and transferred from one treatment to the other at the age of 20 days. Plant fresh mass and flavonoid content were frequently determined. Distribution of flavonoids within the plants was assessed by extraction of different leaf age classes. Continuous exposure to near ambient UV-B induced a strong enhancement in cyanidin and quercetin content and a severe growth reduction, whereas late UV-B treatment merely led to a slight enhancement of quercetin in the absence of any growth response. At the leaf level, continuous UV-B exposure caused a comparable quercetin induction at all three stages of leaf development, whereas late treatment caused a much stronger response in young compared to intermediate and old leaves. These findings indicate that UV-B effects on growth and flavonoid content and pattern of lettuce plants are highly dependent on the leaf and plant developmental stage. We conclude that UV-B exposure throughout seedling development is a more efficient hardening strategy than late short-term exposure.

**Key words.** *Lactuca sativa* – lettuce – growth – flavonoids – UV-B radiation – stage of development – hardening

## Introduction

Ultraviolet-B (UV-B) radiation (280–315 nm), the shortest and most energy-rich solar wavelength range represents an environmental factor of major importance to plants (Paul and Gwynn-Jones 2003). Depending on fluence rate and wavelength, it acts as abiotic stressor (damaging) or developmental signal (non-damaging, Ulm and Nagy 2005). High fluence rates and short wavelength ranges of UV-B radiation cause non-specific stress responses associated with an increase in reactive oxygen species (ROS) and damage to DNA, proteins and membrane lipids (Jansen et al. 1998; Frohnmeyer and Staiger 2003; Jenkins 2009). Low fluence rates and long wavelength spectral parts induce UV-B-specific signal transduction pathways eliciting photomorphogenic responses (Hectors et al. 2007; Jenkins 2009). These specific responses promote UV-B tolerance by induction of genes involved in protection mechanisms such as enhancement of flavonoid biosynthesis (Lois 1994; Schmitz-Hoerner and Weissenbock 2003). Lettuce (*Lactuca sativa* L.*, Asteraceae*), a widespread horticultural crop and a common component of European diet, is known to show an increase in flavonoid content and a growth reduction in response to near-ambient levels of solar UV-B radiation (Krizek et al. 1998; Garcia-Macias et al. 2007; Tsormpatsidis et al. 2008).

Flavonoids comprise a diverse group of plant secondary compounds derived from the flavan or isoflavan skeleton, including e.g. flavonols and anthocyanins, which usually occur as glycosides (Gould and Lister 2006). In addition to constitutive contents of flavonoids present in most tissues, their biosynthesis can be locally enhanced by several biotic and abiotic stressors such as pathogens, temperature changes, hydric stress, high light and UV-B radiation (Chalker-Scott 1999; Treutter 2006). Photoinduction of flavonoids was shown to be mediated by phytochrome, cryptochrome and the putative UV receptor (Mancinelli and Schwartz 1984; Mol et al. 1996; Wade et al. 2001). In red lettuce varieties (red oak leaf, lollo rosso), the most abundant flavonoids are quercetin and cyanidin glycosides, *in vivo* predominantly 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 glycosides, are characterized by a considerable UV-B absorbing capacity and antioxidant activity, both determined by structural features (Rice-Evans et al. 1996; Jordan 2004). These properties as UV shield and radical scavenger are supposed to make epidermally accumulated quercetin an effective UV-B protectant (Caldwell et al. 1983; Kootstra 1994; Lois 1994; Jordan 2004). Anthocyanins, such as cyanidin glycosides, mainly absorb in the green and yellow spectral range between 500 and 600 nm and display a red, violet or blue color (Gitelson et al. 2001; Gould 2004). At high light conditions, anthocyanins may reduce the potential for oxidative damage, photoinhibition and photobleaching of chlorophyll by absorbing excess excitation energy (Smillie and Hetherington 1999; Steyn et al. 2002). Although anthocyanins provide antioxidant activity and some UV absorbance, they are supposed to serve a more general role in photoprotection rather than a UV-B specific function (Beggs and Wellmann 1985; Brandt et al. 1995; Woodall and Stewart 1998; Steyn et al. 2002; Gould and Lister 2006).

Mode and extent of UV-B responses seem to be endogenously determined by the developmental stage of the whole plant as well as of individual leaves (Kubasek et al. 1992; Reifenrath and Müller 2007). Sensitivity to UV-B radiation was reported to be higher in young compared to mature plants, probably due to a lack of substrate availability and chemical protection at early developmental stages (Kuhlmann and Müller 2009a). Within a mature plant, young, developing leaves are usually more responsive than older ones. This is attributed to the higher sensitivity and the higher photosynthetic value of young leaves representing the future photosynthetically active tissues (Lois 1994; Reifenrath and Müller 2007). Yet, systematic studies on the development-dependence of plant UV-B responses are still lacking.

Development-dependence of UV-B responses in lettuce is supposed to be of practical relevance to horticulture. In horticultural production, lettuce is raised in greenhouses and transplanted to the field at the age of two to four weeks. Sudden changes in environmental conditions, such as increased UV-B radiation, may cause severe stress to non-acclimated seedlings (Rozema et al. 1997; Mittler 2006). UV-B acclimation prior to transplantation is supposed to facilitate coping with outdoor conditions by induction of UV-B protection mechanisms (Chalker-Scott and Schott 2004). Therefore, diverse hardening strategies aim at acclimation to ambient or above-ambient levels of UV-B radiation (Del Corso and Lercari 1997; Hoffmann 1999; Teklemariam and Blake 2003; Chalker-Scott and Schott 2004). This is achieved either by a short-term treatment just before transplantation or by continuous UV-B exposure during early seedling development. A new hardening strategy aiming at continuous UV-B exposure is the use of recently developed UV-B transparent films and glasses as greenhouse covering materials (Kuhlmann and Müller 2009b). However, the benefit of continuous compared to late UV-B treatment has not yet been evaluated.

The present study was therefore based on the hypothesis that inclusion or exclusion of the UV-B range from near-ambient solar radiation induces a differential regulation of flavonoid metabolism and biomass accumulation in lettuce seedlings. We furthermore postulated that mode and extent of these responses are determined by the plant's stage of development. Development-dependence of UV-B responses in lettuce might be of particular importance for determining the most advantageous time for UV-B pre-acclimation. In order to test these hypotheses, lettuce plants were raised in greenhouses covered with either a UV-B transmitting (high UV-B) or a UV-B shielding (low UV-B) covering material. At the age of 20 days (d) parts of the populations were transferred from low UV-B to high UV-B and from high UV-B to low UV-B conditions. Plant fresh mass as well as flavonoid content and pattern were frequently determined. Distribution of flavonoids within the plants was assessed by extraction of different leaf age classes.

## Materials and Methods

### *Plant material and cultivation conditions*

Lettuce plants (*Lactuca sativa* L., *Asteraceae,* var. *crispa* L., 'Bughatti', Hild Samen GmbH, Marbach am Neckar, Germany) were grown from seed in trays with 100 soil press pots. The soil press pots were 3.5 × 3.5 × 3.5 cm cubes consisting of pot ground. Eight trays were placed in each greenhouse. Plants were adequately provided with water every morning.

The experiment was performed in newly developed greenhouses particularly designed for examination of plant responses to natural UV-B radiation (design: Gerhard Reisinger, University of Bonn, Germany, construction: Siedenburger Gewächshausbau, Rahden, Germany). Each greenhouse has a ground area of 4.2 × 3.0 m and a height increasing from 2.0 to 3.9 m (mono-pitch roof design). They are aligned in north-south direction with the lower part of the roof directed to the south. In order to avoid shading, the greenhouses are characterized by a light construction and positioned in a row at a distance of 3 m. Plants were placed on tables (85 cm in height) ranged along the walls. For examination of UV-B effects the greenhouses were covered in alternating order with two different cladding materials varying in the degree of UV-B transmission. Ethylene-tetrafluoroethylene film (ETFE film, 100 µm, AGC, Japan) allows for a UV-B transmission of 86.2 %, whereas Float glass (Siedenburger Gewächshausbau, Rahden, Germany) mostly excludes the UV-B range (0.65 % UV-B transmission, Table 1). Since the cut-off wavelength of Float glass (defined as

Table 1. Transmittance of Float glass and ETFE film (280–700 nm).

| Treatment | Covering material | Transmission (%)* | | |
|---|---|---|---|---|
| | | UV-B | UV-A | PAR |
| low UV-B | Float glass | 0.7 | 63.6 | 89.1 |
| high UV-B | ETFE film | 86.2 | 89.4 | 93.1 |

*Mean transmission in the UV-B (280–315 nm), UV-A (315–400 nm) and PAR (400–700 nm) spectral range, given in % of initial radiation level. Transmission data were kindly provided by Centrosolar Glas GmbH & Co. KG, Fürth, Germany.

50 % of mean photosynthetically active radiation [PAR, 400–700 nm] transmission here) is at about 332 nm, the irradiation conditions present under this glass will be designated as 'low UV-B treatment', while irradiation conditions provided by ETFE film will be denoted as 'high UV-B treatment' in the following. Transmission of PAR does not differ substantially between ETFE film and Float glass (Table 1). The transmission was measured with a UV/Vis spectrophotometer (LAMBDA 900, Perkin Elmer, Massachusetts, USA) in the spectral range between 280 and 2500 nm. The greenhouses were installed at Marhof Experimental Station, Wesseling, Germany, located at latitude 50.80° and longitude 6.97°.

On d 21 after sowing, parts of the population grown under low UV-B conditions were exposed to UV-B and high UV-B adapted plants were deprived from UV-B by transferring two trays from each of the two low UV-B houses to both of the high UV-B houses and vice versa. Transfer from high UV-B to low UV-B and from low UV-B to high UV-B at the age of 20 d (which is on d 21) is in the following referred to as 'late' treatment.

A stationary, computer-based system (RAM Electronic GmbH, Norderstedt, Germany) was installed for monitoring temperature and relative humidity. Temperature was comparable under both covering materials accounting for 19.8±0.4 °C (day) and 18.7±0.4 °C (night) under ETFE film and for 20.0±0.3 °C (day) and 18.9±0.4 °C (night) under Float glass (mean±SE). The experiment was conducted from May 19 until June 12, 2008.

### *Sampling*

Plants were sampled on:
- d 16 at 8 a.m. (before transfer)
- d 21 at 8 a.m. (at the time of transfer)
- d 21 at 2 p.m. (6 h after transfer)
- d 22 at 8 a.m. (24 h after transfer)
- d 22 at 2 p.m. (30 h after transfer) and
- d 23 at 8 a.m. (48 h after transfer).

Days are counted from the day of sowing (designated as d 1) onwards.

Total above-ground biomass was cut just above the roots and subsequently frozen in liquid nitrogen for determination of flavonoid content. Samples were pooled from 8 plants on d 16 and from 2 plants on d 21, 22 and 23. Six replicates of these pooled samples were taken from high and low UV-B treatment, 12 replicates from the transferred plants.

In order to assess flavonoid distribution over the plant, different leaf age classes were sampled (d 24). The two oldest (outer) leaves were leathery and not curled, the intermediate fraction was unfolded and curled and the youngest (inner) leaves were still completely folded. Each sample was pooled from six plants; six replicates were taken from continuous treatments (low UV-B and high UV-B) and 12 replicates from switch treatments (low UV-B → high UV-B and high UV-B → low UV-B). The two greenhouses covered with identical material were sampled individually.

### *Determination of plant fresh mass and relative growth rate*

Plant fresh mass (FM) was determined by weighing above-ground tissues of recently harvested lettuce plants. Based on these data relative growth rate (RGR) was calculated according to the following equation:

$$RGR = (\ln(FMt_2) - \ln(FMt_2)) \mid (t_2 - t_1)$$

(FM = fresh mass, t = time point, ln = Logarithmus naturalis)

### *Extraction and HPLC analysis of flavonoids*

Frozen plant material was lyophilised and ground to a fine powder using a mill (MM 2000, Retsch, Haan, Germany). Then, 250 mg of the powder were extracted with 3 mL of aqueous methanol (62.5/37.5, v/v) (AppliChem, Darmstadt, Germany), centrifuged at 4000 rpm for 10 min. and the supernatant was collected. This procedure was repeated with 2 mL aqueous methanol; the supernatants were combined. The extract was washed twice with 4 mL petrol ether (AppliChem, Darmstadt, Germany). For acid hydrolysis of flavonoids 3 mL HCl (5 M) was added to the extracts before incubation for 2 h at 70 °C (see Hertog et al. 1992). Samples were brought up to a volume of 10 mL with methanol. Finally samples were filtered (polypropylene membrane, 0.2 µm, VWR International GmbH, Darmstadt, Germany) and stored at −25 °C until HPLC analysis. Chromatographic analysis was performed on an HPLC 1100 series equipped with a multi-wavelength detector (Agilent, Santa Clara, California, USA). Separation was achieved by use of a C18 column (LiChrosorb RP-18, 125 × 3 mm, 5 µm, Chromatographie Service GmbH, Langerwehe, Germany) and a gradient of 0.1 % formic acid (solvent A) and acetonitile (solvent B, both purchased at AppliChem, Darmstadt, Germany) starting with 15 % solvent B and increasing up to 22 % within 10 min, staying at 22 % for 5 min, going up again from 22 to 27 % within 5 min, remaining at 27 % for 7 min, increasing up to 100 % within 3 min, staying at 100 % for 5 min, decreasing to 15 % within 2 min and finally remaining at 15 % for 5 min. Analyses were run at a flow rate of 0.8 mL $min^{-1}$. Compounds were identified by comparison of retention times and absorption maxima with those of standard substances. Utilized standard substances were cyanidin chloride, kaempferol and quercetin (purchased at Carl Roth GmbH + Co. KG, Karlsruhe, Germany and Fluka AG, Buchs, Switzerland, respectively). Quercetin and kaempferol were detected at 260 nm, cyanidin at 535 nm.

Statistical analysis was performed by means of SPSS software (17.0, SPSS Inc., Chicago, USA). Data were tested by one-way ANOVA followed by a Duncan's post-hoc test.

## Results

### *Plant fresh mass*

Biomass accumulation in lettuce plants was affected by continuous but not by late UV-B treatment (Fig. 1a). Continuous UV-B exposure led to a reduction of above ground fresh mass by 3 to 19 % on d 21 [6], d 22[30] and d 23[48] in comparison with low UV-B grown lettuce plants. The relative growth rate calculated for this time period was 29 % lower at high UV-B compared to low UV-B conditions (Table 2). This reduction in biomass accumulation was accompanied by a more compact plant structure as revealed by visual examination. Late UV-B exposure, in contrast, had no measurable effect on plant fresh mass although relative growth rate was decreased

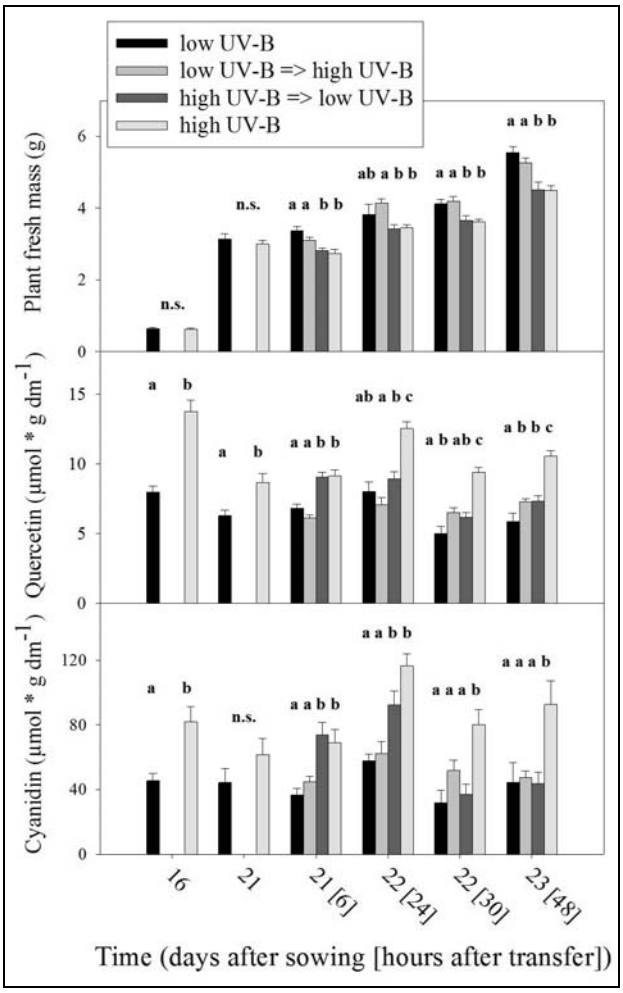



Fig. 1. Mean±SE of total plant fresh mass (a), quercetin content (b) and cyanidin content (c) of lettuce plants grown under different UV-B treatments. Sample size was $n_{lowUV\text{-}B/highUV\text{-}B} = 6$ and $n_{lowUV\text{-}B \rightarrow highUV\text{-}B/highUV\text{-}B \rightarrow lowUV\text{-}B} = 12$ per treatment for flavonoid analysis and n = 12 per treatment for fresh mass determination. Statistical analysis were performed by ANOVA, followed by Duncan's post-hoc test, a–c = significant differences between treatments, n.s. = not significant, $P \leq 0.05$. dm = dry matter.

by 10 % after 48 h compared to plants grown in a UV-B-free environment. Exclusion of UV-B after 20 d (high UV-B → low UV-B treatment) did not affect above-ground biomass accumulation within 2 d (compared to high UV-B treatment).

*Flavonoid content*

The main flavonoid aglyca detected in samples of red oak leaf lettuce were cyanidin and quercetin. Both compounds showed different responses to UV-B treatment throughout the sampling period (Fig. 1b and c). Continuous UV-B exposure led to a stable enhancement in cyanidin and quercetin content by an average of 95 and 62 %, respectively, compared to low UV-B grown plants. Late UV-B treatment induced an increase in quercetin content by 30 % within 30 h. Cyanidin content, in contrast, was not significantly altered upon late UV-B exposure. UV-B exclusion after 21 d caused a decline in cyanidin content by 54 % (d 22 [30 h after transfer]) and in quercetin content by 29 % (d 22 [24 h after transfer]). As a consequence, 24 to 30 h after UV-B withdrawal flavonoid content was comparable to levels found at low UV-B conditions.

*Flavonoid distribution*

The distribution of flavonoids among leaf age classes shows differential responses of the youngest (inner), elder (intermediate) and oldest (outer) leaves under the four irradiation regimes (Fig. 2a and b). Permanent UV-B exposure led to significantly increased quercetin levels in inner (+47 %, not significant), intermediate (+36 %) and outer leaves (+51 %) compared to UV-B exclusion. Late UV-B treatment induced a marked increase in young (+84 %) and intermediate (+30 %) leaves, whereas old leaves were hardly affected (+5 %, Fig. 2a). UV-B exclusion had no significant effect on flavonoid distribution over leaf age classes. Cyanidin content shows a similar variation, although differences between UV-B treatment groups were significant only in young leaves (Fig. 2b). Regarding different leaf developmental stages, independent from UV-B treatment, quercetin content increased in the order young < intermediate < mature leaves.

## Discussion

*Responses to continuous UV-B exposure*

Continuous exposure to near-ambient solar UV-B radiation affected flavonoid metabolism as well as growth of lettuce plants. Quercetin and cyanidin content showed a stable enhancement at high UV-B conditions (Fig. 1b and c). Epidermal accumulation of flavonoids initiated by an enhanced expression of the key enzyme phenylalanine ammonia-lyase (PAL) is the main protection mechanism against UV-B radiation in plants (Lois 1994; Jordan 2002). Flavonols such as quercetin provide UV-B protec-

Table 2. Mean relative growth rate at different UV-B conditions.

| Treatment | low UV-B | low UV-B → high UV-B | high UV-B → low UV-B | high UV-B |
|---|---|---|---|---|
| RGR* | 28.6 | 25.9 | 20.5 | 20.4 |

*From d 21 to 23 (48 h after transfer), based on plant fresh mass.

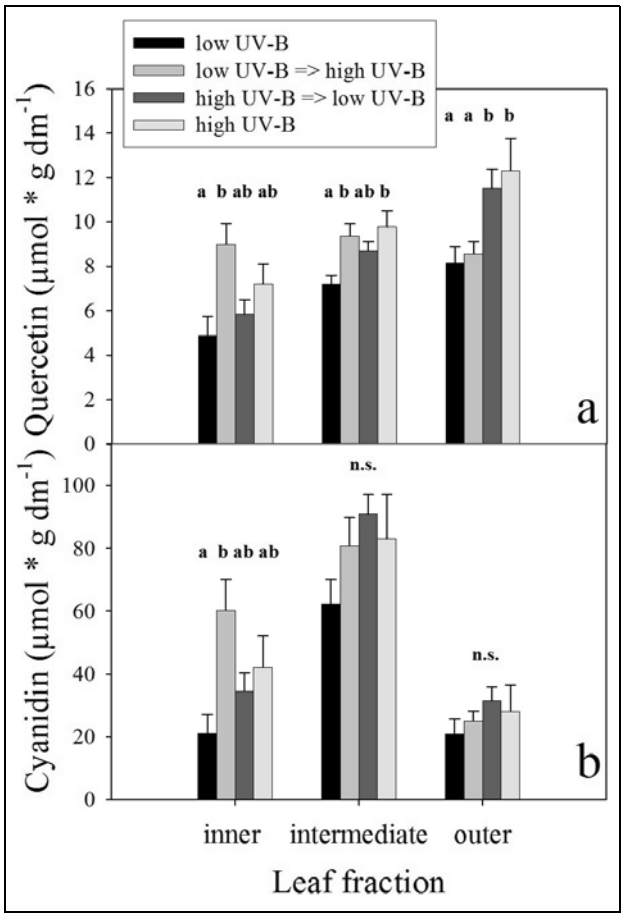



Fig. 2. Mean±SE of quercetin (a) and cyanidin content (b) of inner, intermediate and outer leaf fractions of different UV-B treatment groups on d 24. Sample size was $n_{lowUV\text{-}B/highUV\text{-}B} = 6$, $n_{lowUV\text{-}B \rightarrow highUV\text{-}B/highUV\text{-}B \rightarrow lowUV\text{-}B} = 12$. Treatment effects were analysed with ANOVA, followed by Duncan's post-hoc test, a, b indicate significant differences between UV-B treatments, n.s. = not significant, $P \leq 0.05$. dm = dry matter.

tion by efficiently shielding underlying tissues from UV radiation due to their absorption bands in the UV spectral region (from 250 to 285 and from 320 to 385 nm; Rice-Evans et al. 1996). Anthocyanins such as cyanidin were found to accumulate in response to UV-B (Kakegawa et al. 1991; Klaper et al. 1996) and to reduce photoinhibition and DNA damage (Stapleton and Walbot 1994; Burger and Edwards 1996), although their contribution to total UV absorbance is relatively little (Beggs and Wellmann 1985; Brandt et al. 1995; Woodall and Stewart 1998; Ries et al. 2000).

UV-B protection by quercetin and cyanidin may also be due to their antioxidant activity. *In vitro*, TEAC (Trolox [6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid] equivalent antioxidant activity) values account for 4.7 mM (quercetin) and 4.4 mM (cyanidin; Rice-Evans et al. 1996). *In vivo*, flavonoids are primarily stored in epidermal vacuoles (Charriere-Ladreix and Tissut 1981; Marrs et al. 1995) and are therefore isolated from UV-B induced ROS generation occurring predominantly in the chloroplasts of the palisade mesophyll (Gould et al. 2002). Nevertheless, a minor fraction of flavonoids, located in the cytosol, as well as newly synthesized flavonoids, which have not yet been transported to the vacuole, are well located to reduce oxidative pressure in the cytosol (Olsson et al. 1998; Winkel-Shirley 1999; Gould et al. 2002). At high stress conditions and thus high ROS levels $H_2O_2$ can alternatively diffuse from the mesophyll into the epidermis and from the cytosol into the vacuole, where it is subsequently scavenged (Gould and Lister 2006). Nevertheless, it is not clear, to what extent cytosolic and vacuolar flavonoids are involved in the mitigation of UV-B induced oxidative stress (Gould and Lister 2006).

The observed attenuation in plant fresh mass accumulation induced by continuous UV-B exposure (d 21 [6], d 22[30] and d 23[48]; Fig. 1a) is consistent with previous studies reporting a UV-B induced growth reduction in lettuce plants (Krizek et al. 1998; Garcia-Macias et al. 2007). Rousseaux et al. (2004) could show that this inhibition in biomass accumulation is due to a reduced formation and expansion of leaves. The coincidence of flavonoid induction (d 16) and growth reduction (d 21 [6 h after transfer]) indicates a redirection of carbohydrate substrate from growth to secondary metabolism (Tsormpatsidis et al. 2008; Kuhlmann and Müller 2009a). This resource allocation coincides with the 'growth-differentiation balance hypothesis', postulating a trade-off from growth to defence under stressful conditions (Herms and Mattson 1992). Growth attenuation in response to UV-B is also considered a protection mechanism minimizing exposure to the stressor (Teramura and Sullivan 1994). The assumption that light absorption by anthocyanins could reduce photosynthetic activity and thereby limit growth (Burger and Edwards 1996) presumably doesn't apply for red leafed lettuce, since Tsormpatsidis et al. (2008) found an increase in the photosynthetic performance index of lollo rosso plants grown in the presence of UV-B radiation containing high levels of anthocyanins.

Flavonoid induction as well as growth inhibition are thought to be initiated independent from general stress responses as the treatment was performed using near-ambient solar UV-B radiation with a natural UV-B/PAR ratio. Both responses are known to be elicited by different photoreceptors and specific signal transduction pathways and are therefore defined as true photomorphogenic responses (Kim et al. 1998; Jenkins 2009).

### *Responses to late UV-B exposure and deprival*

Late UV-B exposure led to a 30 % higher quercetin content after 30 h of treatment in comparison with plants kept under low UV-B conditions ($P = 0.019$; Fig. 1b). Cyanidin content, in contrast, was not significantly affected (Fig. 1c). Flavonoid compounds are known to be differentially regulated in response to UV-B exposure according to the need for a particular compound (Olsson et al. 1998; Jordan 2002). The selective induction of quercetin may be due to the fact that it fulfils both functions as an effective UV-B shield and as a strong antioxidant (Caldwell et al. 1983; Kootstra 1994; Lois 1994; Rice-Evans et al. 1996). Cyanidin, lacking the ability to efficiently absorb UV-B radiation, presumably plays a minor role in UV-B protection (Steyn et al. 2002).

The observed restriction of cyanidin induction to young plants is also in agreement with the assumption

that anthocyanins are most important for plant protection during early seedling development (Chalker-Scott 1999). The ability to accumulate anthocyanins was reported to be lost with increasing plant age and decreasing sensitivity to environmental stress (Richter and Hoddinot 1997). Murray and Hackett (1991) found that in ivy the potential for anthocyanin accumulation was coupled to the activity of dihydroflavonol reductase, an enzyme early in the anthocyanin biosynthetic pathway. In young plants diverse elicitors led to an induction of dihydroflavonol reductase activity and accumulation of anthocyanins, whereas mature plants accumulated higher contents of flavonols but no anthocyanins. The increased requirement for anthocyanins in developing tissues may be due to their lower ability to utilize absorbed light energy, leading to an increased risk of photoinhibition and photobleaching (Krause et al. 1995).

At the age of 20 d, UV-B induced flavonoid accumulation seems to be relatively independent from growth, compared to earlier stages of development. Plant fresh mass was hardly affected by late UV-B treatment (Fig. 1a); merely the relative growth rate showed a slight decrease between d 21 and 23 (48 h after transfer) upon late UV-B exposure (Table 2). This may be due to the fact that formation of growth responses takes longer than induction of chemical protection mechanisms (Kuhlmann and Müller 2009a). Yet, it seems more likely that plants of this age are able to regulate defence and growth processes independently as their higher photosynthetic efficiency allows for a more flexible distribution of carbohydrate substrates. At early stages of development, in contrast, seedlings are limited in photosynthetic productivity, carbohydrate resources and efficient protection against environmental stressors. Therefore in young, developing plants the need of UV-B protection can affect resource allocation patterns in terms of a more severe trade-off from growth to defence (Kuhlmann and Müller 2009a). The 'growth-differentiation balance hypothesis' is therefore more applicable to young than to mature plants (Herms and Mattson 1992). It also has to be taken into account, that more mature plants grow at a slower rate and therefore growth responses developing at this stage are more difficult to detect.

UV-B exclusion (high UV-B → low UV-B treatment) led to a decline in cyanidin content by 54 % on d 22 (30 h after transfer; $P = 0.001$) and in quercetin content by 29 % on d 22 (24 h after transfer) compared to high UV-B treatment ($P \leq 0.000$; Fig. 1b and c). These results are in agreement with findings published by Kubasek et al. (1998) indicating that light induction of enzymes involved in flavonoid biosynthesis is a transient response. Abundance of relevant enzymes is flexibly regulated according to the current requirement in a specific situation. Maintenance of a high flavonoid level seems to be a costly protection mechanism, which is down-regulated when there is no particular need for it (Steyn et al. 2002).

The stage of leaf development was shown to determine responsiveness to UV-B treatment as well. Continuous UV-B exposure during early seedling development led to a comparable quercetin induction at all three stages of leaf development, whereas late UV-B treatment caused a much stronger response in young compared to intermediate and old leaves (Fig. 2a and b). UV-B induced accumulation of flavonoids is locally regulated resulting in a heterogeneous distribution within the plant (Lois 1994; Andersen and Jordheim, 2006). In mature plants with high resource availability, young leaves are expected to show the strongest UV-B response since they require particular protection representing the future photosynthetically active sites (Lois 1994; Reifenrath and Müller 2007). Susceptibility to UV-B radiation may be increased in young leaves due to a lack of UV-B absorbing pigments and a stronger UV-B penetration, as shown for young needles of conifers (Delucia et al. 1992).

### *Implications for hardening*

The present findings suggest that continuous exposure to near-ambient solar UV-B radiation during early development of lettuce seedlings is more imprinting and hence more efficient for hardening than late short-term exposure. The strong increase in quercetin and cyanidin content induced by continuous UV-B exposure is supposed to enhance the plant's tolerance towards diverse stressors present in the field (Teramura and Sullivan 1994). The accompanying growth inhibition reduces exposure to adverse environmental conditions. These properties are expected to improve the plant's ability to cope with outdoor conditions (Chalker-Scott and Schott 2004). Late UV-B treatment, in contrast, merely induced a slight enhancement in quercetin content.

### *Conclusions*

The presented results confirm our hypothesis, that inclusion or exclusion of the UV-B range from near-ambient solar radiation induces a differential regulation of flavonoid metabolism as well as biomass accumulation in lettuce seedlings. As postulated these UV-B responses were determined by the stage of plant development. In general, responsiveness to UV-B decreased with increasing leaf and plant age. Due to the development-dependence of UV-B responses, continuous UV-B exposure is thought to be a more efficient hardening strategy in horticultural production apparently leading to a higher stress tolerance than late short-term treatment. The utilization of plastic films and glasses with an increased transparency in the UV-B spectral range may therefore be a convenient tool for hardening. Yet, the concrete benefit of UV-B pre-acclimation concerning stress resistance of seedlings remains to be examined in greater detail in future studies.

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## References

Andersen, Ø.M. and M. Jordheim 2006: The anthocyanins. In: Andersen, Ø.M. and K.R. Markham (eds.): Flavonoids: Chemistry, biochemistry and applications. Taylor & Francis Group, Boca Raton, 471–551.

Beggs, C.J. and E. Wellmann 1985: Analysis of light-controlled anthocyanin formation in coleoptiles of *Zea mays* L.: The role of UV-B, blue, red and far-red light. Photochem. Photobiol. **41**, 481–486.

Brandt, K., A. Giannini and B. Lercari 1995: Photomorphogenic responses to UV radiation III: a comparative study of UVB effects on anthocyanin and flavonoid accumulation in wild-type and aurea mutant of tomato (*Lycopersicon esculentum* Mill.). Photochem. Photobiol. **62**, 1081–1087.

Burger, J. and G.E. Edwards 1996: Photosynthetic efficiency and photodamage by UV and visible radiation, in red versus green leaf *Coleus* varieties. Plant Cell Physiol. **37**, 395–399.

Caldwell, M.M., R. Robberecht and S.D. Flint 1983: Internal filters: Prospects for UV-acclimation in higher plants. Physiol. Plant. **58**, 445–450.

Chalker-Scott, L. 1999: Environmental significance of anthocyanins in plant stress responses. Photochem. Photobiol. **70**, 1–9.

Chalker-Scott, L. and J.D. Schott 2004: Elevated Ultraviolet-B radiation induces cross-protection to cold in leaves of Rhododendron under field conditions. Photochem. Photobiol. **79**, 199–204.

Charriere-Ladreix, Y. and M. Tissut 1981: Foliar flavonoid distribution during *Spinacia* chloroplast isolation. Planta **151**, 309–313.

Del Corso, G. and B. Lercari 1997: Use of UV radiation for control of height and conditioning of tomato transplants (*Lycopersicon esculentum* Mill.). Scientia Horticulturae **71**, 27–34.

Delucia, E.H., T.A. Day and T.C. Vogelman 1992: Ultraviolet-B and visible light penetration into needles of two species of subalpine conifers during foliar development. Plant, Cell Environ. **15**, 921–929.

Frohnmeyer, H. and D. Staiger 2003: Ultraviolet-B radiation-mediated responses in plants. Balancing damage and protection. Plant Physiol. **133**, 1420–1428.

Garcia-Macias, P., M. Ordridge, E. Vysini, S. Waroonphan, N.H. Battey, M.H. Gordon, P. Hadley, P. John, J.A. Lovegrove and A. Wagstaffe 2007: Changes in the flavonoid and phenolic acid contents and antioxidant activity of red leaf lettuce (Lollo Rosso) due to cultivation under plastic films varying in ultraviolet transparency. J. Agric. Food Chem. **55**, 10168–10172.

Gitelson, A., M. Merzlyak and O. Chivkunova 2001: Optical properties and nondestructive estimation of anthocyanin content in plant leaves. Photochem. Photobiol. **74**, 38–45.

Gould, K.S., J. McKelvie and K.R. Markham 2002: Do anthocyanins function as antioxidants in leaves? Imaging of $H_2O_2$ in red and green leaves after mechanical injury. Plant, Cell Environ. **25**, 1261–1269.

Gould, K. 2004: Nature's Swiss army knife: The diverse protective roles of anthocyanins in leaves. Anthocyanins – More than nature's colour **5**, 314–320.

Gould, K. and C. Lister 2006: Flavonoid functions in plants. In: Andersen, Ø.M. and K.R. Markham (eds.): Flavonoids: chemistry, biochemistry and applications. CRC Press LLC, Boca Raton, USA, 397–411.

Hectors, K., E. Prinsen, W. De Coen, M.A.K. Jansen and Y. Guisez 2007: *Arabidopsis thaliana* plants acclimated to low dose rates of ultraviolet B radiation show specific changes in morphology and gene expression in the absence of stress symptoms. New Phytol. **175**, 255–270.

Herms, D. and W. Mattson 1992: The dilemma of plants: to grow or defend. Q. Rev. Biol. **67**, 283–335.

Hertog, M., P. Hollmann and D. Venema 1992: Optimization of a quantitative HPLC determination of potentially anticarcinogenic flavonoids in vegetables and fruits. J. Agric. Food Chem. **40**, 1591–1598.

Hoffmann, S. 1999: Zierpflanzen. Gartenbauwissenschaft **64**, 88–93.

Jansen, M.A.K., V. Gaba and B.M. Greenberg 1998: Higher plants and UV-B radiation: balancing damage, repair and acclimation. Trends Plant Sci. **3**, 131–135.

Jenkins, G.I. 2009: Signal transduction in responses to UV-B radiation. Annu. Rev. Plant Biol. **60**, 407–431.

Jordan, B.R. 2002: Molecular response of plant cells to UV-B stress. Funct. Plant Biol. **29**, 909–916.

Jordan, B.R. 2004: Plant pigments and protection against UV-B radiation. In: Daqvies, K.M. (ed.): Plant pigments and their manipulation. Blackwell Publishing Ltd., Oxford, 275–287.

Kakegawa, K., E. Hattori, K. Koike and K. Takeda 1991: Induction of anthocyanin synthesis and related enzyme activities in cell cultures of *Centaurea cyanus* by UV-light irradiation. Phytochemistry **30**, 2271–2273.

Kim, B., D. Tennessen and R. Last 1998: UV-B-induced photomorphogenesis in *Arabidopsis thaliana*. The Plant Journal **15**, 667–674.

Klaper, R., S. Frankel and M.R. Berenbaum 1996: Anthocyanin content and UVB sensitivity in *Brassica rapa*. Photochem. Photobiol. **63**, 811–813.

Kootstra, A. 1994: Protection from UV-B-induced DNA damage by flavonoids. Plant Mol. Biol. **26**, 771–774.

Krause, G.H., A. Virgo and K. Winter 1995: High susceptibility to photoinhibition of young leaves of tropical forest trees. Planta **197**, 583–591.

Krizek, D.T., S.J. Britz and R.M. Mirecki 1998: Inhibitory effects of ambient levels of solar UV-A and UV-B radiation on growth of cv. 'New Red Fire' lettuce. Physiol. Plant. **103**, 1–7.

Kubasek, W., B. Shirley, A. McKillop, H. Goodman, W. Briggs and F. Ausubel 1992: Regulation of flavonoid biosynthetic genes in germinating *Arabidopsis* seedlings. The Plant Cell Online **4**, 1229–1235.

Kubasek, W.L., F.M. Ausubel and B.W. Shirley 1998: A light-independent developmental mechanism potentiates flavonoid gene expression in *Arabidopsis* seedlings. Plant Mol. Biol. **37**: 217–223.

Kuhlmann, F. and C. Müller 2009a: Development-dependent effects of UV radiation exposure on broccoli plants and interactions with herbivorous insects. Environ. Exp. Bot. **66**, 61–68.

Kuhlmann, F. and C. Müller 2009b: Independent responses to ultraviolet radiation and herbivore attack in broccoli. J. Exp. Bot. **60**, 3467–3473.

Llorach, R., A. Martinez-Sanchez, F.A. Tomas-Barberan, M.I. Gil and F. Ferreres 2008: Characterisation of polyphenols and antioxidant properties of five lettuce varieties and escarole. Food Chem. **108**, 1028–1038.

Lois, R. 1994: Accumulation of UV-absorbing flavonoids induced by UV-B radiation in *Arabidopsis thaliana* L. Planta **194**, 498–503.

Mancinelli, A.L. and O.M. Schwartz 1984: The photoregulation of anthocyanin synthesis IX. The photosensitivity of the response in dark and light-grown tomato seedlings. Plant Cell Physiol. **25**, 93–105.

Marrs, K.A., M.R. Alfenito, A.M. Lloyd and V. Walbot 1995: A glutathione S-transferase involved in vacuolar transfer encoded by the maize gene Bronze-2. Nature **375**, 397–400.

Mittler, R. 2006: Abiotic stress, the field environment and stress combination. Trends Plant. Sci. **11**, 15–19.

Mol, J., G. Jenkins, E. Schäfer, D. Weiss and V. Walbot 1996: Signal perception, transduction, and gene expression involved in anthocyanin biosynthesis. Crit. Rev. Plant Sci. **15**, 525–557.

Murray, J. and W. Hackett 1991: Dihydroflavonol reductase activity in relation to differential anthocyanin accumulation in juvenile and mature phase *Hedera helix* L. Plant. Physiol. **97**, 343–350.

Olsson, L.C., M. Veit, G. Weissenböck and J.F. Bornmann 1998: Differential flavonoid response to enhanced UV-B radiation in *Brassica napus*. Phytochemistry **49**, 1021–1028.

Paul, N.D. and D. Gwynn-Jones 2003: Ecological roles of solar UV radiation: Towards an integrated approach. Trends Ecol. Evol. **18**, 48–55.

Reifenrath, K. and C. Müller 2007: Species-specific and leaf-age dependent effects of ultraviolet radiation on two Brassicaceae. Phytochemistry **68**, 875–885.

Rice-Evans, C.A., N.J. Miller and G. Paganga 1996: Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radic. Biol. Med. **20**, 933–956.

Richter, C. and J. Hoddinot 1997: UV-B effects on growth, pigments and electrolyte leakage in conifer seedlings. Plant Physiol. **98**, 114–120.

Ries, G., W. Heller, H. Puchta, H. Sandermann, H.K. Seidlitz and B. Hohn 2000: Elevated UV-B radiation reduces genome stability in plants. Nature **406**, 98–101.

Rousseaux, M.C., S.D. Flint, P.S. Searles and M.M. Caldwell 2004: Plant responses to current solar Ultraviolet-B radiation and to supplemented solar Ultraviolet-B radiation simulating ozone depletion: An experimental comparison. Photochem. Photobiol. **80**, 224–230.

Rozema, J., J. van de Staaij, L.O. Björn and M. Caldwell 1997: UV-B as an environmental factor in plant life: stress and regulation. Trends Ecol. Evol. **12**, 22–28.

Schmitz-Hoerner, R. and G. Weissenbock 2003: Contribution of phenolic compounds to the UV-B screening capacity of developing barley primary leaves in relation to DNA damage and repair under elevated UV-B levels. Phytochemistry **64**, 243–255.

SMILLIE, R. and S. HETHERINGTON 1999: Photoabatement by anthocyanin shields photosynthetic systems from light stress. Photosynthetica **36**, 451–463.
STAPLETON, A.E. and V. WALBOT 1994: Flavonoids can protect maize DNA from the induction of ultraviolet radiation damage. Plant Physiol. **105**, 881–889.
STEYN, W.J., S.J.E. WAND, D.M. HOLCROFT and G. JACOBS 2002: Anthocyanins in vegetative tissues: a proposed unified function in photoprotection. New Phytol. **155**, 349–361.
TEKLEMARIAM, T. and T. BLAKE 2003: Effects of UVB preconditioning on heat tolerance of cucumber (*Cucumis sativus* L.). Environ. Exp. Bot. **50**, 169–182.
TERAMURA, A. and J. SULLIVAN 1994: Effects of UV-B radiation on photosynthesis and growth of terrestrial plants. Photosynth. Res. **39**, 463–473.
TREUTTER, D. 2006: Significance of flavonoids in plant resistance: a review. Environmental Chemistry Letters **4**, 147–157.
TSORMPATSIDIS, E., R.G.C. HENBEST, F.J. DAVIS, N.H. BATTEY, P. HADLEY and A. WAGSTAFFE 2008: UV irradiance as a major influence on growth, development and secondary products of commercial importance in Lollo Rosso lettuce 'Revolution' grown under polyethylene films. Environ. Exp. Bot. **63**, 232–239.
ULM, R. and F. NAGY 2005: Signalling and gene regulation in response to ultraviolet light. Curr. Opin. Plant Biol. **8**, 477–482.
WADE, H.K., T.N. BIBIKOVA, W.J. VALENTINE and G.I. JENKINS 2001: Interactions within a network of phytochrome, cryptochrome and UV-B phototransduction pathways regulate chalcone synthase gene expression in *Arabidopsis* leaf tissue. The Plant Journal **25**, 675–685.
WINKEL-SHIRLEY, B. 1999: Evidence for enzyme complexes in the phenylpropanoid and flavonoid pathways. Physiol. Plant. **107**, 142–149.
WOODALL, G.S. and G.R. STEWART 1998: Do anthocyanins play a role in UV protection of the red juvenile leaves of *Syzygium*? J. Exp. Bot. **49**, 1447–1450.

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