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Interactive effects of PAR and UV radiation on the physiology, morphology and leaf optical properties of two barley varieties

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ABSTRACT

The interactive effects of photosynthetically active radiation (PAR) on plants' acclimation to ultraviolet (UV) radiation were examined under field conditions in two barley varieties (Barke, Bonus). Plants, pre-treated under UV exclusion and low PAR intensities, were subsequently exposed to four radiation treatments representing the combination of low [−] and high [+] UV and PAR intensities. Selective UV and PAR filters were used for UV exclusion and reduction of PAR to ca 25% of ambient irradiance. A system of modulated lamps was used to enhance UV to ca 200% of ambient.

Changes in flavonol and chlorophyll content, chlorophyll fluorescence, gas-exchange and leaf hyperspectral reflectance were studied during seven days of acclimation to the new treatments. At the end of this period morphological analysis of aboveground biomass was carried out.

The [UV+PAR−] treatment significantly reduced the photosynthetic activity of barley leaves; the reduction was more pronounced in old than young leaves and greater in the variety Barke than Bonus. Whereas, [PAR+] treatment triggered photoprotective mechanisms which partially ameliorated the UV effects on photochemistry and carbon assimilation. The [PAR+] treatment induced accumulation of flavonols, mainly in young leaves, whereas in old leaves UV-induced accumulation was more pronounced. An inverse proportion was found between flavonol content and specific leaf area irrespective of barley variety and UV/PAR treatment. Enhanced UV radiation reduced the final leaf length, particularly in [PAR−] plants, in young leaves and in variety Barke. However, [PAR+] mitigated the morphological effects induced by the [UV+] treatment, particularly changes in SLA.

1. Introduction

Ultraviolet-B radiation (UV-B; 280–315 nm) is an important component of the environment acting as an ecophysiological factor with the potential to alter plant growth and photosynthesis (reviewed in Caldwell et al., 2007; Ballaré et al., 2011). Plant response to UV-B is effectively regulated by photoprotective mechanisms that dissipate excess radiation. At the physiological level these photorepair mechanisms and antioxidant responses mop up reactive oxygen species, UV-B screening compounds and adaptation in leaf morphology stop UV-B from reaching the chloroplasts. These mechanisms contribute to a co-ordinated response to UV-B, some of which can be enhanced by the UV-A portion of the photosynthetically active radiation (PAR).

UV-B affects various aspects of photosynthesis (Jansen et al., 2010). Photosystem (PS) II, rather than PS I, is considered to be the most vulnerable target of UV-B (e.g., Tyystjärvi, 2008). Reductions in CO_2 assimilation rate may be further mediated through reduction in light-harvesting complexes, disruption of thylakoid membrane integrity, and/or degradation and inactivation of Rubisco (reviewed in Takeuchi et al., 2002). Several studies have also shown that reduction in CO_2 assimilation is caused by UV-induced changes in stomatal conductance (Jansen and van den Noort, 2000; Urban et al., 2006).

In general, plant response to UV-B depends on the biologically effective dose applied (Götz et al., 2010; Kotilainen et al., 2011) and interactions with other environmental stimuli (Caldwell et al., 2007). In particular, the spectral balance between PAR (400–700 nm), UV-A (320–400 nm) and UV-B has been shown to be important in determining plant sensitivity in field studies (e.g.,

Abbreviations: A_{max}, light-saturated CO_2 assimilation rate; G_s, light-saturated stomatal conductance; F_V/F_M, maximum quantum yield of photosystem II; PAR, photosynthetically active radiation; UV, ultraviolet radiation; SLA, specific leaf area; VI, vegetation index.

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Krizek, 2004). In several studies, elevated UV-B radiation has produced negligible or even stimulating effects on plant growth and/or photosynthesis when PAR was also high (Searles et al., 2001; Nithia et al., 2005).

There is general agreement that PAR can alleviate some of the negative effects of enhanced UV-B radiation. Blue light stimulates the production of photolyases which are involved in the repair of UV-induced cyclobutane pyrimidine dimers (CPD) of DNA (Mazza et al., 1999; Ballaré et al., 2011). Some of those UV-screening compounds accumulated in plant leaves due to UV radiation also response to PAR (e.g., flavonoids, ferulic acids, hydroxycinnamic acids etc.; reviewed in Burchard et al., 2000; Searles et al., 2001; Jansen et al., 2008). Whereas under low PAR intensities, UV-A can be particularly effective in mitigating UV-B damage (Burchard et al., 2000; Jenkins, 2009). However, when PAR is high, effective UV-B damage mitigation seems to proceed irrespective of UV-A dose. Götz et al. (2010) found that high PAR intensities confer basic UV protection through quercetin accumulation, which is enhanced when UV radiation is also received by the plant. Some phenolic compounds are constitutively synthesized in the leaf, others respond to UV-A while a third strata respond only when UV-B is also present (Kotilainen et al., 2009). On the other hand, Pfündel et al. (1992) found that high PAR intensities together with enhanced UV-B leads to inhibition of violaxanthin de-epoxidation that represents an important part of plant photoprotection.

UV-B radiation induces a range of morphogenic responses including epidermal and leaf thickening and curling, inhibition of hypocotyl, stem, and leaf elongation, axillary branching, and shifts in the root-shoot ratio (reviewed in Jansen, 2002). Photomorphogenic responses are mediated by UV-B-specific signalling compounds (e.g., UV RESISTANCE LOCUS 8) and pathways stimulating the expression of genes involved in UV-protection and hence promoting plant survival under UV-B (Jenkins, 2009).

Only a little is known about the relative importance of constitutive (i.e., genetic) and inducible UV protection in plants (Rizzini et al., 2011). However, it has been reported that variation in constitutive UV protection is small as compared to the amplitude of environmentally induced changes in UV protection (Jansen et al., 2010). In addition to inherent UV protection, Fedina et al. (2009) reported that the effects of UV-B radiation on barley plants are related to the developmental stages of their photosynthetic apparatus.

Barley represents a relatively sensitive crop species to UV-B (Reuber et al., 1996; Hideg et al., 2006) and light-induced oxidative stress (Wu and von Tiedemann, 2004; Štroch et al., 2008). The UV-B tolerance of barley varieties is reportedly associated with genotypic differences affecting those mechanisms that confer physical protection (UV screening), biochemical defence (scavenging of free radicals), and CPD photolyase activity, that lead to a decrease in Rubisco content (Reuber et al., 1996; Mazza et al., 1999; Hideg et al., 2006; Fedina et al., 2009). The impact of UV-B on leaf traits also differs with the timing of UV-exposure during development (Reifenrath and Müller, 2007). However, it is not well understood how interactive effects of genotype, leaf-age and PAR intensity influence the acclimation of barley to UV-B radiation. The aim of this study was to obtain greater mechanistic insight into the relative importance of the above-mentioned factors that confer resistance to UV-B and how they interact to produce either an amplification or attenuation of the established UV-B effects on photosynthesis, growth and accumulation of UV-shielding compounds. This aim was addressed by the experimental manipulation of solar radiation to produce contrasting doses of UV and PAR to achieve clear responses which could be used to evaluate all the interactions among the variables examined. The following hypotheses were tested in our model field experiment: (1) high PAR intensities can induce accumulation of UV-shielding compounds under UV exclusion, (2) PAR is involved in both, physiological photosynthetic acclimation and leaf morphological acclimation to enhanced UV-B, and (3) acclimation to UV-B radiation is modified by the leaf age and plant genotype. The experiment was done on two barley varieties of contrasting sensitivity to photo-oxidative stress (Wu and von Tiedemann, 2004; Štroch et al., 2008).

2. Material and methods

2.1. Plant material

The experiment was done during August 2010, in a field trial in the park of the Global Change Research Centre AS CR (Brno, CZ). Two barley varieties differing in their sensitivity to light-induced oxidative stress (Wu and von Tiedemann, 2004; Štroch et al., 2008) were studied: Barke (sensitive) and Bonus (tolerant). The seeds of both varieties were pre-germinated at room temperature on wet filter paper for 48 hours. Only germinating seeds were then transplanted into small pots (5 cm diameter) filled with the mixture (1:1) of horticultural substrate and substrate for potted houseplants (Agro CS, Ceska Skalice, CZ). Three seeds were transplanted into each pot in a triangular spatial distribution to avoid mutual shading of plants during early growth. Sixteen replicates (pots) of each variety were grown in each UV/PAR treatment. Uniform watering was ensured by the capillary action from plastic trays.

The plants were pre-treated under conditions of low PAR intensities, UV-A and UV-B exclusion. Neutral density filters 0.6ND (Lee Filters, Hampshire, UK) were used to reduce PAR intensity to 25% of natural sunlight. Clear plastic filters Lee U.V. 226 (Lee Filters, UK) were used for UV-A and UV-B exclusion with a very low reduction (up to 10%) of PAR (see www.leefilters.com for detailed spectral filter characteristics). After 14 days, the first leaf (hereafter reported also as older) of both varieties had fully developed, the second leaf (middle) had almost completed its development and the third leaf (younger) was emerging. Subsequently, the barley plants were transferred into individual UV/PAR treatments.

2.2. UV and PAR treatments

Barley plants were grown under four treatments representing combinations of UV exclusion [UV−] or enhanced [UV+] UV radiation, and reduced [PAR−] or ambient [PAR+] PAR; hereafter reported as [UV−PAR−], [UV+PAR+], [UV−PAR+] and [UV+PAR−]. The barley plants (48 plants per treatment) were exposed to the individual treatments for seven days. Individual treatments were provided by open-sided chambers (area 1 m^2, height 50 cm). The UV and PAR filters covered the top and upper part of the side walls (20 cm down from the top).

Lee U.V. 226 and Lee 0.6ND filters (Lee Filters, UK) were used for the exclusion of UV radiation and reduction of PAR intensity to 25% of natural sunlight, respectively. Li-190SA sensors (Li-Cor, USA) were used to monitor ambient and filtered PAR intensities. A modulated illumination system of similar design to that reported by Šprtová et al. (1999) was used to achieve enhanced UV intensities. The system consists of two UV-A (TL 20 W/10 SLV; Philips) and three UV-B (TL 20 W/12 RS SLV; Philips) fluorescent lamps. The UV lamps were wrapped in pre-solarised (8 h) 0.13 mm thick cellulose diacetate film to avoid transmission of residual UV-C radiation (<280 nm). The system monitored incident UV intensity and UV intensity under the lamp-bank using broad-band UV-A (SKU 420; Skye Instruments Ltd, Powys, UK) and UV-B sensors (SKU 430; Skye Instruments Ltd, UK). See www.skyeinstruments.com for detailed spectral characteristics of the UV sensors used. The outputs from all radiation sensors were recorded using a data logger DL2e (Delta-T Devices Ltd., Cambridge, UK). The lamp output was

adjusted to provide total UV irradiance of 200% of incident UV using a feedback-and-amplification circuit (Konel, Zlín, CZ). The twice ambient increase in UV irradiance was chosen with respect to the prevailing weather conditions (cloudy), with the aim of achieving a dose similar to the summer maxima of UV-B that is typical for this latitude, and to ensure contrasting effects on photosynthesis, growth and the accumulation of UV-screening compounds.

A spectroradiometer SM 9000 (PSI, Brno, CZ) was used to measure the emission spectrum of the UV lamps in the range 200–980 nm. Daily biologically effective UV-B doses (Table 1) were calculated using Green's formulation of the generalized plant action spectrum normalized to 300 nm (Green et al., 1974) and the measured irradiance spectrum of cellulose diacetate filtered lamps (following Kotilainen et al., 2011).

2.3. Physiological measurements

The *in vivo* contents of epidermal flavonols and chlorophylls (Chl $a+b$) were determined daily around noon by Dualex 4 Flav (Force-A, Orsay, F). After seven days of treatment leaf hyperspectral reflectance was measured using the spectroradiometer FieldSpec3 (ASD Inc., Boulder, CO, USA) coupled with an integrating sphere (ASD Inc., USA). The spectral signature ranged from 350 to 2500 nm with a sampling interval of 1 nm and spectral resolution with full width at half maximum 2.4 nm. All measurements were done on the middle part of fully developed leaves.

An open gas-exchange system Li-6400 (Li-Cor, Lincoln, NE, USA) was used to estimate the light-saturated (1200 $\mu mol\,m^{-2}\,s^{-1}$) CO_2 assimilation rate (A_{max}) and stomatal conductance (G_S). All measurements were performed on the intact leaves of five plants per treatment under constant microclimatic conditions (leaf temperature: $25 \pm 1\,^{\circ}C$, relative air humidity: $55 \pm 3\%$) and under ambient CO_2 concentration ($385 \pm 5\,\mu mol\,mol^{-1}$). Simultaneously, measurements were made of maximum quantum yield of chlorophyll fluorescence (F_V/F_M) of dark adapted (25 min) leaves by FluorPen FP 100 (PSI, CZ). All the measurements were done around noon. A sequence of gas-exchange and fluorescence measurements was performed before and during (1st, 4th, and 7th day) exposure to the UV/PAR treatments. In addition, the light-saturated rate of *in vivo* Rubisco carboxylation (V_{Cmax}) was determined on 4th day of acclimation using Li-6400 (Li-Cor, USA). V_{Cmax} values were calculated on the basis of light-saturated (1200 $\mu mol\,m^{-2}\,s^{-1}$) rates of CO_2 assimilation at non-saturating CO_2 concentrations (385, 200, and 50 $\mu mol\,mol^{-1}$) using the equations of Farquhar et al. (1980).

2.4. Morphological analyses

After seven days of treatment, five plants from each treatment were used for destructive morphological analyses of aboveground biomass. The leaf length, width, and leaf area were estimated using a portable leaf area meter (LI-3000A, Li-Cor, USA). Subsequently, plants were dried to a constant weight at 60 °C, and the specific leaf area (SLA; ratio of projected leaf area to dry weight) of individual leaves was determined.

2.5. Data analysis

Before the analysis of variance (ANOVA), the normality of data for individual parameters was tested using a Kolgomorov–Smirnov test. For the general analysis of UV, leaf-age and barley variety effects, the data were grouped according to PAR and separately analysed within the [PAR+] and [PAR−] groups using a three-way fixed-effect ANOVA model. The homogeneity of variances was tested using a Levene test, and where necessary a square root or reciprocal transformation was used to improve the homogeneity of variances.

A two-way ANOVA followed by multiple range test was performed to investigate the effects of PAR/UV treatments and leaf-age classes on physiological and morphological parameters within the individual barley cultivars. Tukey's post hoc ($p = 0.05$) test was used to test for significant differences between treatments.

An analysis of covariance (ANCOVA) was employed to test for differences in the relationships between UV and PAR treatments, leaf age or barley varieties. In cases where the relationships were non-linear, variables were log-transformed to meet the assumption of linearity needed for ANCOVA. Differences among slope coefficients were tested first and if there was no significant difference, tests for differences between intercepts were carried out.

All statistical tests were done in Statistica 9 software (StatSoft, Tulsa, USA).

3. Results

3.1. Environmental conditions during UV/PAR treatments

Changes in weather conditions from cloudy (daily sum of PAR up to $2.3\,MJ\,m^{-2}\,day^{-1}$) to clear skies (daily sum of PAR up to $5\,MJ\,m^{-2}\,day^{-1}$) tended to also change the other microclimatic parameters (Table 1). Ambient UV-B doses, measured by the broadband SKU 430 sensor (Sky Instruments), reached daily maxima up to only $0.35\,W\,m^{-2}$ during cloudy days; however, up to $1.4\,W\,m^{-2}$ was registered during the sunny days. Daily sums of biologically effective UV-B doses calculated from different action spectra are summarized in Table 1. Daily courses of ambient and enhanced (200% of ambient) UV-B intensities during two contrasting days (partly cloudy/overcast) are shown in Fig. 1.

Table 1
Microclimatic conditions and biologically effective UV-B doses ($UV\text{-}B_{BE}$) estimated for [UV+] and [PAR+] treatments. UV radiation in treatments [UV−] was almost completely filtered out and the $UV\text{-}B_{BE}$ can be considered as zero. The sum of PAR in treatments [PAR−] reduced to 25% of ambient PAR. The $UV\text{-}B_{BE}$ doses were calculated from the irradiance spectrum of cellulose diacetate filtered lamps and action spectra related to the expected UV effects on plants. FLAV—action spectrum for flavonoid accumulation (Ibdah et al., 2002); PG—action spectrum for plant growth inhibition (Flint and Caldwell, 2003); GEN—generalized plant action spectrum (Green et al., 1974); LMT—local mean time; T_{air}—mean air temperature; ΣPAR—daily sum of photosynthetically active radiation.

Day (12:00–12:00 LMT)	T_{air} (°C)	ΣPAR ($MJ\,m^{-2}\,day^{-1}$)	$UV\text{-}B_{BE}$		
			FLAV ($kJ\,m^{-2}\,day^{-1}$)	PG ($kJ\,m^{-2}\,day^{-1}$)	GEN ($kJ\,m^{-2}\,day^{-1}$)
1	24.4	4.72	25.41	19.96	4.76
2	18.2	2.67	17.72	13.92	3.31
3	14.4	4.97	26.12	20.51	4.88
4	16.0	3.24	20.18	15.85	3.77
5	12.9	2.28	14.73	11.57	2.76
6	13.3	3.59	18.30	14.37	3.42
7	15.3	4.51	23.40	18.38	4.38

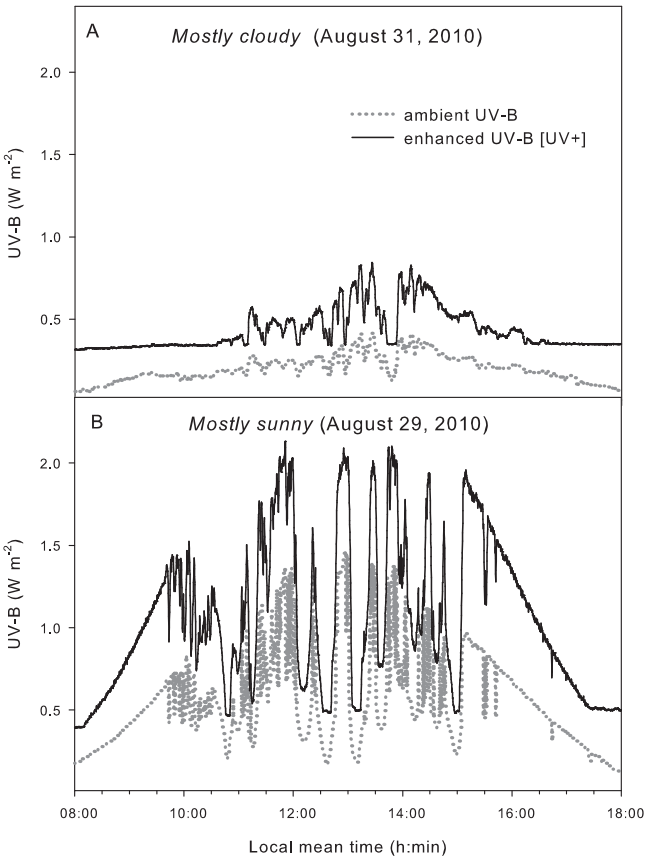

Fig. 1. Daily courses of ambient (dotted line) and enhanced (solid line) UV-B intensity ([UV+]) produced by the lamp system during two days with contrasting sky conditions: August 31, 2010–mostly cloudy (A), August 29, 2010–mostly sunny (B).

3.2. Dynamics of flavonol accumulation during UV/PAR treatments

There were similar trends in the accumulation of flavonols in individual leaf-age classes determined during UV/PAR treatments in both varieties—Barke (Fig. 2A) and Bonus (Fig. 2B). Under the [UV−PAR−] treatment, the flavonol content remained low and only increased in the younger (3rd) leaves (Fig. 2). In comparison to the older (1st) leaf, the flavonol content was higher by 6–9% in the middle (2nd) leaf and 73–82% higher in the younger (3rd) leaf. The constitutive accumulation of flavonols, under the [UV−PAR−] treatment, was higher by 13–77% in variety Bonus as compared to Barke (Fig. 2; Table 3).

The [UV+PAR−] treatment mainly caused flavonols to increase in older and middle (1st and 2nd) leaves (by 134% and 185% in Barke and 68% and 104% in Bonus), whereas the increase was smaller in younger (3rd) leaves, only 70% (Barke) and 9% (Bonus). On the contrary, the [UV−PAR+] treatment mainly increased flavonol content in younger leaves (137% in Barke and 73% in Bonus: Fig. 2). Thus the [UV+PAR+] treatment raised flavonol accumulation in all leaf-age classes, particularly in the middle (2nd) leaf (by 252% in Barke and 127% in Bonus: Fig. 2), as compared to [UV−PAR−] treatment (Fig. 2). Irrespective of barley variety and leaf age, the intensity of leaf reflectance in the spectral range 378–400 nm linearly decreased with increasing leaf flavonol content. The highest correlation ($R^2 = 0.61$; $p < 0.01$) was found for the spectral reflectance at 387 nm (data not shown). However, vegetation indices (VIs), based on the simple ratios of spectral reflectance, produced higher correlations with flavonol content than with simple spectral reflectance. The correlation matrix shows that the highest values of the linear coefficient of determination ($R^2 = 0.7–0.8$) for simple ratio VIs are in the range $R_{390–410}/R_{410–450}$ and a somewhat lower linear coefficient ($R^2 = 0.6–0.7$) in the range of $R_{660–680}/R_{680–685}$ (Fig. 3).

3.3. Effects of UV/PAR treatments on photosynthetic parameters

Changes in total chlorophyll content induced by UV/PAR treatments were more pronounced in variety Barke than Bonus (Table 2). The [UV+PAR−] treatment reduced the chlorophyll content mainly in older and middle (1st and 2nd) leaves, whereas the [UV+PAR+] treatment caused a reduction in chlorophyll content only in the younger (3rd) leaf. Changes in A_{max} in leaves of different age classes caused by UV/PAR treatments are shown in Fig. 4. The A_{max} values gradually decreased with an increasing leaf age irrespective of treatment and barley variety. There was a statistically significant decrease in A_{max} (35–52%) in variety Barke after one-day of exposure to [UV+PAR−] as compared to [UV−PAR−] conditions. A similar tendency for decline in A_{max} (2–24%) was recorded in the variety Bonus; however, it was statistically non-significant. After seven days of [UV+PAR−] treatment, there was a statistically significant reduction in A_{max} for both Barke (44–71%) and Bonus (32–54%) as compared to the [UV−PAR−] treatment. Visual symptoms of leaf damage accompanied these reductions in A_{max} which were more pronounced and appeared first in older rather than younger leaves. These necrotic spots were observed during the fourth day of [UV+PAR−] treatment in the variety Barke, while there was an approximately two-day delay before their appearance in the variety Bonus. There were negligible differences in A_{max} between the [UV−PAR−] and [UV−PAR+] treatments, in both barley varieties and all leaf-age classes. In addition, there were no significant changes in A_{max} between [UV+PAR+] and [UV−PAR+] treatments during the whole experiment, irrespective of leaf-age and variety. On the contrary, significantly lower A_{max} values were present in plants exposed to [UV+PAR−] as compared to [UV−PAR−] conditions, in particular in variety Barke (Fig. 4; Table 3).

Effects of UV/PAR treatments on F_V/F_M values were similar to trends for A_{max} (Fig. 5; Table 3). However, the changes were less pronounced and not always statistically significant. Seven days of exposure to [UV+PAR−] led to a decrease in F_V/F_M as compared to [UV−PAR−] by 29% and 7% in the variety Barke and by 19% and 4% in the variety Bonus in the 2nd and 3rd leaf, respectively. Similarly to A_{max}, most changes in F_V/F_M due to enhanced UV were not statistically significant under the [PAR+] treatments.

Under all treatments, the slope of relationship between stomatal conductance and CO_2 assimilation rate under saturating irradiance was significantly modified (log-transformed data; ANCOVA; $p < 0.01$) by leaf age (Fig. 6). There was no significant effect of PAR on these relationships ($p = 0.39$). Although the impact of enhanced UV on the shape of this relationship was small (Fig. 6A) there was a significant effect on the slope ($p < 0.01$). Enhanced UV treatments resulted in stomatal closure that consequently led to a decline in the CO_2 assimilation rate. In addition, the [UV+PAR−] treatment caused a decline in A_{max} at corresponding G_S ($\approx$0.1 mol m^{-2} s^{-1}) as compared to other treatments, due to decreases in photochemical efficiency (Fig. 5) and Rubisco carboxylation activity *in vivo* (V_{Cmax}; Fig. 7). The linear relationships between V_{Cmax} and A_{max} showed tight correlations ($R^2 = 0.93–0.98$; $p < 0.01$) irrespective of leaf age and barley genotype and the slope of these relationships was significantly affected by UV and PAR treatments (ANCOVA; $p = 0.03$) (Fig. 7).

3.4. Morphological responses to UV/PAR treatments

After the seven-day acclimation period, leaf length was the most sensitive morphological parameter to UV/PAR treatment, though

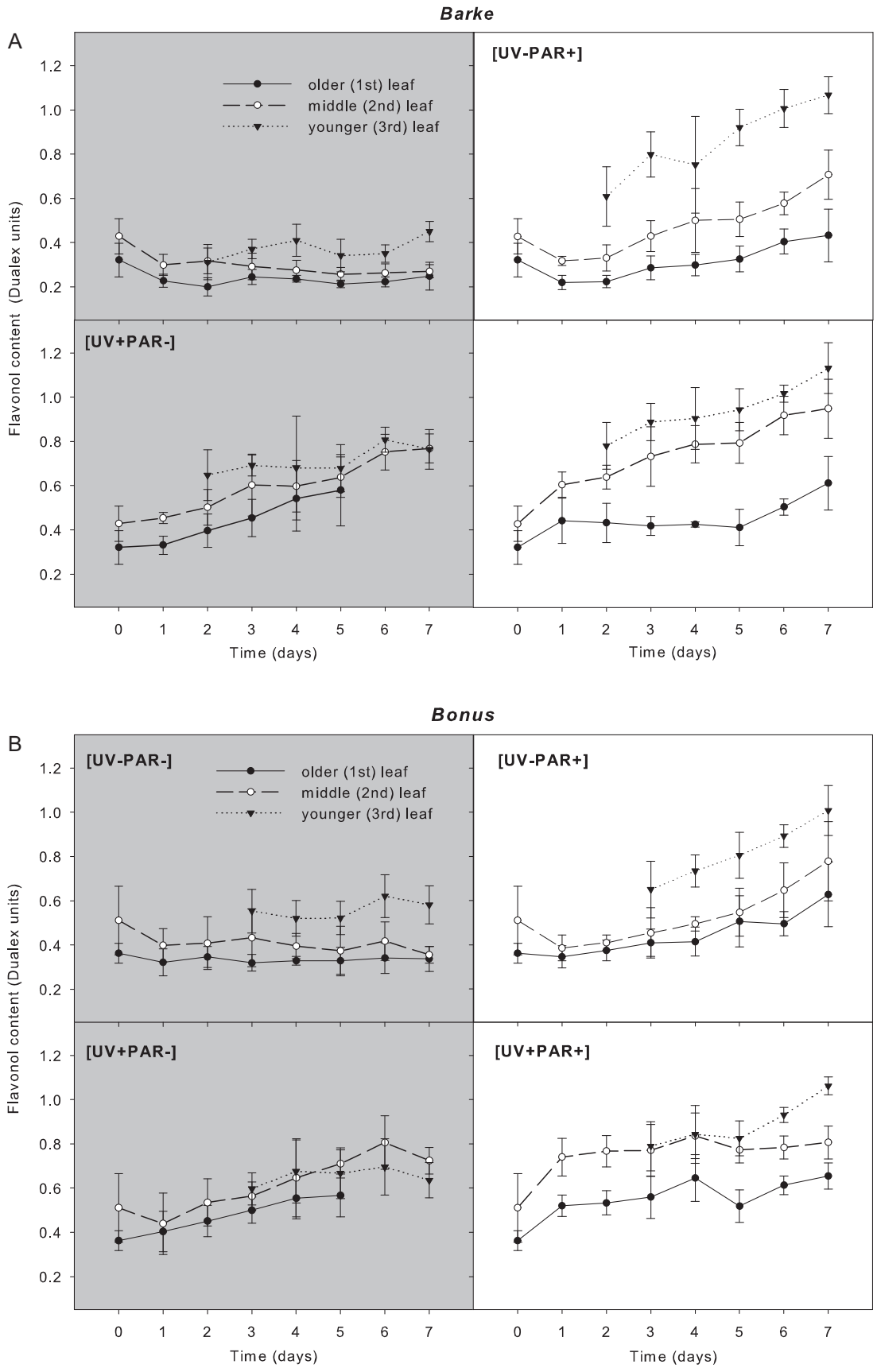

Fig. 2. Changes in flavonol content measured *in vivo* using the instrument Dualex 4 Flav during the acclimation of barley plants to individual UV/PAR treatments. Data are reported separately for each leaf age and variety Barke (A) and Bonus (B). Means (symbols) and standard deviations (vertical bars) are presented ($n \geq 5$).

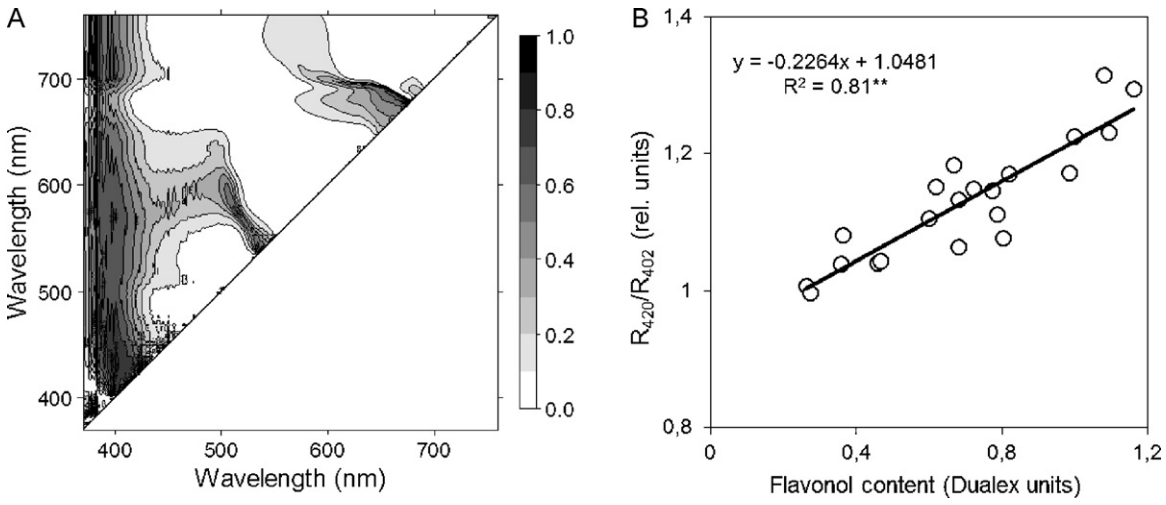

Fig. 3. (A) Matrix of coefficients of determination (R^2) for the linear relationships between flavonol content (measured after seven days of treatment) and simple ratios of reflectance intensity (R_x/R_y) in the spectral range from 350 to 750 nm irrespective of UV/PAR treatment, leaf-age and barley genotype. (B) The linear relationship with highest coefficient of determination (R^2 = 0.81**) between R_{420}/R_{402} and flavonol content. **Denotes statistically significant relationship at $p < 0.01$.

the effect of UV-B was modified by the leaf age and genotype (Fig. 8A and B; Table 3). In respect to leaf length, the 1st leaves of both varieties were fully developed, while the 2nd leaves were partly developed and the 3rd leaves were only emerging at the start of the experiment. While the leaf length remained unchanged in the older (1st) leaves, in the both middle (2nd) (23% in Barke, 10% in Bonus) and younger (3rd) leaves (44% in Barke, 24% in Bonus) the final leaf length was significantly smaller due to [UV+PAR−] treatment compared to the [UV−PAR−]. Comparing [UV−PAR−] and [UV−PAR+] plants, high PAR irradiances led to a slight reduction (statistically non-significant) in the leaf length of all leaf-age classes. On the contrary, the [UV+PAR+] treatment resulted in a significant reduction in the leaf length of the younger (3rd) leaf (developing during the experiment) in variety Barke (23%) as

Table 2
Changes in total chlorophyll contents (Chl $a+b$; $\mu g\,cm^{-2}$) during the acclimation of two barley varieties (Barke, Bonus) to individual UV/PAR treatments. Means and standard deviations (SD) are reported ($n \geq 5$).

	Treatment	1st Day				4th Day						7th Day					
		Older (1st) leaf		Middle (2nd) leaf		Older (1st) leaf		Middle (2nd) leaf		Younger (3rd) leaf		Older (1st) leaf		Middle (2nd) leaf		Younger (3rd) leaf	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Barke	[UV−PAR−]	24.5	2.1	24.0	2.0	23.8	1.5	23.4	3.2	24.7	1.3	22.5	3.4	20.1	2.5	23.8	2.8
	[UV+PAR−]	19.3	1.7	19.8	1.8	15.5	3.4	19.4	0.9	14.2	3.0	–		15.7	1.0	18.3	2.9
	[UV−PAR+]	22.7	1.4	22.0	0.5	22.6	1.0	24.1	1.3	21.8	2.2	19.2	2.1	22.6	1.3	20.9	1.4
	[UV+PAR+]	21.2	2.0	22.3	1.8	21.4	1.7	23.5	1.5	18.4	3.3	18.9	1.9	20.9	1.4	17.6	1.0
Bonus	[UV−PAR−]	21.2	1.7	19.0	1.8	22.8	2.1	22.7	1.7	18.4	2.4	18.8	2.1	18.7	2.4	17.2	1.5
	[UV+PAR−]	20.6	2.7	19.3	2.6	19.2	2.2	17.8	6.9	16.2	2.9	–		18.7	3.3	16.0	1.7
	[UV−PAR+]	21.9	1.3	19.5	1.7	19.8	0.3	20.1	2.3	14.6	3.1	18.3	4.1	20.4	1.6	14.4	1.4
	[UV+PAR+]	19.8	1.9	19.2	2.2	19.9	1.5	20.2	2.1	19.0	2.6	17.4	1.4	19.3	1.4	17.6	0.8

Table 3
Summary of significance levels (p-values of the multi-way ANOVA) for the effects of UV treatment (UV), barley genotype (G), and leaf-age (A) on the physiological and morphological parameters under [PAR−] and [PAR+] treatments. Significant interactions ($p < 0.05$) are indicated in bold. * and ** denote significant effects at $p < 0.05$ and $p < 0.01$ respectively. Flav—flavonols content *in vivo*; Chl $a+b$—total chlorophylls content *in vivo*; A_{max}—light-saturated rate of CO_2 assimilation; G_S—light-saturated stomatal conductance; F_V/F_M—maximum quantum yield of photosystem II; LL—leaf length; LA—leaf area; SLA—specific leaf area.

		Flav	Chl $a+b$	A_{max}	G_S	F_V/F_M	LL	LA	SLA
PAR−	UV	**<0.01****	**<0.01****	**<0.01****	**<0.01****	**<0.01****	**<0.01****	**<0.01****	**<0.01****
	G	0.61	**0.03***	0.50	0.42	**<0.01****	**<0.01****	**<0.01****	0.40
	A	**<0.01****	0.51	**<0.01****	**0.03***	**<0.01****	0.56	0.12	0.25
	UV × G	**<0.01****	**<0.01****	**0.02***	0.24	**0.01***	**<0.01****	**<0.01****	0.38
	UV × A	**<0.01****	0.45	0.12	0.36	**<0.01****	**<0.01****	**<0.01****	**<0.01****
	G × A	0.63	**<0.01****	0.13	0.45	0.06	0.25	0.38	0.05
	UV × G × A	0.12	0.99	0.94	0.52	0.26	0.14	0.09	0.21
PAR+	UV	**0.01***	**0.04***	**<0.01****	**<0.01****	**<0.01****	**<0.01****	**<0.01****	0.90
	G	0.17	**<0.01****	0.61	**<0.01****	0.81	**<0.01****	**<0.01****	0.73
	A	**<0.01****	**<0.01****	**0.03***	0.46	**<0.01****	0.21	0.38	**<0.01****
	UV × G	0.13	**<0.01****	0.50	0.07	0.22	**<0.01****	**<0.01****	0.07
	UV × A	0.30	0.05	0.83	0.23	**0.03***	0.18	0.42	0.84
	G × A	0.69	**0.04***	0.64	0.05	0.54	0.15	0.08	0.08
	UV × G × A	0.17	**<0.01****	0.41	0.30	0.13	0.33	0.12	0.42

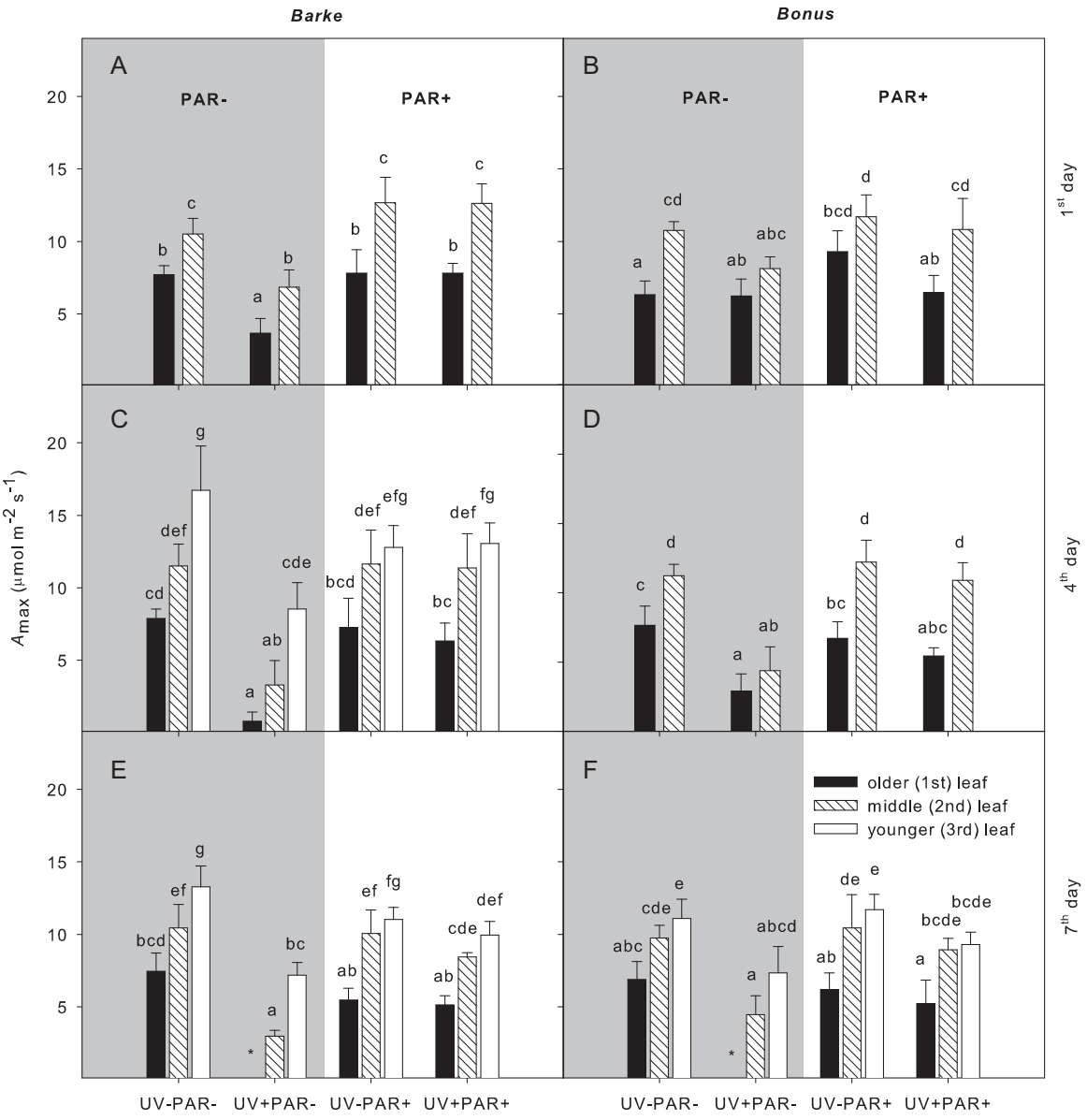

Fig. 4. Changes in light-saturated rate of CO_2 assimilation (A_{max}) during seven-day acclimation to individual UV/PAR treatments: 1st day (A and B), 4th day (C and D), 7th day (E and F). Measurements were done on older (1st – black), middle (2nd – shaded), and younger (3rd – white column) leaves of both barley varieties—Barke (A, C and E) and Bonus (B, D and F). Means (columns) and standard deviations (vertical bars) are presented ($n = 5$). Different letters denote statistically significant differences between treatments and leaves within individual varieties ($p < 0.05$). Asterisks denote damaged leaves which were not measurable.

compared to [UV−PAR+] treatment, whereas any equivalent reduction was negligible in Bonus.

PAR intensity (comparing [UV−PAR−] and [UV−PAR+]) had no significant effect on the pattern of leaf area development (Fig. 8C and D). The [UV+PAR−] treatment led to a significant reduction in the leaf area, primarily as a result of reduced leaf width (data not shown), as compared to [UV−PAR−], in all leaf-age classes and in both the varieties studied (45–65% in Barke and 26–48% in Bonus). Similarly to the leaf length, the UV effect was most pronounced in the younger (3rd) leaf and in the variety Barke. Comparing [UV−PAR+] and [UV+PAR+] treatments, a statistically significant reduction in leaf area was only present for the younger (3rd) leaf of variety Barke (by 32%).

The response of SLA to UV was different in the [PAR+] and [PAR−] treatments (Fig. 8E and F). There was no effect of enhanced UV treatment in the [PAR+] plants of either variety for all leaves. On the contrary, the [UV+PAR−] treatment led to a significant reduction in SLA as compared to the [UV−PAR−] treatment. The highest, statistically significant, decline was in the older (1st) leaves of the both varieties (54% in Barke and 48% in Bonus), and the younger (3rd) leaves of both varieties studied followed a similar tendency, however, in their case this was not statistically significant.

4. Discussion

The field experiment was designed to test the hypotheses that (1) accumulation of UV-shielding compounds can be induced by high PAR intensities, (2) PAR is involved in plant acclimation to enhanced UV-B at both physiological and morphological levels, and (3) acclimation to UV-B radiation is modified by the leaf age and plant genotype (i.e., by constitutive UV protection).

By investigating the time course of acclimation to UV radiation during leaf development, and the interactions of UV with PAR, leaf age, and genotype, we went beyond previous studies, which only considered the interactions of only two factors; i.e., UV-B vs. PAR (Götz et al., 2010), UV-B vs. leaf age (Kakani et al., 2004), or UV-B vs.

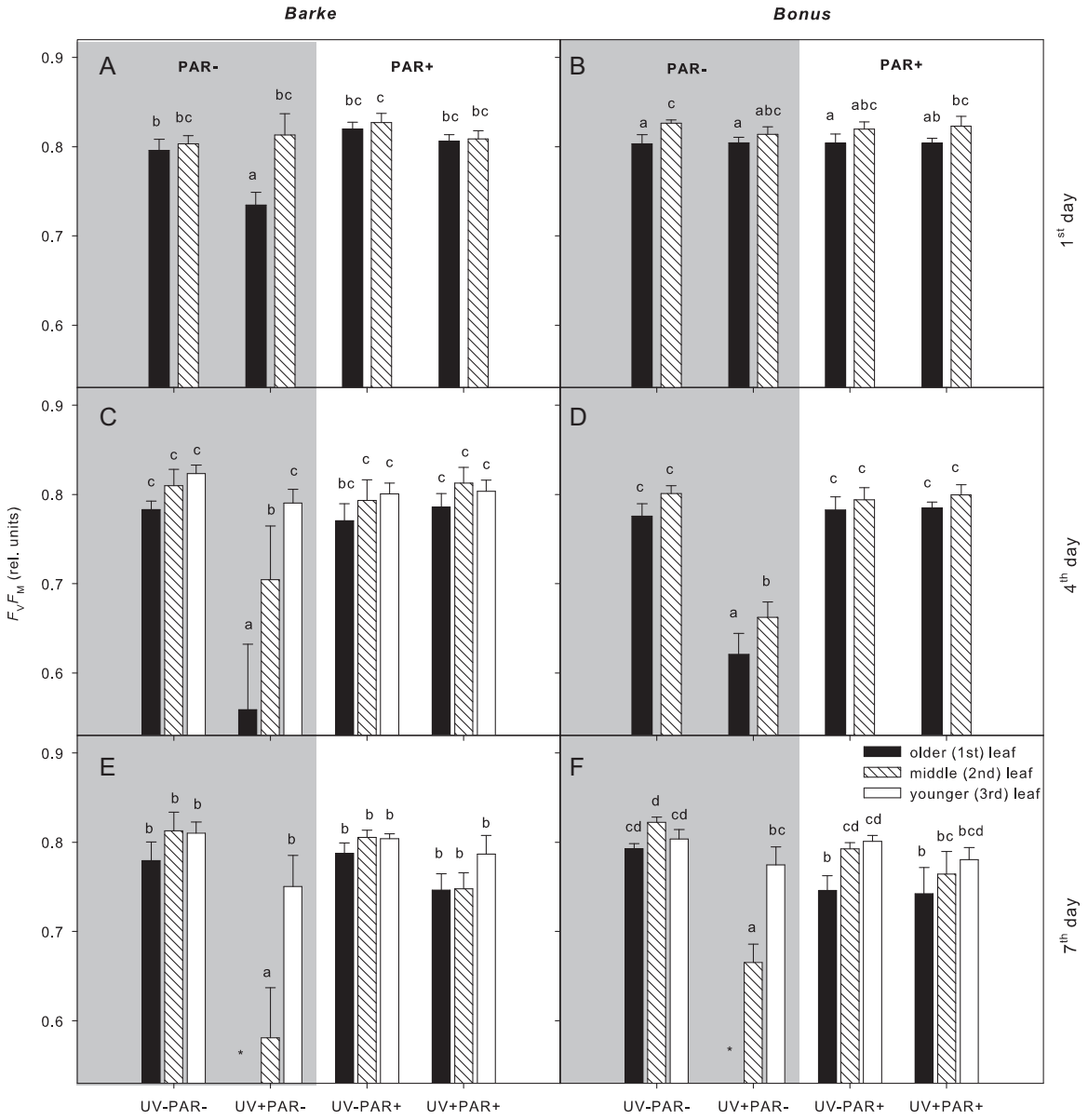

Fig. 5. Changes in the maximum quantum yield of photosystem II (F_V/F_M) during seven-day acclimation to individual UV/PAR treatments: 1st day (A and B), 4th day (C and D), 7th day (E and F). Measurements were done on older (1st – black), middle (2nd – shaded), and younger (3rd – white column) leaves of both barley varieties—Barke (A, C and E) and Bonus (B, D and F). Means (columns) and standard deviations (vertical bars) are presented ($n = 5$). Different letters denote statistically significant differences between treatments and leaves within individual varieties ($p < 0.05$). Asterisks denote damaged leaves which were not measurable.

genotype (Mohammed and Tarpley, 2011). To determine the constitutive accumulation of UV-shielding compounds independently from UV/PAR induction, plants were pre-cultivated under excluded UV and reduced PAR conditions ([UV−PAR−]).

4.1. Is the accumulation of UV-shielding compounds induced by PAR?

The UV-induced and PAR-induced accumulation of UV-shielding compounds in leaves was investigated in older (1st), middle (2nd) as well as younger (3rd) leaves acclimated to [UV−PAR−] conditions. This enabled us to distinguish the effect of ontogenesis from the influence of UV-B or PAR on the accumulation of flavonols. We proved that high PAR irradiances can induce the accumulation of flavonols in barley leaves irrespective of UV treatment (Fig. 2). While high PAR irradiances mainly stimulated flavonol biosynthesis in younger leaves, enhanced UV radiation stimulated the accumulation of flavonols in older leaves.

Likewise, Götz et al. (2010) showed that high PAR triggers flavonoid biosynthesis, in particular quercetin, in *Arabidopsis* plants exposed to low biologically effective UV irradiances (25 mW m^{-2}). The combination of high UV and PAR irradiances leads, not only to further increases in the quantity (Meijkamp et al., 2001; Götz et al., 2010), but also in the quality (e.g., increased ratio between quercetin and kaempferol), of UV-screening metabolites (Rozema et al., 2002; Jansen et al., 2008).

In general, epidermally located flavonoids and hydroxycinnamic acid esters prevent the penetration of short solar wavelengths (280–450 nm) into leaves (DeLucia et al., 1992; Burchard et al., 2000). However, Agati et al. (2009) demonstrated that acclimation to contrasting UV and PAR irradiances also leads to changes in flavonoid distribution within mesophyll and epidermal

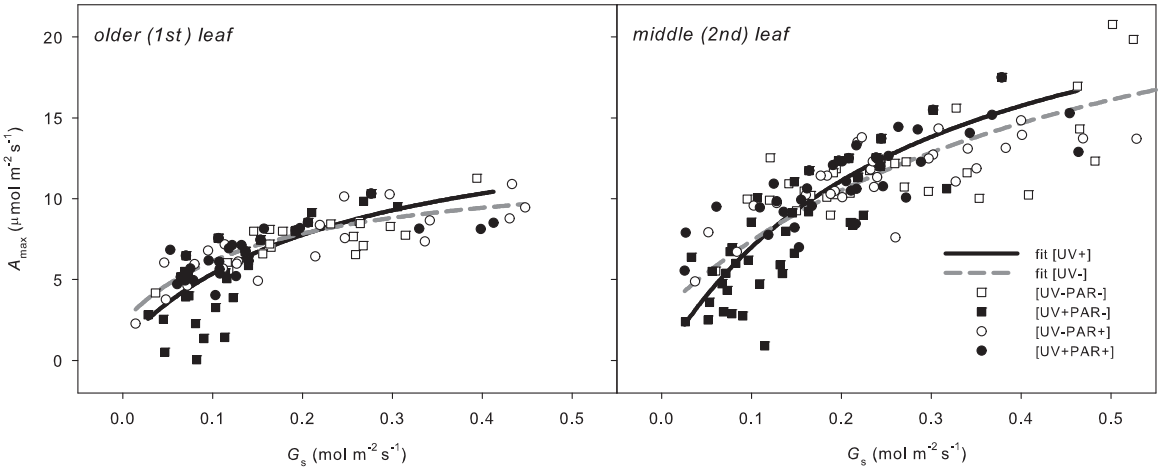

Fig. 6. Relationship between CO_2 assimilation rate (A_{max}) and stomatal conductance (G_S) estimated under saturating irradiance (1200 μmol m^{-2} s^{-1}) in the older (1st) (A) and middle (2nd) barley leaf (B) during the whole seven-day experiment. Open circles represent leaves exposed to reduced UV [UV−], closed circles represent leaves exposed to enhanced UV radiation [UV+] irrespective of PAR treatment and barley variety. A rectangular hyperbolic function [$y = y_0 + ax/(b+x)$] was fitted to the data. Coefficients of determination (R^2) were 0.539** [UV+] and 0.706** [UV−] for the older (1st) leaf and 0.655** [UV+], 0.654** [UV−] for the middle (2nd) leaf. **Denotes statistically significant relationship at $p < 0.01$. ANCOVA was used to determine whether UV, PAR and leaf age affects the relationships. Data were log-transformed to meet the assumption of linearity. Relationships did not significantly differ between PAR treatments ($p = 0.39$), but leaf age and UV significantly affected the slope ($p < 0.01$).

cells. Accumulation of quercetin and luteolin derivatives in mesophyll cells in the absence of UV wavelengths leads to the hypothesis that flavonoids, in particularly UV-inducible quercetin (Rozema et al., 2002), also play a key role in countering light-induced oxidative stress (Jansen et al., 2008; Agati et al., 2009).

We have shown that the flavonol content of leaves can be estimated from spectral reflectance using the R_{420}/R_{402} ratio, or potentially based on VIs from the spectral region around 680 nm (Fig. 3). As far as we know, flavonol content has not been estimated non-destructively using VIs before. Previously, simple reflectance data were related to UV effects, where higher $R_{280-300}$ and lower $R_{300-380}$ have been reported in the sun-adapted leaves of *Quercus ilex* from higher altitudes (exposed to higher UV doses) as compared to lower altitudes (Filella and Peñuelas, 1999).

4.2. Does PAR influence photosynthetic and morphological acclimation to enhanced UV?

It is commonly acknowledged that high-PAR can alleviate the negative effects of enhanced UV-B radiation, though the evidence for this hypothesis in the literature is contradictory (Pfündel et al., 1992; Sullivan et al., 2003; Nithia et al., 2005; Jansen et al., 2010). In our study, [PAR+] treatment mitigated reductions in A_{max} caused by [UV+] (Fig. 4). The acclimation of CO_2 assimilation rate to combined UV/PAR treatments operates on several structural levels encompassing changes in chlorophyll content and changes in stomatal function (reviewed in Caldwell et al., 2007; Jansen et al., 2010; Ballaré et al., 2011).

In our study, the total chlorophyll content significantly decreased in [UV+PAR−] treated plants; however, UV-induced changes in Chl $a+b$ were not present in [PAR+] plants at any leaf developmental stages for either of the genotypes studied (Table 2). Similarly, Jordan et al. (1994) reported a reduction in chlorophyll content (mainly in Chl a) by 20% in green pea plants after seven days of UV-B treatment; however, UV-B-induced changes were dependent upon the leaf developmental stage.

Both UV radiation and excessive PAR particularly target PS II. In our study, the [UV+] treatment led to the decrease of F_V/F_M in [PAR−] plants, while it remained almost unchanged in [PAR+] plants (Fig. 5). *In vitro* studies have shown that simultaneous illumination by PAR and UV-B impairs PS II activity to a smaller extent to that expected when they are independently illuminated (e.g., Tyystjärvi, 2008). This protective effect was pronounced only at low PAR irradiances but becomes negligible at high irradiances.

Several studies have attributed reduced A_{max} after enhanced UV-B treatment to a reduction in Rubisco content and/or decrease in its carboxylation activity (Jordan et al., 1992; Šprtová et al., 1999; Takeuchi et al., 2002). Based on tight linear correlations between V_{Cmax} and light-saturated A_{max} (Fig. 7), the [UV+] treatment led to a decrease in Rubisco activity in [PAR−] acclimated plants, while its effect was negligible in [PAR+] acclimated plants. Thus, the [PAR+] treatment prevents negative UV effects on the extent of light absorption (Table 2), photochemical efficiency (Fig. 5) as well as carboxylation activity (Fig. 7).

Finally, UV radiation led to a decrease in stomatal conductance, particularly in [PAR−] acclimated plants (Fig. 6). This result is in

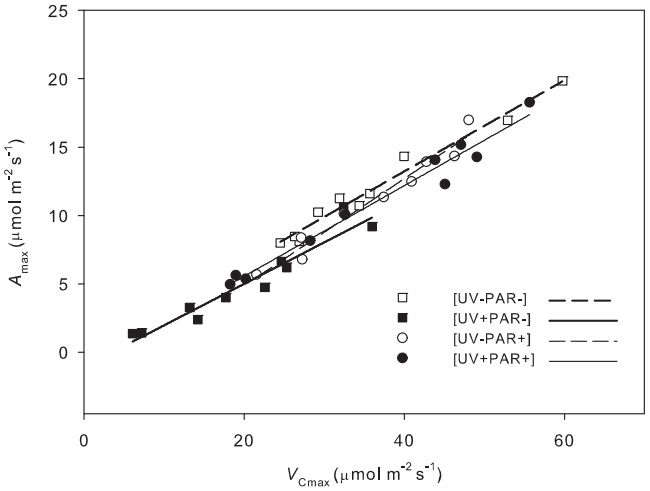

Fig. 7. Relationship between maximum carboxylation rate *in vivo* (V_{Cmax}) and light-saturated rate of CO_2 assimilation (A_{max}). The measurements were done after four-day exposure to individual UV/PAR treatments. The linear functions were fitted to the data of the individual UV/PAR treatments (R^2 ranged from 0.927** to 0.975**), irrespective of barley variety and leaf age. **Denotes statistically significant relationship at $p < 0.01$. ANCOVA showed the significant effect of UV and PAR treatments on the slope of relationships ($p < 0.01$).

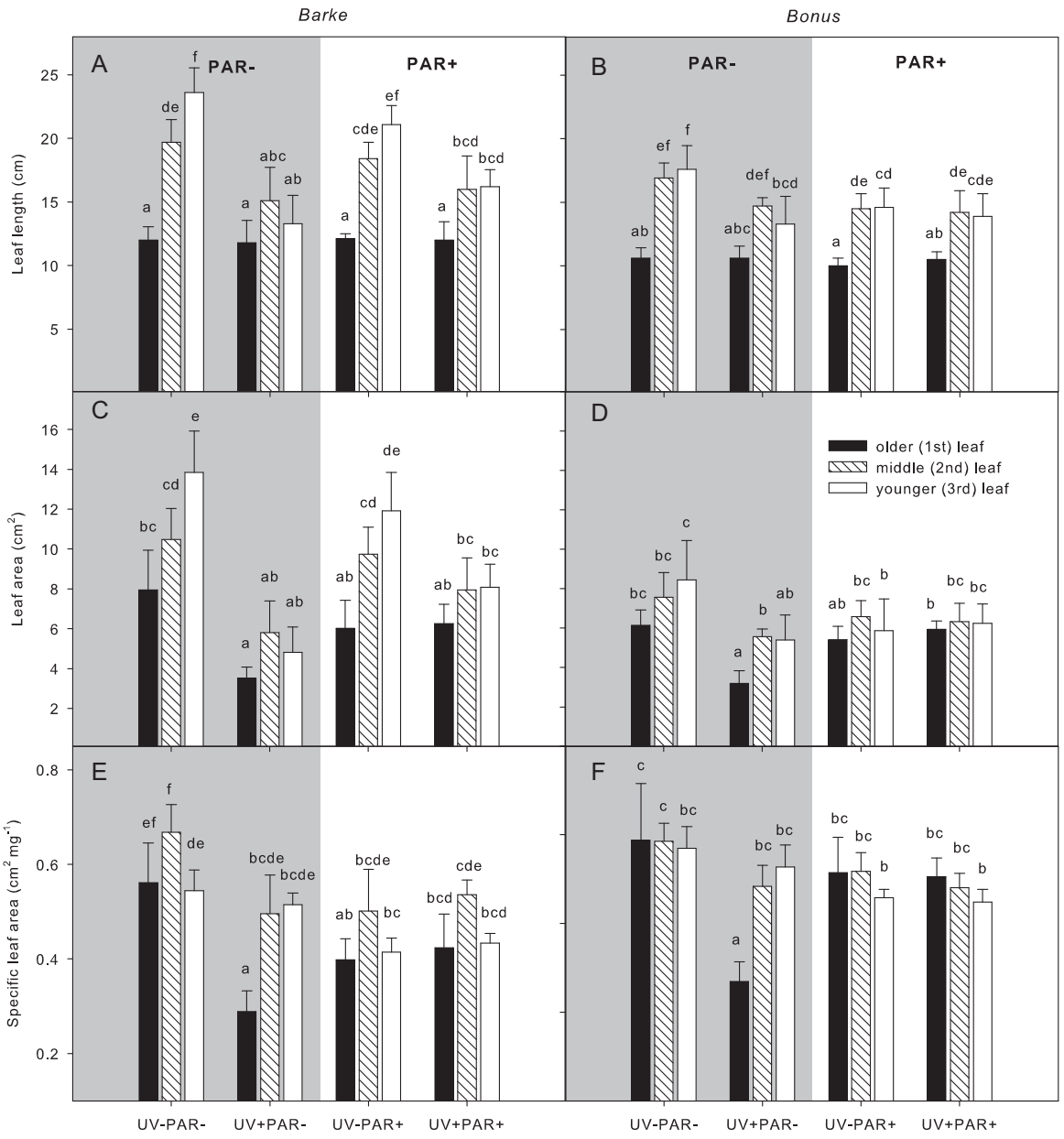

Fig. 8. Leaf length (A and B), leaf area (C and D) and specific leaf area (E and F) after seven-day exposure to UV/PAR treatments. Measurements were done on the older (1st – black), middle (2nd – shaded), and younger (3rd – white column) leaves of both barley varieties—Barke (A, C and E) and Bonus (B, D and F). Means (columns) and standard deviations (vertical bars) are presented ($n = 5$). Different letters denote statistically significant differences between treatments and leaves within individual varieties ($p < 0.05$).

accordance with findings of other authors (Sullivan et al., 2003; Urban et al., 2006); however, increased G_S due to enhanced UV has also been reported (Nithia et al., 2005). Reduced G_S leads to decreases in intercellular CO_2 concentration and consequently in A_{max}. Based on the relationship between A_{max} and G_S, estimated under saturating PAR intensity (Fig. 6), we can conclude that the variations in A_{max} between individual UV/PAR treatments in the middle (2nd) leaves were mainly caused by the variations in G_S rather than physiological limitations. On the contrary, in the older (1st) leaves the decrease in A_{max}, under the [UV+PAR−] treatment, was caused by the combination of G_S reduction, reduction in PS II efficiency (Fig. 5) and Rubisco activity (Fig. 7).

Of the UV effects on morphology, growth inhibition (decreased elongation due to photo-oxidative destruction of the indole acetic acid), activation of leaf thickening and axillary branching are regarded as typical UV-induced responses (Jansen et al., 1998; Jansen, 2002; Krizek, 2004). In our study, leaf length and leaf area were decreased, whereas leaf thickness, measured as SLA, was increased by the [UV+PAR−] treatment. On the contrary, UV-induced changes in morphological parameters were largely mitigated by [PAR+] conditions (Fig. 8). This is consistent with a general agreement that high PAR intensities may compensate for the negative effects on plant morphology induced by UV radiation (Jansen et al., 1998; Jansen, 2002; Krizek, 2004). Both PAR and UV-B may lead to increased leaf thickening, which can contribute to reduced penetration of UV-B into the leaf interior (Burchard et al., 2000) and thus protects the photosynthetically active mesophyll cells (Meijkamp et al., 2001).

We have shown that SLA is inversely proportional to flavonol content in leaves (Fig. 9). Recently, it has been shown that flavonoids are internal modulators of indole acetic acid transport and its oxidation rates (Jansen, 2002). Thus, UV-induced changes in flavonoid accumulation and auxin homeostasis could contribute to changes in plant morphology. Previously, Mutikainen et al. (2002)

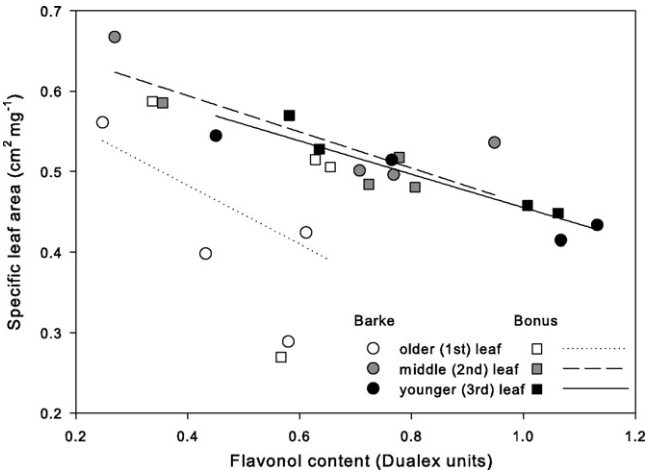

Fig. 9. Relationship between specific leaf area (SLA) and flavonol content estimated *in vivo* in two barley varieties studied (Barke – circles, Bonus – squares). There was a tight correlation between SLA and flavonols for the middle ($R^2 = 0.67$; $p < 0.01$) and younger ($R^2 = 0.9$; $p < 0.01$) leaves of both barley varieties studied, whereas the correlation was insignificant in older leaves ($R^2 = 0.21$; $p > 0.05$). ANCOVA revealed that barley variety did not have a significant effect ($p = 0.57$) on the relationship, but that leaf age significantly affected the intercept ($p < 0.01$).

found the negative correlations between the content of flavonol glycosides and height and relative growth of *Betula pendula*.

4.3. Is the acclimation to UV radiation modified by the leaf age?

In general, younger leaves have higher photosynthetic capacity as compared to older leaves (Fig. 4). Since they have a higher value to the plant (Reifenrath and Müller, 2007), we expected young leaves to have more efficient protection against [UV+] treatments than old leaves. This hypothesis was confirmed since the [UV+] treatments led to higher production of flavonols in younger (3rd) leaves (Fig. 2), as well as less pronounced damage to PS II activity (Fig. 5) and a smaller reduction in A_{max} (Fig. 4) than in the middle (2nd) or older (1st) leaves.

In accordance with our findings, the accumulation of flavonoids during [UV+] exposure was faster in younger as compared to older leaves of several other crops (Kakani et al., 2004; Reifenrath and Müller, 2007) and tree species (Ibanez et al., 2008; Sun et al., 2010). Using a fluorescence imaging system, Štroch et al. (2008) identified faster development of UV-shielding compounds in tissues at younger developmental stages along the leaf blade.

Although UV-B radiation is regarded as an environmental factor inducing early leaf senescence (Jordan et al., 1994), the evidence for an interactive effect of UV radiation and leaf age in the literature is contradictory. Bassman et al. (2002) reported no interaction between UV treatments and leaf age on gas exchange variables in Douglas-fir, whereas significant interactions were evident between UV-B and leaf age for photosynthesis and stomatal conductance in cotton leaves (Kakani et al., 2004).

However, it is unclear whether the UV-induced decline in A_{max} is attributable to age-related modifications in foliar morphology, stomatal conductance, biochemical and/or photochemical potentials. In agreement with the findings of Kakani et al. (2004) in cotton, we report an interactive effect of [UV+PAR−] treatment on total chlorophyll content only in the older (1st) leaves (Table 2). However, this is contrary to the findings of Day et al. (1996) in pea. Also, although there was a reduction in maximum quantum yield of PS II under [UV+PAR−] treatment in the older leaves, it was almost unchanged in younger leaves (Fig. 5). On the contrary, Naidu et al. (1993) found that enhanced UV-B significantly decreased the F_V/F_M ratio only in the most recent fully expanded needles of loblolly pine.

It has been previously shown that Rubisco is actively synthesized right until the leaf is fully expanded. Rubisco content then decreases with increasing leaf age and its nitrogen is reallocated into newly developing leaves (Takeuchi et al., 2002). This leads to the typical age-related distribution of assimilation capacity (Fig. 4) that is limited under the light-saturating irradiances by the Rubisco carboxylation activity (Fig. 7). The reduction in A_{max} due to the [UV+PAR−] treatment was more pronounced in older leaves (up to 88%; in the 1st leaf of Barke) than in the younger leaf (up to 47% in the 3rd leaf of Barke). Takeuchi et al. (2002) identified that supplemental UV-B radiation significantly accelerated the degradation of Rubisco around the time of leaf maturation in the two cultivars of rice. Moreover, synthesis of Rubisco was suppressed during the early leaf developmental stages under enhanced UV-B.

In spite of decreasing stomatal conductance with leaf age (e.g., Kakani et al., 2004), the relationships between A_{max} and G_S (Fig. 6) revealed that the age-related decrease in A_{max} was primarily caused by the reduced Rubisco content and/or activity irrespective of UV treatment. In accordance with other studies (Kakani et al., 2004; Urban et al., 2006), we observed a reduction in G_S due to [UV+] treatment, particularly in older (1st) leaves; however, Jansen and van den Noort (2000) found that UV-B radiation may induce either stomatal opening or closing in *Vica faba*.

In the present study, the UV-B effect on leaf length decreased with increased leaf age. In the older leaves, leaf length extension was almost completed at the start of the experiment, which explains the small response to UV. On the contrary, there were no significant differences in plant height and leaf length of *Citrus aurantifolia* in relation to development stage between plants grown with and without solar UV-B radiation (Ibanez et al., 2008). Here, the UV-B effect on leaf thickness was more pronounced in the mature leaves (1st and 2nd) in comparison with the younger (3rd) leaf (see SLA values in Fig. 8E and F). Because younger leaves are attenuating less UV-B radiation than mature leaves (DeLucia et al., 1992), accumulation of flavonoids probably has a more important role in protection against UV-B radiation in newly developed leaves than morphological adjustment. Ruhland and Day (1996) also concluded that UV-screening effectiveness is not necessarily related to concentration of UV-absorbing compounds, but also due to anatomical changes within the epidermis (thickening of the cuticle) that occur with leaf age.

4.4. How does plant genotype influence its acclimation to UV radiation?

Much less is known about constitutive differences than inducible differences in UV protection (Jansen et al., 2010). In our study, we have demonstrated that plant genotype significantly modulates photosynthetic acclimation to UV/PAR treatments (Figs. 4 and 5; Table 3). Variety Barke, regarded as sensitive to light-induced oxidative stress (Wu and von Tiedemann, 2004), had higher sensitivity to [UV+] treatments, whereas the responses to UV/PAR treatments were less pronounced and in most cases non-significant in variety Bonus. This corresponds to lower constitutive accumulation of UV-screening compounds in variety Barke. Significant genotypic variability in UV-B responsiveness has likewise been reported in other barley varieties (Hideg et al., 2006) and also in cowpea (e.g., Singh et al., 2008), soybean (e.g., Koti et al., 2007) or rice (e.g., Mohammed and Tarpley, 2011).

Under the [UV−PAR−] treatment, the more tolerant variety Bonus had higher constitutive quantities of UV-screening compounds than variety Barke, particularly in younger leaves (Fig. 2). This is in accordance with Sun et al. (2010), who proved that young leaves of *Ginkgo biloba* have higher constitutive as well as UV-induced contents of flavonoids than old leaves. Despite of lower constitutive accumulation of UV-screening compounds in variety

Barke, the UV/PAR induced accumulation was similar in both varieties or even higher in variety Barke (in younger leaves). The final content of induced flavonols was several times higher than those present constitutively. Similarly, Jansen et al. (2010) found that variation in constitutive UV protection is small compared to the amplitude of environmentally induced changes in UV protection. These findings support the conclusions of Mohammed and Tarpley (2011) that the tolerance of rice cultivars to UV-B is preferentially given by UV-induced increases in phenolic content.

Variability in photosynthetic response to UV treatment is often associated with genotypic differences in CPD photolyase activity, which catalyses the reversal of UV-damaged DNA, and may consequently lead to the decrease in Rubisco content (Fedina et al., 2010). In addition, studying two clones of *Calamagrostis* species, Urban et al. (2006) report different limitations to photosynthesis in *Calamagrostis arundinacea* and *Calamagrostis villosa* exposed to enhanced UV-B radiation. While the decrease in A_{max} was caused only by reduced G_S in *C. arundinacea*, both G_S and V_{Cmax} decreased in *C. villosa* due to enhanced UV-B treatment.

In response to enhanced UV-B radiation, intraspecific variation in morphological parameters has been determined in many crop species, such as barley (Mazza et al., 1999), rice (Mohammed and Tarpley, 2011) and soybean (Li et al., 2002). In the present study, morphological changes due to UV-B in [PAR+] were only present in variety Barke (Fig. 8). In addition, although the effect of UV-B in [PAR−] was significant for both varieties, the greater reduction in morphological parameters in variety Barke also illustrated a higher sensitivity of this variety to UV-B compared with variety Bonus.

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