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Anatomical, physiological, and biochemical traits involved in the UV-B radiation response in highbush blueberry

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Abstract

The effects of a long-term simulated spring-summer UV-B daily course on some anatomical, physiological, and biochemical features were studied in new and old leaves of blueberry (*Vaccinium corymbosum* L.) cultivars Legacy, Brigitta, and Bluegold. The results show that under UV-B exposure, leaf thickness increased in Bluegold due to an increased intercellular cavities. By contrast, Brigitta maintained its leaf thickness. The net photosynthetic rate was not significantly affected by the UV-B radiation in any of the cultivars; however, Brigitta presented a better photosystem II performance, since this cultivar had more efficient photochemistry under the UV-B radiation. In addition, Brigitta also maintained enhanced total phenol and total anthocyanin content compared to the other cultivars. In conclusion, Brigitta was more resistant to the UV-B radiation than the other two cultivars.

Additional key words: antioxidants, carotenoids, chlorophyll content, chlorophyll fluorescence, net photosynthetic rate, *Vaccinium corymbosum.*

Introduction

Incident solar ultraviolet (UV) radiation (including UV-B 280 - 320 nm) is composed of non-photosynthetically active wavelengths. This UV-B radiation penetrates the ozone (O_3) layer of the stratosphere (Caldwell and Flint 1997, Jones *et al.* 2003, Wang *et al.* 2008). This radiation has changed due to depletion of the stratospheric ozone resulting in an impact on vegetation, particularly at higher altitudes and latitudes in zones where the net depletion of O_3 is greatest (Madronich 1992, Madronich *et al.* 1998). In southern Chile, due to the extension of the Antarctic ozone hole, higher UV-B doses have been reported than in the Northern Hemisphere, especially in spring-summer (Huovinen *et al.* 2006).

UV-B radiation adversely affects physiological processes and anatomical features in plants (*e.g.*, growth, leaf thickness, photosynthesis, *etc.*) (Jansen *et al.* 2001, Rozema *et al.* 2002, Turunen and Latola 2005). The photosynthetic apparatus and particularly photosystem (PS) II can be UV-damaged (Jansen *et al.* 1998) with negative effects on carbon assimilation, growth, and biomass production (Jordan 1996). Moreover, the UV-B radiation decreases amount of chlorophylls (Chls) and carotenoids (Cars) with a simultaneous reduction in photosynthetic rate (Pfündel *et al.* 1992, Rozema 1999). Additionally, due to the increase in production of reactive oxygen species (ROS), oxidative stress has also been associated with UV-B radiation injury in plants (Mackerness *et al.* 2001, Hollósy 2002, Stratmann 2003,

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Abbreviations: Φ_{PSI} - effective quantum yield of photosystem II; Cars - carotenoids; Chl - chlorophyll; DPPH - 2.2-diphenyl-1-picrylhydrazyl; F_v/F_m - variable to maximum fluorescence ratio; NPQ - non-photochemical quenching; PAR - photosynthetically active radiation; P_N - net photosynthetic rate; PS - photosystem; ROS - reactive oxygen species; RSA - radical scavenging activity.

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Selvakumar 2008, Lazarova *et al.* 2014). Oxidative stress induces lipid peroxidation of cell membranes, which causes breakdown of their structure and function (Murphy and Vu 1996).

Plants have developed some strategies against the deleterious effects of UV-B radiation, including morphological and anatomical adaptations like an altered leaf angle, thicker leaves, and increased reflectance of leaf surfaces by hairs or epicuticular wax crystals (Krauss *et al.* 1997, Rozema 1999, Fukuda *et al.* 2008). The increase or decrease in leaf epidermis thickness under high UV-B doses depends on species or cultivar (Jansen *et al.* 1998, Kakani *et al.* 2003). In this way, Phoenix *et al.* (2001) reported that the leaf thickness of *Vaccinium myrtillus* increases during exposure to elevated UV-B radiation, but Inostroza-Blancheteau *et al.* (2014) observed a short-term reduction in leaf thickness in a UV-B resistant *V. corymbosum* genotype.

Plants have also developed biochemical adaptations to protect themselves against UV-B radiation. The accumulation of UV-B absorbing metabolites, such as phenolic compounds, which act as photoprotective compounds, appears to be the main mechanism against the negative effects of UV-B (Caldwell and Flint 1997, Burchard *et al.* 2000, Kolb *et al.* 2001, Katerova *et al.* 2009). The UV-B-absorbing compounds are photostable and strongly absorb radiation in the range of 220 to 320 nm (Harborne 1986, Day 1993). Another important feature of these compounds is that they do not impair the transmission of the photosynthetically active radiation (PAR) into leaves (Ruhland and Day 1996, Burchard *et al.* 2000). They include specific flavonoids, hydroxycinnamic acid, and hydroxycinnamic acid esters which accumulate mainly in the epidermal layer and in the leaf hairs (Harborne 1986, Day 1993, Tattini *et al.* 2005). It has been demonstrated that UV-B-absorbing metabolites and antioxidant compounds counteract the deleterious effect of UV-B radiation on plant growth (Agarwal 2007, Ibañez *et al.* 2008). Phenolic compounds (especially flavonols) not only have the capacity of absorbing UV-B radiation, but also a high antioxidant activity which can play a key role against the potential oxidative stress induced by UV-B.

There are differences among plant species and ecotypes with respect to the sensitivity to UV-B depending on their phenological stage, seasonality, and evolved UV-B protective mechanisms (Teramura and Sullivan 1994). In fact, also differences in responses of new and old leaves to UV radiation have been reported (Reifenrath and Müller 2007). New leaves have a greater photosynthetic capacity than old leaves, and therefore they should be better protected against biotic and abiotic factors (Iwasa *et al.* 1996, Reifenrath and Müller 2007, Ibañez *et al.* 2008).

Highbush blueberry is deciduous fruit shrub, native to the Northern Hemisphere, that in recent years has been positioned as important crop in Chile due to its high fruit antioxidant activity and a good economic yield (Lyrene and Muñoz 1997, Ribera *et al.* 2010). It is cultivated on a large scale in south-central Chile with different soil types (*e.g.*, volcanic acid soils) and climatic conditions (including UV radiation). However, studies about the anatomical, physiological, and biochemical responses of this crop to some environmental features prevalent under Chilean conditions are limited (Reyes-Díaz *et al.* 2009, 2010, Inostroza-Blancheteau *et al.* 2011, 2014, Rojas-Lillo *et al.* 2014).

In the present study, we investigated the long-term effects of UV-B radiation on some anatomical, physiological, and biochemical features in new and old leaves of three blueberry cultivars under maximum UV-B levels expected in summer.

Materials and methods

Two-year-old saplings of three highbush blueberry (*Vaccinium corymbosum* L.) cultivars (Legacy, Brigitta, and Bluegold), frequently cultivated in southern Chile (Guerrero 2006, Reyes-Díaz *et al.* 2009), were provided by a commercial farm "Berries San Luis" located in Lautaro, La Araucanía Region, Chile (38º 29` S, 72º 23` W). They were grown in a substrate of oat shell + sawdust + pine needles at a 1:1:1 proportion. Afterwards, saplings of similar height were conditioned in plastic boxes filled with Hoagland's nutrient solution (Hoagland and Arnon 1959) for 40 d in a greenhouse under the conditions mentioned below. After conditioning, the plastic boxes were separated in two groups: 1 group of 9 plastic boxes with 6 plants each (total 54) was used as control without UV-B radiation, and another group with the same number of plastic boxes and plants was subjected to the UV-B radiation treatment (+UV-B), using a panel with a set of UV-B lamps (*Q-Panel 313*, Cleveland, Ohio, USA). The lamps were programmed with dimming ballasts (*Transtar*, Hebburn, UK) simulating a natural spring-summer mean UV-B daily course in south-central Chile (Fig. 1). These lamps emit 57 % of UV-B and only 5.7 % of UV-A radiation. To prevent UV-C radiation, the lamps were wrapped with 0.08 mm thick cellulose diacetate film placed at 40 cm from the top of plants. The duration and dose of UV-B radiation were determined using a portable spectroradiometer (*Li-Cor 1800*, Lincoln, NE, USA) as described by Inostroza-Blancheteau *et al.* (2014). The UV-B values represented biologically effective UV-B radiation according to the generalized curve described by Caldwell (1968). The hydroponic solutions were aerated continuously and changed every three days, whereas the pH of solutions were monitored with a portable pH meter (*Hi-Tech-Instruments*, Shanghai, China) and adjusted daily to 5.2 using 0.1 M HCl. The greenhouse conditions were: a 16-h photoperiod, day/night temperatures of

25/20 °C, a relative humidity of 80 %, and a mean photosynthetic active radiation (PAR) at midday of around 300 µmol m^{-2} s^{-1}.

At each interval, *in vivo* measurements of photosynthetic performance were taken in new and old leaves (see below) from six plants per treatment. Additionally, equivalent leaf samples were harvested and stored at -80 ºC until biochemical analyses. Completely unfolded leaves of second to third node from the apex were considered as new leaves and those from seventh to eighth node as adult leaves.

The experiment was arranged on a split-plot design with three cultivars × two treatments (-UV-B and +UV-B) × two leaf ages × 6 replicates × 5 evaluation times (1, 5, 7, 15, and 40 d) for the physiological and biochemical determinations. The boxes with plants were changed in position every day to minimize any positional effects. In order to study specific effects on anatomical characteristics of new and old leaves of each cultivar under long-term UV-B exposure, samples were taken at the beginning and the end of the experiment.

Changes in leaf anatomy were observed by optical microscopy. Center sections of fresh leaves were quickly fixed in formaldehyde, acetic acid, and ethanol (FAA) for 48 h and preserved in 70 % (v/v) ethanol. Leaf cross-sections of 8 mm were stained with safranin-fast green and mounted in water-glycerol and finally examined with a microscope (*SZX7*, *Olympus*, Tokyo, Japan). The leaf anatomical measurements were analyzed using the *Q-Capture Pro v. 5.0* software. The width of the adaxial epidermis, mesophyll, intercellular cavities, and abaxial epidermis were measured after 40 d of the treatment with or without UV-B radiation (0.12 W m^{-2}).

Chl *a* fluorescence from new and old leaves was measured using a portable pulse-amplitude modulated fluorimeter (*FMS 2*, *Hansatech Instruments*, King's Lynn, UK) to determine the photochemical efficiency of PS II according to Reyes-Díaz *et al.* (2009). The maximum quantum yield characterized by the variable to maximum fluorescence ratio (F_v/F_m) and the effective quantum yield (Φ_{PSII}) were calculated as described by Maxwell and Johnson (2000).

The net photosynthetic rate (P_N) was determined *in vivo* on attached leaves using a portable CO_2 infrared gas analyzer (*Licor LR6400*, *LI-COR,* Lincoln, NE, USA) equipped with a cuvette which controlled irradiance (300 µmol m^{-2} s^{-1}), temperature (20 ºC), humidity (80 %), CO_2 concentration (360 µmol mol^{-1}), and flow rate of 200 cm^3 min^{-1} (for details see Reyes-Díaz *et al.* 2011).

Chls and Cars were extracted with 96 % (v/v) ethanol, measured with a spectrophotometer (*Spectronic UNICAM* model *Genesys 5*, New York, USA) at 663, 646, and 470 nm, and pigments content were calculated according to Lichtenthaler and Welburn (1983).

Total phenols were quantified with the Folin-Ciocalteu method and measured by a spectrophotometer at 765 nm using chlorogenic acid as standard (Slinkard and Singleton 1977). Total anthocyanins were analyzed by the method described by Chang *et al.* (2002). The absorbances of anthocyanin extracts were determined at 530 and 657 nm. Total anthocyanin content was expressed as mg(cyanidin-3-glucoside equivalent) g^{-1}(f.m.).

Radical scavenging activity (RSA) of roots and leaves was assayed using the 2.2-diphenyl-1-picryl-hydrazyl (DPPH) scavenging method as described by Chinnici *et al.* (2004). Absorbance was measured at 515 nm using *Trolox* as standard.

For statistical analyses, reported values correspond to the average of six individual replicates for each cultivar, UV-B treatments, leaf age, and time. All data passed the normality and equal variance Kolmogorov-Smirnov tests. The data were subjected to a three-way analysis of variance (*ANOVA*) (where the factors were UV-B exposure time, cultivar, or leaf age). Tukey's test was used to identify those values with significant differences. Both analyses were performed with the *Sigma Stat v. 2.0* software (*SPSS*, Chicago, IL, USA). Differences between the values were considered significant at $P \leq 0.05$.

Results

The most evident effect of the UV-B exposure on leaf anatomy was around 2.5-fold increase in mesophyll (palisade plus spongy parenchyma) thickness of new Legacy leaves (Table 1, Fig. 2). This increase in mesophyll thickness was lower (1.4-fold) in old leaves. The mesophyll in new Bluegold leaves and in both Brigitta new and Brigitta old leaves exhibited no differences between the UV-B treated and untreated leaves. Old leaves of Bluegold showed a slight increase in mesophyll and total leaf thicknesses. The UV-B radiation resulted in a greater total leaf thickness of both Legacy leaf types (Table 1, Fig. 2).

Practically no differences were found between new and old leaves in the light response curves of P_N under the UV-B radiation of each cultivar (Fig. 3) except that old leaves had somewhat broader ranges during the time course of UV-B exposure than new leaves. However, Legacy showed a tendency to higher P_N after 1 d of the treatment in both leaf types than exhibited at other times (Fig. 3*E*,*F*). At the end of the experiment, Bluegold P_N values for old leaves slightly exceeded the values at other times of the UV-B exposure (Fig. 3*D*). Old leaves from the three cultivars and new leaves from Legacy presented generally higher light saturation points of P_N (around 1000 µmol m^{-2} s^{-1}) than new leaves from Brigitta and Bluegold (around 700 µmol m^{-2} s^{-1}) during the UV-B exposure (Fig. 3).

The maximum quantum yield (F_v/F_m) of PS II in new and old leaves of the studied cultivars practically did not vary under the UV-B exposure staying between 0.7 - 0.8 (not shown), which is in the value range for healthy leaves (Björkman and Demmig 1987). The effective

Table 1. Differences in thickness of leaf tissues and total leaf thickness [µm] of three blueberry cultivars subjected to UV-B radiation simulating a summer daily course (9:00 to 20:00 with maximum of 0.12 Wm^{-2} at midday) for 40 d. Means ± SEs, $n = 6$. Different *lower case letters* indicate statistically significant differences between new and old leaves; different *upper case letters* between treatments, and different *numbers* among cultivars ($P \leq 0.05$).

	Brigitta		Bluegold		Legacy	
	control	UV-B	control	UV-B	control	UV-B
New leaves						
Upper epidermis	18.0±0.7aA	18.3±1.2aA	18.9±1.9aA	19.9±1.2aA	14.6±4.7aA	15.7±2.2aA
Mesophyll	153.7±2.2aA^1	166.8±9.9aA^1	120.6±2.2aA^2	132.9±10.7aA^2	99.9±1.1aA^2	249.5±6.1aB^3
Lower epidermis	9.8±1.2aA	15.3±2.2aB	10.9±3.3aA	11.8±2.6aA	11.7±1.9aA	12.7±2.6aA
Leaf thickness	181.5±1.9aA^1	200.4±10.7aB^1	150.5±6.5aA^2	164.6±11.4aA^2	126.2±6.7aA^3	278.0±7.7aB^3
Old leaves						
Upper epidermis	18.1±2.2aA	23.5±2.5bB^1	16.1±2.4aA	14.9±3.4aA^2	14.6±2.8aA	17.8±1.2aA^2
Mesophyll	213.2±7.0bA^1	201.5±5.7bA^1	122.1±4.5aA^2	155.4±8.9bB^2	177.9±4.6bA^3	242.4± 0.5aB^3
Lower epidermis	12.8±0.1bA	17.8±1.1aB^1	12.2±1.4aA	12.1±2.2aA^2	11.9±0.3aA	12.1± 0.5aB^3
Leaf thickness	244.1±9.1bB^1	242.8±5.0bB^1	150.4±4.4aA^2	182.4±8.4aB^2	204.3±7.2bA^3	272.3±1.8aB^3

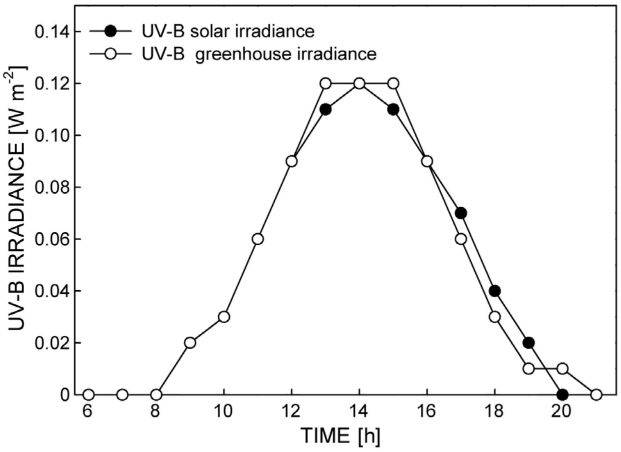

Fig. 1. Typical course of biologically effective UV-B radiation ($W m^{-2}$) under outdoor conditions on a summer day (January) in southern Chile (*closed symbols*) and simulated course in a greenhouse (*open symbols*).

quantum yield of PS II (Φ_{PSII}) increased significantly in old Legacy leaves (Fig. 4*B*) and new Brigitta leaves at the end of the UV-B treatment compared to the control (Fig. 4*A*), whereas in Bluegold there were practically no differences in Φ_{PSII} between the UV-B treated and untreated leaves (Fig. 4*A*,*B*). It also appears that in both the leaf types of Legacy and Bluegold, the Φ_{PSII} was affected by the UV-B radiation on the first day of the treatment showing statistically significant decreases ($P \leq 0.05$) and then a subsequent increase (Fig. 4*A*,*B*). The greatest decreases in Φ_{PSII} were in new Legacy leaves (34.2 %) and old leaves of Bluegold (57.8 %) (Fig. 4*A*,*B*). Statistically significant interactions for Φ_{PSII} between the cultivars and the UV-B exposure were found in new leaves ($P \leq 0.001$). In Brigitta Φ_{PSII} was significantly higher in new than in old leaves ($P \leq 0.05$) at 40 d, whereas Legacy and Bluegold showed no differences between the two leaf types (Fig. 4*A*,*B*).

Decreases in non-photochemical quenching (NPQ) were found during the UV-B exposure in both Bluegold leaf types and in old Brigitta leaves compared with the untreated leaves (Fig. 4*C*,*D*). For NPQ an interaction between the cultivar and the UV-B time exposure were found only for old leaves. For this parameter and for each leaf type, statistically significant differences were found among the cultivars between both Brigitta and Bluegold and Brigitta and Legacy ($P \leq 0.01$), whereas Legacy and Bluegold did not exhibit significant differences. Significant differences were found between new and old Brigitta leaves ($P < 0.05$) when plants were exposed to UV-B from day 1 to day 40 (Fig. 4*C*,*D*).

The total Chl (*a*+*b*) content of the three cultivars varied widely in new and old leaves during the UV-B treatment. At the beginning of the experiment, new Legacy leaves had a significantly lower amount of Chl (*a*+*b*) than Brigitta and Bluegold (Fig. 6*A*). Nonetheless, new Legacy leaves showed the highest Chl (*a*+*b*) content on the 5th day, remaining constant afterwards. Brigitta and Bluegold new leaves had around 60 % less Chl (*a*+*b*) content than Legacy. Old Legacy leaves showed the lowest pigment content especially in the final days of the treatment (Fig. 6*B*). The Chl *a*/*b* ratio of the non-UV-B treated leaves exhibited similar values among cultivars (Fig. 6*C*,*D*). This is in contrast to the UV-B treatment where Bluegold showed the highest Chl *a*/*b* values at both leaf ages and Legacy and Brigitta the lowest (Fig. 6*C*,*D*). The total Cars content of new leaves from the three cultivars decreased significantly in the first five days of the UV-B treatment (Fig. 6*E*). Afterwards, an increase in Cars in Bluegold and Brigitta was shown without reaching the control values at the end of the experiment. By contrast, in Legacy the decline was dramatic showing the lowest Cars content among the cultivars ($P \leq 0.05$). Even if old and new Legacy leaves had the lowest Cars content at the end of the UV-B treatment, a pronounced rise was found in the first days of the UV-B exposure in old leaves ($P \leq 0.05$). Old Brigitta and Bluegold leaves showed a behavior similar to new leaves at the end of the treatment (Fig. 6*E*,*F*). Significant interactions between the UV exposure time and the leaf age were found in the three cultivars for Chl (*a*+*b*), and Chl *a*/*b* ($P \leq 0.05$) but not for Cars.

New leaves from all the three cultivars showed a strong increase in RSA in the first seven days of the UV-B treatment followed by a decrease until the end of the UV-B treatment (Fig. 5*A*). This decrease was higher in Bluegold than in Brigitta (23.6 *vs*. 17.3 %, respectively), whereas Legacy had a 30.2 % decrease (Fig. 5*A*).

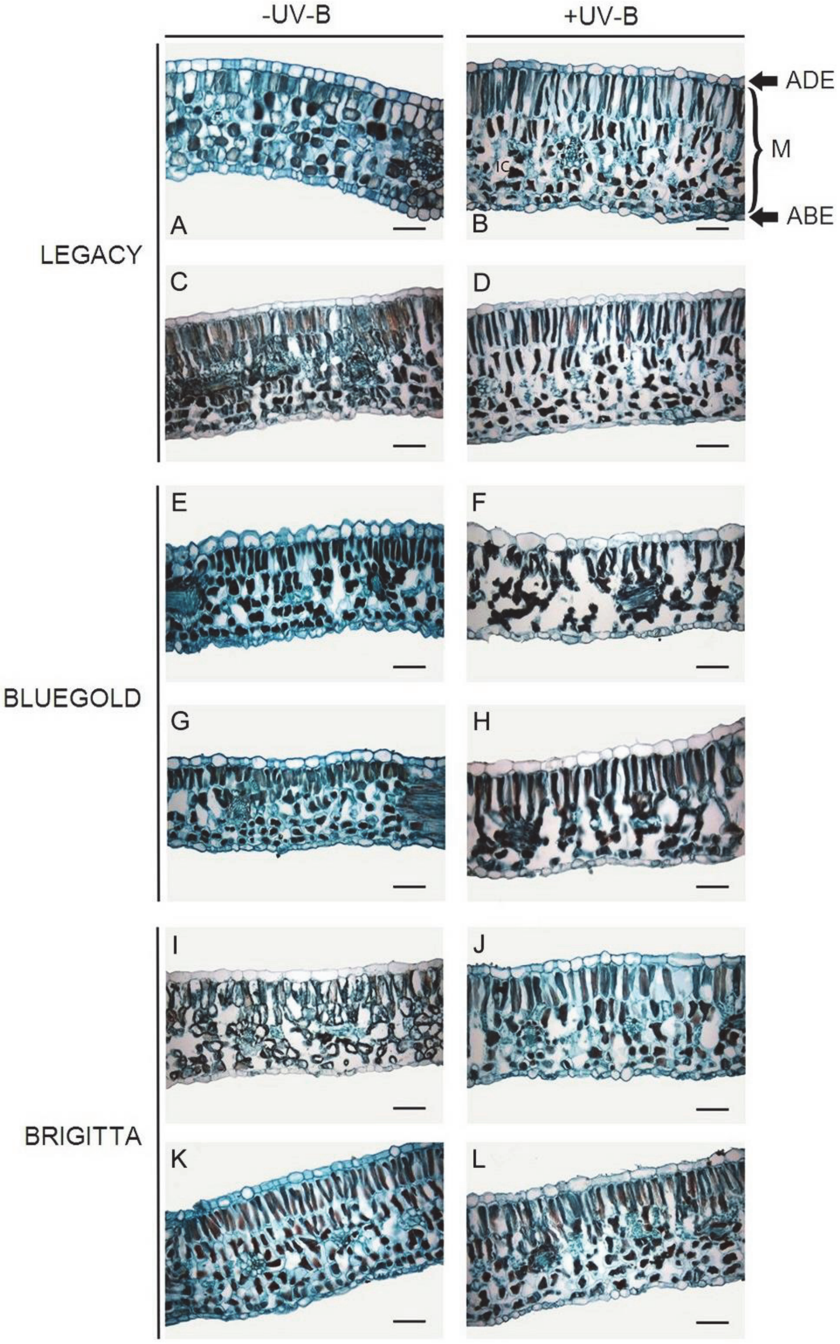

Fig. 2. Leaf anatomical structure of three blueberry cultivars subjected for 40 d to UV-B radiation (simulating a summer daily course) in a greenhouse. *A* and *B*: new Legacy leaves (-UV-B and +UV-B), *C* and *D*: old Legacy leaves (-UV-B and +UV-B); *E* and *F*: new Bluegold leaves (-UV-B and +UV-B), *G* and *H*: old Bluegold leaves (-UV-B and +UV-B); *I* and *J*: new Brigitta leaves (-UV-B and +UV-B), *K* and *L*: old Brigitta leaves (-UV-B and +UV-B). Observations were made on six different cuts from new and old leaves using a *Zeiss* optical microscope. *Scale bars* 50 µm.

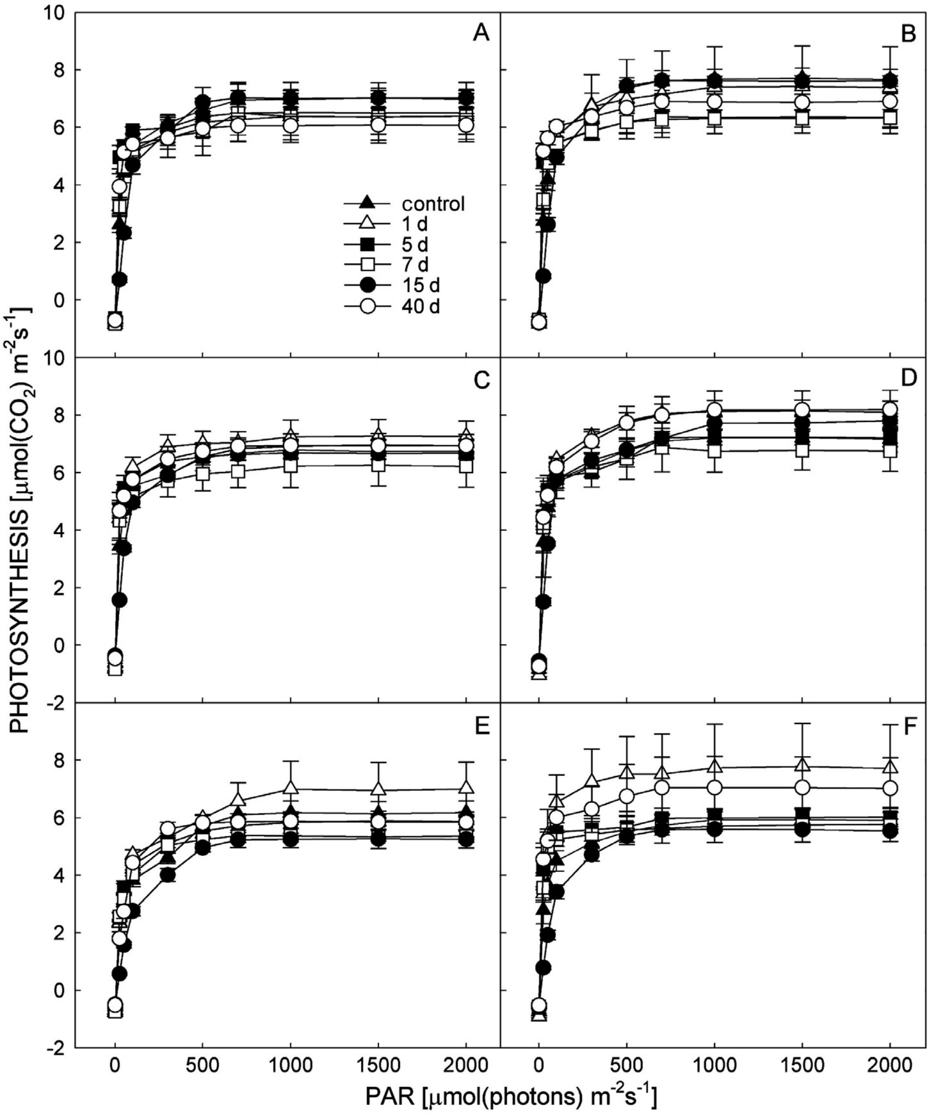

Fig. 3. Light response curves of P_N in new and old leaves of *V. corymbosum* cultivars exposed to UV-B radiation for different times. Brigitta new (*A*) and old (*B*) leaves; Bluegold new (*C*) and old (*D*) leaves; Legacy new (*E*) and old (*F*) leaves. Means ± SEs, *n* = 6.

Furthermore, RSA in old Legacy and Bluegold leaves showed an initial increase and then a strong decrease at day 5, reaching values below those of untreated leaves. These values were maintained until the end of the UV-B exposure. In Brigitta, RSA values practically did not vary in the first days of the treatment, decreasing afterwards until the end of the UV-B treatment (Fig. 5*B*). For RSA a statistically significant interaction between the cultivar and the time of UV-B exposure was found in new ($P = 0.017$) and old ($P = 0.002$) leaves. In relation to this parameter, significant differences between cultivars were found ($P \leq 0.001$) (Fig. 5*A*, *B*).

The total phenol content in new leaves of all the cultivars and in old Brigitta and Bluegold leaves increased in 1 to 7 d of the exposure to UV-B compared to the controls, decreasing afterwards in Bluegold (Fig. 5*C*,*D*). The highest increase in phenol content was found in new Bluegold (3-fold) and Legacy (2.2-fold) leaves and in old Bluegold leaves (2-fold) (Fig. 5*C*,*D*). At the end of the UV-B exposure, the phenol content of new leaves from each cultivar was similar to the controls. The same trend was observed in old Bluegold and Legacy leaves but not in Brigitta, where the phenol content remained unchanged up to day 7. Both leaf types of Brigitta exhibited higher phenol content than those of Bluegold and Legacy at 40 d of the UV-B treatment (Fig. 5*C*,*D*). Significant interactions between the cultivars and the treatments were found for both leaf types ($P \leq 0.001$). The course of total anthocyanin content of new and old leaves from the three cultivars rose in the first seven days of the UV-B treatment, remaining generally constant until the end, with the exception of new Legacy leaves where a sustained increase was observed (Fig. 5*E*,*F*). In Bluegold the anthocyanin content during the UV-B exposure was different depending on the age of leaves. Although there was an increase in this compound in both leaf types until day seven of the UV-B treatment, in old leaves there was a steep decline at the end of the UV-B treatment (Fig. 5*E*,*F*). At the end of the experiment, new Brigitta and Legacy leaves showed a higher anthocyanin content (52 and 34 %, respectively) than old leaves (Fig. 5*E*,*F*). In the three cultivars, a statistically significant interaction among the time of UV-B exposure and the leaf age was shown for anthocyanin content ($P = 0.001$). New leaves of the three cultivars demonstrated statistically significant differences among them ($P \leq 0.001$). For old leaves, significant differences were found between Bluegold and Legacy ($P \leq 0.001$) and Bluegold and Brigitta ($P \leq 0.001$) but not between Legacy and Brigitta ($P = 0.524$).

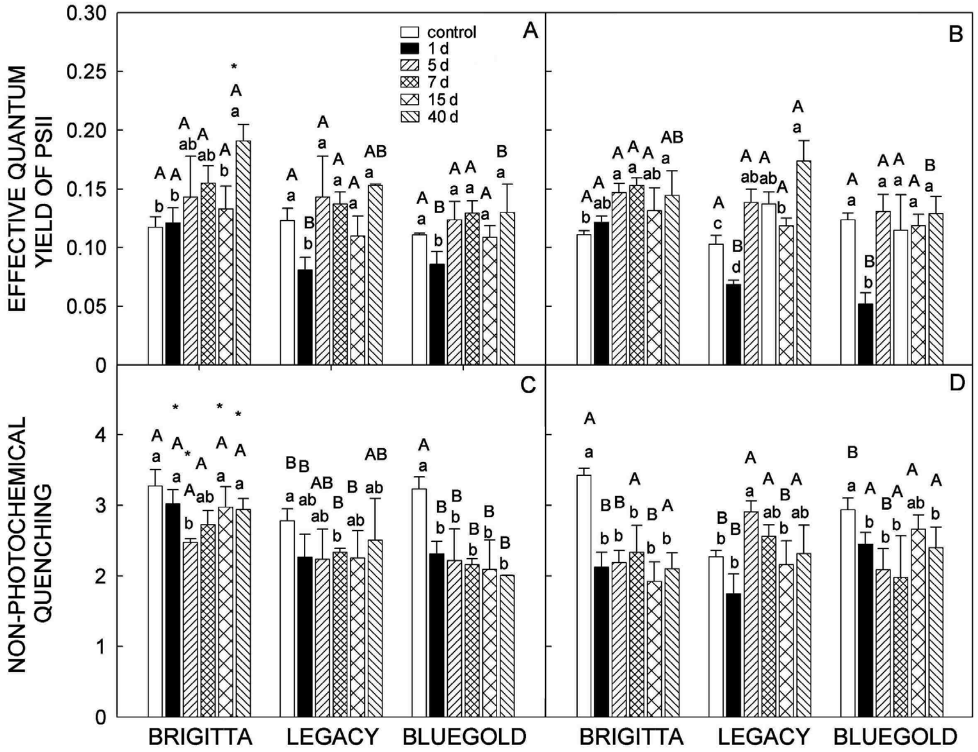

Fig. 4. Chlorophyll *a* fluorescence parameters in leaves from *V. corymbosum* exposed to UV-B radiation for different times. The effective quantum yield of photosystem II in new (*A*) and old (*B*) leaves and non-photochemical quenching in new (*C*) and old (*D*) leaves. Means ± SEs, $n = 6$. Different *upper case letters* indicate significant differences among cultivars, different *lower case letters* among times, and *asterisks* between leaf types ($P \leq 0.05$).

Discussion

In this study, anatomical changes were observed in blueberry plants under long-term UV-B exposure. The total leaf thickness exhibited a statistically significant increase ($P \leq 0.05$) in both leaf types of Legacy followed by Bluegold (Table 1). In Bluegold the increase in leaf thickness was due to an increase in intercellular cavities. This characteristic can be explained by a greater disorganization of the mesophyll layer (Table 1, Fig. 2). This suggests that this cultivar is sensitive to UV-B radiation. In contrast, Brigitta maintained its leaf thickness under the long-term UV-B exposure suggesting a greater resistance to UV-B radiation than the other cultivars. Similar results were observed in Bluegold under a short-term UV-B exposure (Inostroza-Blancheteau *et al.* 2014). It is widely reported that an increased leaf thickness is an important mechanism to avoid UV-B radiation reaching the photosynthetic structures. In a two year experiment under field conditions, Johanson *et al.* (1995) reported that UV-B radiation increases leaf thickness around 6 % in *Vaccinium vitis-idaea*. Otherwise, in *V. myrtillus*, *V. uliginosum*, and *V. vitis-idaea*, there are no significant effects of UV-B on leaf thickness and adaxial and abaxial cuticle thickness after seven years of exposure to UV-B (Semerdjieva *et al.* 2003). However, there has been no clear evidence that another environmental factor could be responsible for this different behavior. Rozema *et al.* (1997) indicated that an increased leaf thickness can be related to the UV-B resistance of plant species. A similar response in *Solanum tuberosum* cv. Désirée was reported by Santos *et al.* (2004). By contrast, Inostroza-Blancheteau *et al.* (2014) reported that a short-term UV-B exposure differentially decreases total leaf thickness in Brigitta and Bluegold genotypes apparently indicating a different defense strategy against increasing doses of UV-B radiation. These controversial results indicate that this change is not a good indicator of UV-B resistance in plants.

Our results demonstrate that the light curves of P_N did not show any statistically significant differences under the UV-B exposure (Fig. 3). This could be explained by an efficient nitrogen nutrition provided by the nutrient solution similarly as occurs in *Phaseolus vulgaris* (Riquelme *et al.* 2007). According to these investigators, a negative effect of UV-B on CO_2 assimilation was found only when accompanied by nitrogen restriction and a concomitant reduction in Rubisco activity. Lau *et al.* (2006) reported that the photosynthetic apparatus in *Zea mays* leaves exposed to UV-B radiation easily tolerates ambient UV-B when the plants are adequately fertilized.

The effective quantum yield of PS II (Φ_{PSII}) in both types of Brigitta leaves was enhanced by UV-B (Fig. 4*A*,*B*) even though their P_N did not vary. Considering that Φ_{PSII} measures the proportion of light absorbed by Chl associated with PS II and used in photochemistry (Maxwell and Johnson 2000), we can speculate that Brigitta is able to conduct a more efficient photochemistry and therefore a better PS II performance under UV-B exposure than the other cultivars. Based on this parameter, Brigitta appears to perform better at coping with UV-B radiation than the other cultivars ($P \leq 0.05$). Nevertheless, new leaves from this cultivar did not maintain the content of Chl (*a*+*b*) under the UV-B exposure, but old leaves maintained their Chl (*a*+*b*). In this sense, our results are consistent with the study performed by Greenberg *et al.* (1997). Despite showing the highest content of Chl (*a*+*b*) in new leaves (Fig. 6*A*) under the UV-B radiation, Legacy did not show a better PS II performance than Brigitta. Pradhan *et al.* (2006) reported a relatively higher loss in Chl *b* content for

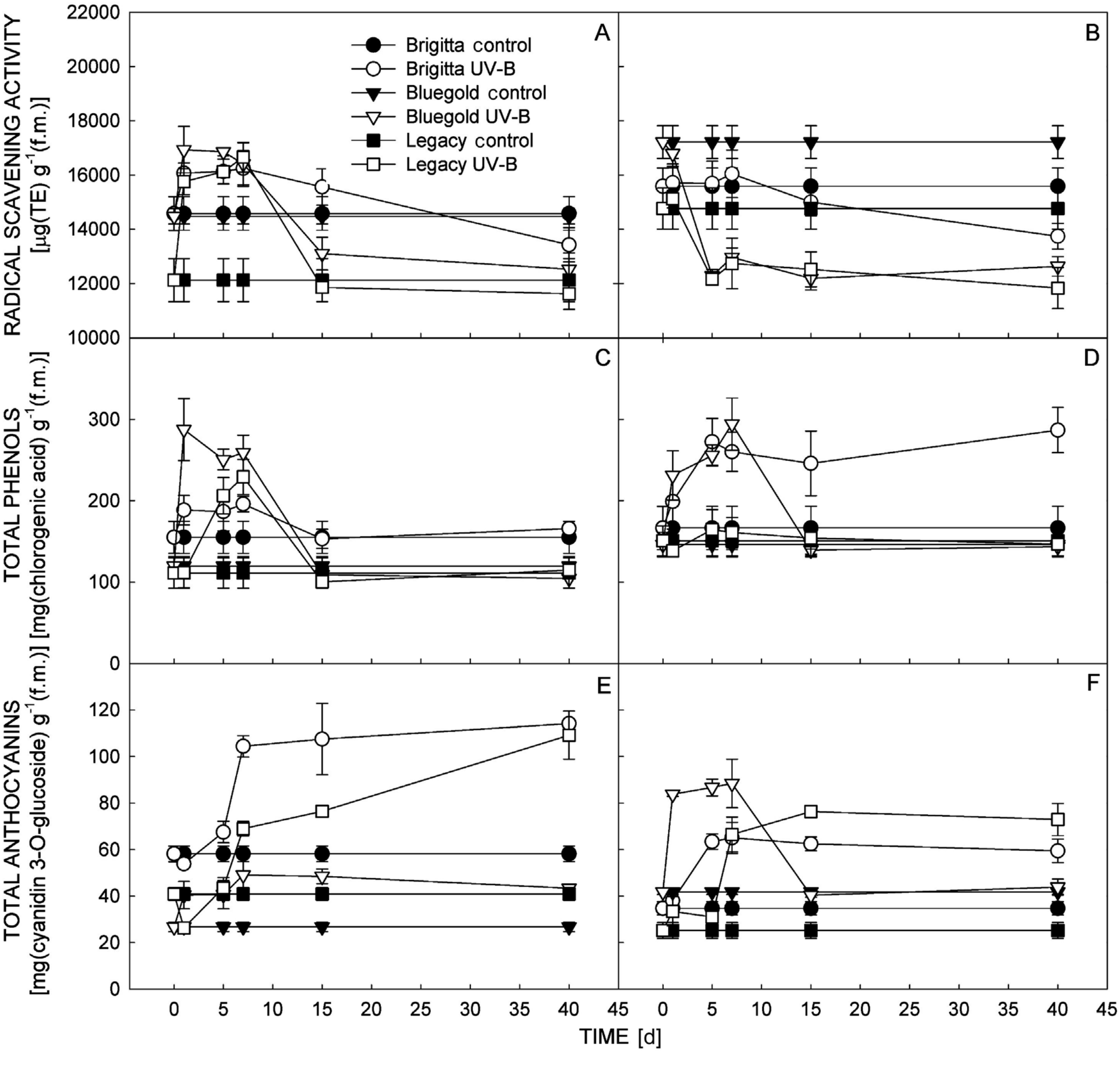

Fig. 5. Changes in leaf radical scavenging capacity (*A*, *B*), phenol content (*C*, *D*), and anthocyanin content (*E*, *F*) in new and old leaves, respectively, of cultivars of *V. corymbosum* exposed to UV-B radiation for different times. Means ± SEs, $n = 6$.

wheat leaves in UV-B treated samples inferred from the Chl *a*/*b* ratio indicative of disorganization in the peripheral antenna system. The loss of Chls is generally attributed to the activation of membrane-bound chlorophyllase because of the thylakoid membrane disorganization (Nayak *et al*. 2003). Brigitta and Legacy had a lower Chl *a*/*b* ratio than Bluegold in new and old leaves exposed to the UV-B radiation at the end of the experiment. This suggests a greater stability of Chl *b* relative to Chl *a* in these two cultivars (Fig. 6*C*,*D*). A Chl content decrease under UV-B exposure was reported in weed species (Wang *et al*. 2007). In relation to leaf Cars, Brigitta and Legacy had a similar behavior in the first days of the UV-B treatment. However, in both Legacy leaf types, Cars content decreased significantly towards the end of the UV-B exposure (Fig. 6*E*,*F*). This behavior in Legacy is consistent with Pradhan *et al.* (2006), who reported that the Cars content in wheat leaves subjected to UV-B increases nearly 100 % during the first three days of the experiment and then drops back to the initial values.

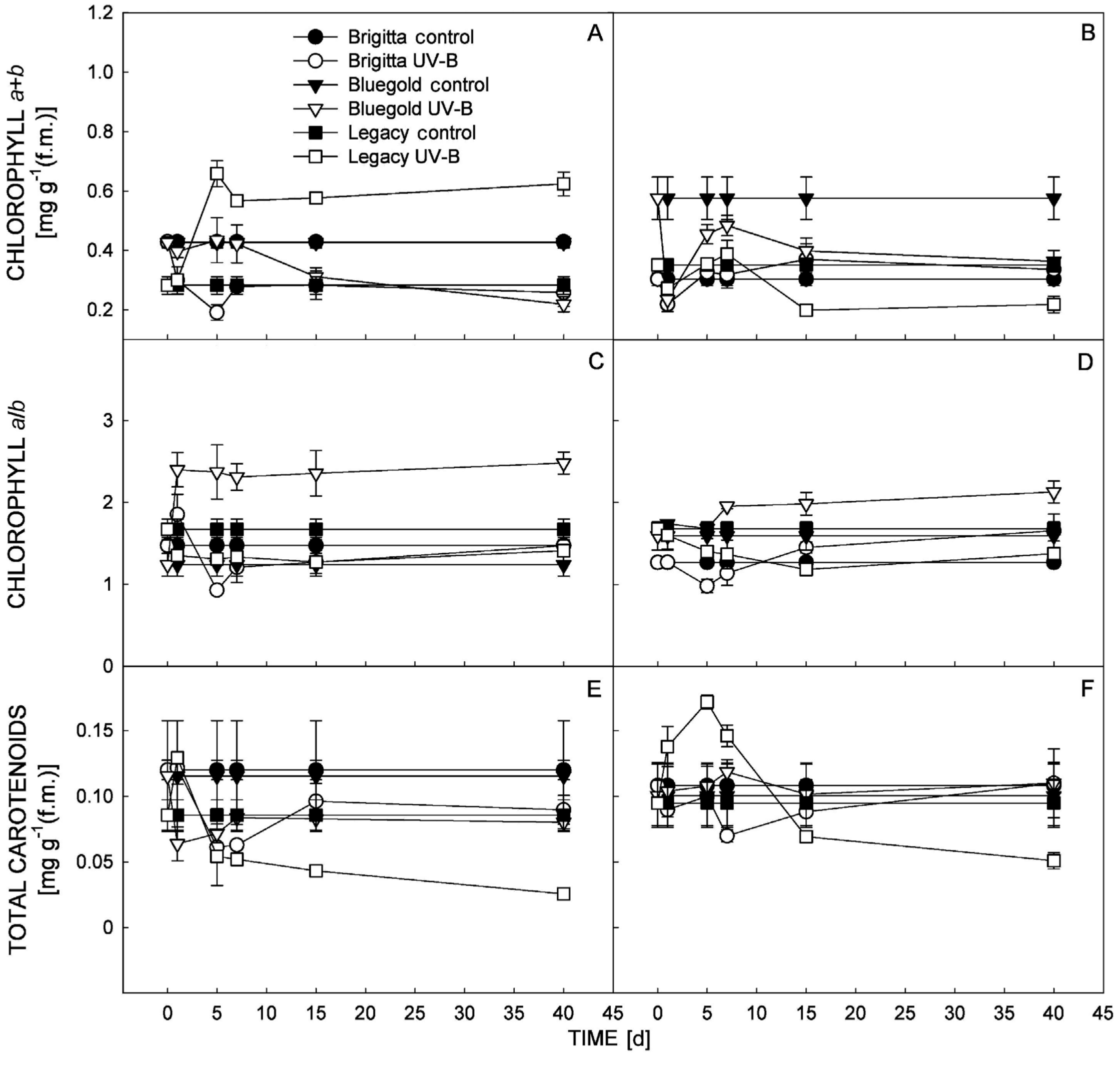

Fig. 6. Photosynthetic pigments in new and old leaves of *V. corymbosum* cultivars exposed to UV-B radiation for different times. Chl (*a*+*b*) content in new and old leaves (*A*, *B*); Chl *a*/*b* of new and old leaves (*C*, *D*), and Cars content of new and old leaves (*E*, *F*). Means ± SEs, *n* = 6.

Non-photochemical quenching (NPQ) gives information about the heat dissipation of excess energy in PS II. It was reported that an increased NPQ is associated with Cars, specifically with the de-epoxidation state of xanthophyll pigments, which is known as effective mechanism against UV-B in some higher plants such as *Pisum sativum* (Bolink *et al.* 2001) and marine picoplankton species *Nannochloropsis gaditana* (Sobrino *et al.* 2005). By contrast, our results revealed an obvious decrease of NPQ, with no relationship to Cars content, during the UV-B exposure in both Bluegold leaf types and old Brigitta leaves (Fig. 3*C*,*D*). Studies performed on *Arabidopsis thaliana*, *Solanum lycopersicum*, and *Cucumis sativus* provided similar results concerning NPQ, and the authors suggested that NPQ decrease could be related to the inhibition of violaxanthin de-epoxidase activity (Moon *et al.* 2011). In *Picea abies* subjected to long-term UV-B radiation, Trošt and Gaberščik (2008) found no clear evidence of NPQ response claiming that unaltered NPQ is related to UV-B tolerance of this species due to the high content of UV-B absorbing compounds.

In addition, it is known that UV-B radiation also increases production of ROS leading to an enhanced oxidative stress in plants. To counteract this effect, plants have developed different protecting pigments and enzymatic and non-enzymatic antioxidants depending on species and cultivar (Shao *et al.* 2008, Takshak *et al.* 2014). Non-enzymatic antioxidants also include phenolic compounds such as flavonoids (Skórska and Szwarc 2007, Merzlyak *et al.* 2008). In our work, new Brigitta leaves showed a strong increase of RSA under the UV-B stress in the initial days of the UV-B treatment and maintained this afterwards (Fig. 5*A*). This increase in RSA could be explained by the high phenol content in new leaves of Brigitta. This response could enable an efficient protection of the photochemical efficiency of PS II under UV-B radiation in this cultivar (Fig. 4). In this respect, Reinfenrath and Müller (2007) reported differential effects of UV-B radiation between new and old leaves. These authors indicated that young leaves have a higher photosynthetic activity offering a more efficient protection and stronger responses to short-term exposure to UV-B treatment in *Nasturtium officinale* and *Sinapis alba*.

Many studies report that phenolic compounds increase in species of *Ericaceae* and *Cucurbitaceae* families by solar radiation (Teklemariam and Blake 2003, Jaakola *et al.* 2004, Eichholz *et al.* 2011). In our case, the three studied cultivars showed an increased phenol content during the first days of the UV-B treatment in both the leaf types and decreasing afterwards (Fig. 5*C*,*D*). However, Brigitta was able to maintain a greater phenol content than the other two cultivars (Fig. 5*C*,*D*). A similar behavior was found in *Hypericum retusum* plants exposed to long-term UV-B radiation (Namli *et al.* 2014). In new Brigitta leaves a strong increase in anthocyanin content was found followed by Legacy compared to the untreated plants, whereas old leaves from the three cultivars increased this compound only in the first days of the treatment. An increase in anthocyanin accumulation due to UV-B radiation was also found in *Lactuca sativa* leaves (Guo *et al.* 2008).

In conclusion, our study revealed no significant differences between the leaf types subjected to the long-term UV-B exposure. However, the most important differences were found among the cultivars. In Bluegold the UV-B radiation negatively affected leaf thickness and inner organization, whereas Brigitta maintained this trait and its internal cell organization. These results are also supported by an improved PS II performance in Brigitta since this cultivar was able to conduct a more efficient photochemistry under the UV-B radiation than the other cultivars. In addition, Brigitta was able to maintain a greater phenol and anthocyanin content than the other cultivars. Therefore, our results suggest that Brigitta has a greatest resistance to UV-B radiation. Further molecular analyses will contribute to an increase of the knowledge of the impact of long-term UV-B exposure on this woody plant.

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