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Response of selected antioxidants and pigments in tissues of *Rosa hybrida* and *Fuchsia hybrida* to supplemental UV-A exposure

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The effect of supplemental UV-A (320–400 nm) radiation on tissue absorption at 355 nm, levels of various antioxidants (ascorbate, glutathione, carotenoids and flavonoids) and of antioxidant scavenging capacity were investigated with leaves and petals of *Rosa hybrida*, cv. Honesty and with leaves, petals and sepals of *Fuchsia hybrida*, cv. Dollarprinzessin. Supplemental UV-A did not result in visible changes in plant morphology of either species. In leaves it induced small increases in levels of chlorophylls *a* and *b*, the carotenoids antheraxanthin, lutein and β-carotene, and high increases in the flavonols quercetin and kaempferol. Petals hardly responded, while the coloured sepals of fuchsia showed an increase in quercetin derivatives. HPLC of unhydrolysed flavonoids showed that individual quercetin derivatives in leaves of both species and kaempferol derivatives in rose leaves increased 2-fold. Some kaempferol derivatives in fuchsia leaves were more than 2-fold enhanced or were newly induced by supplemental UV-A. Increases in L-ascorbic acid levels in fuchsia leaves, and decreases in rose leaves as result of supplemental UV-A were observed, but differences appeared statistically not significant, while L-ascorbate levels remained unchanged in the other tissues investigated. Anthocyanins and reduced glutathione levels were unaffected in all tissues. The combined UV-A induced increases in concentrations of these antioxidant species, did not lead to significant increases in antioxidant capacity of tissues, measured as Trolox equivalents in 50%-ethanol extracts. Light absorption at 355 nm of leaf extracts was significantly increased upon UV-A exposure. Our results indicate that the major protection towards UV-A exposure, in particular in the leaves, will originate from absorption of irradiation, and not from scavenging reactive oxygen species.

Introduction

The increasing exposure of plants and animals to UV radiation by air pollution-induced ozone depletion has led to physiological research in the protective mechanisms by which plants react to this anthropogenic source of stress. Since UV-B (280–320 nm) is the wavelength range affected by changes in stratospheric ozone depletion, most research has focused on UV-B. In addition to DNA damage, UV-B exposure has been reported to result in growth inhibition and in increased levels of compounds, mainly flavonoids, which have the capacity to not only shield the tissue by UV absorption, but also to scavenge the reactive oxygen species generated (Day and Demchik 1996, Cuadra et al. 1997, Burchard et al. 2000, Harborne and Williams 2000). In general, plant response to UV-B is highly variable, and is dependent on environmental conditions and plant source, i.e. species, cultivar (Rao et al. 1995, Ambasht and Agrawal 1998, Searles et al. 2001).

The response of plants to UV-A (320–400 nm) is far less intensively investigated, but seems much more uniform

Abbreviations – ABTS, 2,2′-Azinobis-(3-ethylbenzothiazoline-6-sulphonic acid).

than the response to UV-B. In contrast to UV-B, UV-A is not usually regarded as a potentially damaging stress factor, although Turcsanyi and Vass (2000) reported a UV-A induced impairment of photosystem II. Supplementation of photosynthetically active radiation (PAR, 400–700 nm) with UV-A may enhance the levels of pigments such as chlorophyll, carotenoids, and UV-absorbing compounds, including antioxidants, and has been reported to stimulate growth in general (Foyer et al. 1994, Ehling-Schultz et al. 1997, Doehler 1998, Jahnke 1999, Lingakumar et al. 1999, Shiozaki et al. 1999). UV-A irradiation is also reported to mitigate the damaging effect of UV-B (Flint and Caldwell 1996). These positive effects of UV-A offer the possibility to use low levels of UV-A to increase the concentration of antioxidant compounds in plant species which are believed to also have a health-promoting activity when present in human and animal food sources (Steinmetz and Potter 1996). For ornamental plants UV-A and UV-B-induced changes of coloured pigments and thus of tissue coloration, may lead to a higher commercial value (Hoffman 1999). Also in red-leafed lettuce the anthocyanin levels increased upon UV-A irradiation (Voipio and Autio 1995). Despite the relatively large number of reports on UV-induced accumulation of antioxidant compounds, its effect on the antioxidant scavenging capacity of exposed tissues has, to our knowledge, hitherto not been described.

Given our interest in ornamental crops, we studied the effects of UV-A exposure of leaves and petals of *Rosa hybrida* and *Fuchsia hybrida* with special emphasis on the levels of L-ascorbic acid, glutathione, flavonoids, anthocyanins and carotenoids, compounds which may also function as antioxidants. In fuchsia sepals were also analysed since their pink colour, which has a strong impact on ornamental value, suggests a high anthocyanin content. We also studied the effect of UV-A irradiation on the photosynthetic pigments chlorophylls *a* and *b*. These compounds are involved in either plant vitality, scavenging or generation of oxygen stress and/or may absorb UV-A light. In addition, we investigated whether UV-A exposure may lead to tolerance towards photo-oxidative stress via alteration of the capacity of the tissue to scavenge reactive oxygen species or via absorption of the UV radiation itself.

Materials and methods

Plant material

Plants of *Fuchsia hybrida* cv. Dollarprinzessin, and *Rosa hybrida* cv. Honesty, were grown from cuttings. After one growth cycle of about 6 weeks all aboveground tissues were removed before the start of the experiments. Subsequently, plants were grown for 6–7 weeks at a photoperiod of 12 h at a constant temperature of 20°C and a relative humidity of 75%. Photosynthetically active radiation (400–700 nm) was provided by Philips HPI-T lamps at 227 $\mu mol\, m^{-2}\, s^{-1}$, in the absence (control) or presence of supplementary UV-A radiation at 15.9 $\mu mol\, m^{-2}\, s^{-1}$ using Philips Black light TL-tubes ($\lambda_{max} = 348$ nm, range 320–400 nm). UV-A radiation from HP-I lamps in control treatments was reduced to 2.7 $\mu mol\, m^{-2}\, s^{-1}$, restricted to the 370–400 nm range, by transparent glass plates. Photon fluxes were measured using a Li-Cor Li 1800 spectroradiometer (Li-Cor Inc., Lincoln, NE, USA).

Detached petals and young, unshaded and unfolded leaves of both species (and from fuchsia also the intensely pink coloured sepals) were harvested, immediately frozen in liquid nitrogen, homogenized with a mortar and pestle and stored at −80°C until further analysis.

Extraction

For all analyses 4 aliquots of 200 mg ± 5 mg FW from independent tissue samples were weighed out while the sample was kept under liquid nitrogen. If not mentioned otherwise, the aliquots were extracted with 2.0 ml solvent. Further homogenization was achieved by sonication for 15 min at room temperature. Final results were corrected for the weighed amounts.

Dry matter

Dry matter percentage was determined after overnight lyophilization of plant tissues, followed by heating for 24 h at 105°C. No further weight loss was observed upon longer heat treatment.

Carotenoids and chlorophylls

For analyses of carotenoids and chlorophylls tissue was extracted in 1.8 ml 100% acetone, containing 0.1% *t*-butylated hydroxytoluene (BHT) and 20 mg calcium carbonate (Gilmore and Yamamoto 1991) and washed with 2 ml of the same solution. Both supernatants were combined and cleared over 0.2 µm-filters. Aliquots of 20 µl from the combined extract were analysed using a Dionex (Bavel, the Netherlands) HPLC, equipped with an ODS-1 5 µm non-endcapped C_{18}-pre-column (7.5 × 4.6 mm) and analytical column (250 × 4 mm) (Alltech, Deerfield, IL, USA), a Dionex AS50 autosampler and a Dionex GP50 Gradient pump. Pigments were separated using a solvent programme modified from Gilmore and Yamamoto (1991) at 1 ml min^{-1} starting isocratically for 10 min with acetonitrile-methanol-0.1 M Tris HCl pH 8.0 (72:8:3, by vol. + BHT), followed by a linear gradient to methanol-hexane (4:1, v/v + BHT) at 15 min, an isocratic elution in this solvent until 25 min, a return to the initial solvent at 30 min and column equilibration for 5 min until the next analysis. A Gynotek UVD170S (Germering, Germany) was used to quantitatively detect the eluting compounds. All carotenoids and chlorophyll *b* were separated in this system, although lutein overlapped with chlorophyll *a*, as shown by detection

at 440 nm. Chlorophyll *a* did not absorb at 450 nm. Therefore, carotenoids were quantified after HPLC separation at 450 nm and chlorophylls *a* and *b* were quantified in the crude acetone extracts according to Inskeep and Bloom (1985) by absorption at 645 and 663 nm. Plotting HPLC- versus A_{645}/A_{663}-measurements for chlorophyll *b* showed a linear regression with a slope of 1.09 and $R^2 = 0.92$. Carotenoids were measured using authentic standards for calibration, when available. Specific extinction coefficients of violaxanthin, antheraxanthin and neoxanthin were considered to be identical to that of zeaxanthin (Gilmore and Yamamoto 1991).

Antioxidant capacity and tissue light-absorption

For analyses of antioxidant capacity, of absorption at 355 nm, as a measure of total flavonols (Gislefoss et al. 1992), and of absorbtion at 535 nm, as a measure of total anthocyanin content (Voipio and Autio 1995), tissues were extracted with 1.8 ml of 50% ethanol, containing 0.1% citric acid. The residues were washed with 2.0 ml of the same solvent. Both supernatants were combined and cleared over 0.2 µm-filters. For A_{535}-assays 5 µl of 6 M HCl were added. A_{355}- and A_{535}- values were measured with a Lambda 10 UV-Vis spectrophotometer (Perkin Elmer, Nieuwerkerk a/d IJsel, the Netherlands).

Antioxidant capacity of ethanol/citric acid-extracts was measured according to Re et al. (1999), modified for 96-well plate analyses. Briefly, 10 µl-aliquots of 50-fold diluted samples were mixed with 90 µl of a 2-mM $ABTS^{\bullet+}$-solution [ABTS: 2,2′-Azinobis-(3-ethylbenzothiazoline-6-sulphonic acid)] in phosphate buffered saline pH 7.4, generated by adding $K_2S_2O_8$. After exactly 1 min rates of decrease in $ABTS^{\bullet+}$-radicals were measured as absorption at 415 nm. Antioxidant capacity of extracts was expressed as Trolox-equivalents by comparison with sequentially diluted Trolox (0.5–2.5 mM) in 50% ethanol/0.1% citric acid.

HPLC of flavonoids and anthocyanins

Aliquots of 10 µl of the ethanol-citric acid extracts were analysed using an HPLC system, equipped with a 5-µm Novapack C_{18} column (160 × 3.9 mm, Waters), kept at 30°C, as the stationary phase, a Waters 600E Multisolvent Delivery System (Waters Chromatography, Etten-Leur, the Netherlands) and a Promis autoinjector (Separations Analytical Instruments). Pigments were separated by elution at a flow rate of 1.0 ml min^{-1} using a gradient of acetonitrile, containing 0.1% TFA, increasing linearly from 5 to 25% in 30 min, then to 30% in 5 min, and to 50% in 2 min, followed by isocratic elution for 3 min at 50% acetonitrile. Finally, the acetonitrile concentration was brought to the initial concentration in 5 min and the column was equilibrated for 6 min until the next analysis. A photodiode array detector (Waters Chromatography, type 996) was used to record spectra which were processed by Millenium32 software from Waters Chromatography. Flavonols and anthocyanins were quantitatively detected at 355 and 535 nm, respectively.

Since HPLC patterns of flavonols are usually very complex, showing many different derivatives for which standards are not available, quantification is often limited to the aglycones. For measurement of flavonol aglycones, the ethanol extracts were acidified by addition of 0.32 ml of 3 M HCl, followed by hydrolysis at 90°C for 1 h with a reported yield of 85% for quercetin derivatives (Hertog et al. 1992). After dilution with methanol (1:1), subsequent sonication for 10 min and filtration over 0.2 µm-membranes, 10 µl-aliquots were analysed by HPLC as described above for flavonoids and anthocyanins with the exception that isocratic elution was performed for 14 min with 25% acetonitrile in 0.1% TFA at a flow rate of 0.9 ml min^{-1}. Aglycones were quantified by absorption measurement at 370 nm using quercetin and kaempferol as external standards (0–50 µg ml^{-1}).

L-Ascorbic acid

Tissues were extracted with 2 ml of 5% HPO_3. *L*-Ascorbic acid in the extracts was analysed by the HPLC system described for flavonoids and anthocyanins using a 5-µm PRO C_{18}-column (250 × 4.6 mm) at 30°C as the stationary phase. Elution was performed with 50 mM phosphate buffer pH 4.4 at a flow rate of 0.5 ml min^{-1} for 15 min. Ascorbic acid was detected at 260 nm. The column was washed with 50% acetonitrile in 50 mM phosphate pH 4.4 for 5 min and equilibrated in the original solvent for 10 min before the next analysis.

Low-molecular weight thiols

Low-molecular weight thiols, often predominantly reduced glutathione (GSH), were extracted and quantified as described by De Vos et al. (1994). Shortly, tissue samples were lyophilized overnight before extraction in 2 ml of 200 mM sulfosalicylic acid, containing 5 mM diethylenetriamine pentaacetic acid. Low-molecular weight thiols were quantified as GSH equivalents at 412 nm, assuming a molar extinction coefficient of 13 600.

Statistical analysis

Results were statistically analysed using a one-way ANOVA, followed by a Student's *t*-test for comparison of means. Inspection of residuals after ANOVA suggested that, given the low amount of replications (n = 4), the normal distribution was not violated. A normal distribution may be anticipated due to the nature of the data, being dry matter proportions and concentrations of chemical compounds.

Results

Morphological parameters

Plant architecture, including plant height, which was about 10 cm for *Fuchsia hybrida* and 60 cm for *Rosa*

Table 1. Effect of UV-A exposure, during the entire photoperiod of 12 h, on dry matter content and levels of L-Ascorbate and low-molecular weight (LMW) thiols, expressed as GSH equivalents, in tissues of *Rosa hybrida* cv. Honesty and *Fuchsia hybrida* cv. Dollarprinzessin. ND = not detectable; detection limit for GSH equivalents = 2 μg g FW^{-1}. Data represent the mean of four samples ± SD. Values for each pair-wise comparison of control versus UV-A followed by the same letter were not significantly different using a Student's *t*-test ($P < 0.05$).

	Dry matter		L-Ascorbate		LMW thiols	
	Control	UV-A	Control	UV-A	Control	UV-A
	% of FW		μg g FW^{-1}		GSH equiv. mg FW^{-1}	
Rosa hybrida						
Leaves	27.6 ± 1.4a	27.1 ± 1.2a	298 ± 55a	259 ± 92a	42 ± 18a	32 ± 3a
Petals	15.5 ± 1.2a	14.3 ± 0.5a	40 ± 3a	41 ± 9a	23 ± 5a	20 ± 3a
Fuchsia hybrida						
Leaves	14.9 ± 0.3a	15.1 ± 0.3a	63 ± 18a	108 ± 17b	74 ± 4a	76 ± 5a
Petals	7.1 ± 0.2a	6.9 ± 0.5a	7 ± 7a	7 ± 5a	ND	ND
Sepals	5.3 ± 0.4a	5.3 ± 0.4a	23 ± 2a	25 ± 9a	7 ± 2a	6 ± 2a

hybrida, did not change with supplemental UV-A irradiation. Dry matter proportion of the tissues investigated was also unaffected by exposure to UV-A (Table 1). The only visible difference as a result of UV-A exposure was a slightly more intense pink coloration of the petal margin in rose flowers (not shown).

Water-soluble antioxidants

From the water-soluble antioxidants L-ascorbate significantly increased in fuchsia leaves upon UV-A exposure ($P < 0.05$), while in rose leaves the levels of ascorbate and low-molecular weight thiols decreased slightly, but differences were not significant (Table 1). For sepals of fuchsia and petals of both species the levels of both antioxidants were unaffected by UV-A irradiation. Low-molecular weight thiols were also unaffected in fuchsia leaves and below the detection level in fuchsia petals.

Chlorophyll and carotenoids

Photosynthetic pigments were only detectable in leaves, and not in petals or sepals (detection limit was 1 μg g^{-1} FW). Both chlorophylls *a* and *b* in leaves increased by about 25% in rose ($P < 0.05$) and 10% for fuchsia (not significant; $P = 0.06$ for chlorophyll *a* and $P = 0.13$ for chlorophyll *b*) upon UV-A exposure (Table 2). The effect of UV-A supplementation on carotenoids was variable, ranging from no increase for zeaxanthin in rose leaves to a 2-fold rise for antheraxanthin in rose. Only the increase in antheraxanthin was significant in both species ($P < 0.01$ for rose; $P < 0.05$ for fuchsia).

Flavonoids

Kaempferol and quercetin derivatives were the only flavonol types observed in acid hydrolysates of ethanol extracts from leaves, petals and sepals of rose and fuchsia. These two flavonol types were quantified as their aglycones. A 4-fold increase in kaempferol derivatives was observed upon UV-A exposure for fuchsia leaves ($P < 0.001$) where the rise was 2-fold for quercetin conjugates ($P < 0.001$, Table 3). Rose leaves showed a 2-fold increase for both quercetin ($P < 0.01$) and kaempferol derivatives ($P < 0.01$). In rose petals a decrease was observed which was significant for kaempferol ($P < 0.05$) but not for quercetin aglycones ($P = 0.08$), while fuchsia sepals showed a 50% increase, which was not significant ($P = 0.12$), for only quercetin flavonoids. Flavonol aglycones in fuchsia petals and kaempferol derivatives in sepals were unaffected by UV-A.

HPLC of non-hydrolysed extracts showed no free kaempferol or quercetin in any of the tissues investigated

Table 2. Effect of UV-A exposure on concentrations of chlorophylls, xanthophyll cycle pigments and other carotenoids in leaves of *Rosa hybrida* and *Fuchsia hybrida*. For further details see Table 1.

	Rosa hybrida		*Fuchsia hybrida*	
	Control	UV-A	Control	UV-A
	μg g FW^{-1}			
Chlorophylls				
Chlorophyll *a*	1650 ± 261a	2104 ± 70b	2080 ± 164a	2235 ± 64a
Chlorophyll *b*	571 ± 88a	714 ± 20b	616 ± 48a	677 ± 20a
Xanthophyll cycle carotenoids				
Violaxanthin	34.7 ± 4.3a	36.7 ± 2.5a	39.7 ± 4.2a	40.7 ± 2.9a
Anterheraxanthin	8.8 ± 2.0a	14.5 ± 1.1b	14.7 ± 1.6a	18.1 ± 1.6b
Zeaxanthin	1.5 ± 0.2a	1.5 ± 0.4a	3.7 ± 1.6a	5.1 ± 1.6a
Other carotenoids				
Neoxanthin	13.3 ± 2.9a	14.1 ± 1.8a	12.5 ± 1.1a	13.4 ± 0.9a
Lutein	108 ± 20a	135 ± 18a	146 ± 30a	151 ± 8a
β-Carotene	87 ± 10a	103 ± 12a	116 ± 6a	122 ± 17a

Table 3. Effect of UV-A exposure on levels of kaempferol and quercetin flavonoids in tissues of *Rosa hybrida* and *Fuchsia hybrida*. Flavonoids were measured as their aglycones after extraction and acid hydrolysis. For further details see Table 1.

	Kaempferol		Quercetin	
	Control	UV-A	Control	UV-A
	$\mu g\ g\ FW^{-1}$			
Rosa hybrida				
Leaves	29 ± 5a	56 ± 8b	686 ± 57a	1211 ± 136b
Petals	4196 ± 735a	3161 ± 353b	234 ± 85a	142 ± 16a
Fuchsia hybrida				
Leaves	152 ± 11a	627 ± 32b	585 ± 93a	1281 ± 107b
Petals	37.0 ± 3.0a	41.0 ± 3.6a	1249 ± 175a	1287 ± 78a
Sepals	15.6 ± 2.5a	15.6 ± 1.7a	183 ± 39a	260 ± 76a

(data not shown). HPLC patterns of rose leaf extracts showed two major peaks absorbing at 355 nm. Based on their absorption spectra one corresponds to ellagic acid, while the other was a quercetin glycoside. Furthermore, a number of small peaks corresponding to quercetin and kaempferol glycosides were observed as judged from their absorption spectra. Upon UV-A exposure rose leaves showed an increase, which was similar for all flavonol derivatives. In fuchsia leaves the patterns of kaempferol and quercetin glycosides showed several compounds (Fig. 1). As a result of UV-A supplementation all quercetin glycosides increased about 2-fold, but the kaempferol glycoside levels showed a differential rise: some increased 2-fold which is similar to the increase observed for quercetin glycosides, while for others the increase was much more pronounced (Fig. 1, arrows).

Antioxidant capacity of tissues

In leaves and petals of rose a decrease was observed for antioxidant capacity upon UV-A irradiation, although the differences were not significant (Table 4). Values for antioxidant capacity in fuchsia tissues were not significantly affected, but as in rose they also showed a tendency to decrease when exposed to UV-A.

Tissue light absorption and HPLC of anthocyanins

A significantly higher absorption at 355 nm was observed for ethanol extracts from leaves of rose ($P < 0.01$) and fuchsia ($P < 0.001$) and from fuchsia sepals ($P < 0.01$), while rose petals showed lower A_{355}-values when irradiated with UV-A ($P < 0.05$, Table 4). Absorption at 355 nm of fuchsia petals did not change.

Light absorption of acidified ethanol extracts at 535 nm did not increase significantly in any of the tissues investigated (Table 4). As expected from their more intense coloration the A_{535}-values for fuchsia tissues were much higher than those for rose.

HPLC analysis of these extracts showed only one major (>90% of total A_{535}) compound which absorbed

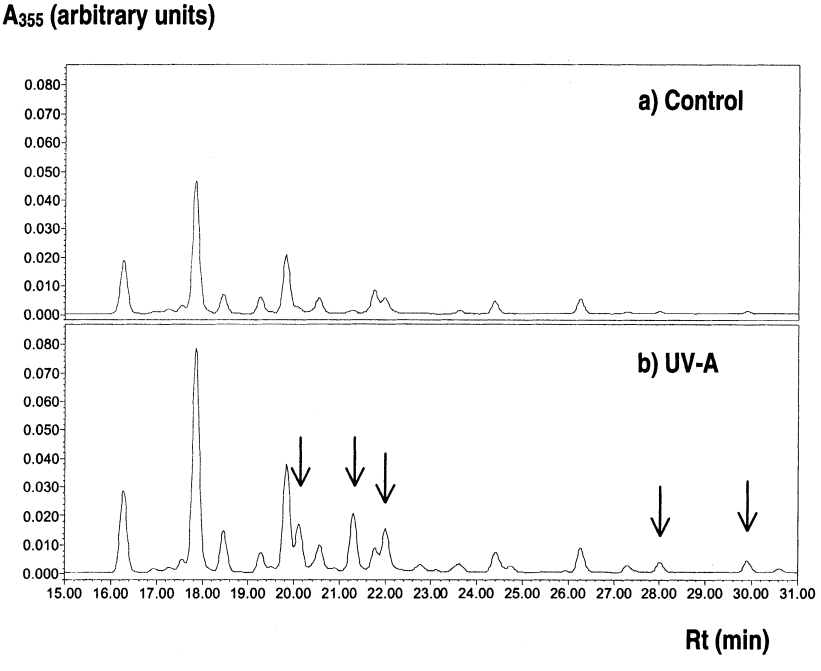

Fig. 1. HPLC-pattern of non-hydrolysed ethanol-extracts of leaves of *Fuchsia hybrida*, cv. Dollarprinzessin: a) exposed to white light (Control) and b) exposed to white light supplemented with UV-A. Arrows indicate kaempferol derivatives, that are strongly enhanced by UV-A irradiation. No significant peaks were observed before 15 min and beyond 30 min.

Table 4. Effect of UV-A exposure on antioxidant capacity, expressed as Trolox equivalents, and A_{355}- and A_{535}-values in 50% ethanol-extracts from tissues of *Rosa hybrida* and *Fuchsia hybrida*. For further details see Table 1.

	Antioxidant capacity		Absorption at 355 nm		Absorption at 535 nm	
	Control	UV-A	Control	UV-A	Control	UV-A
	μmol Trolox g FW^{-1}		A_{355} g FW^{-1} ml^{-1} cm^{-1}		A_{535} g FW^{-1} ml^{-1} cm^{-1}	
Rosa hybrida						
Leaves	261 ± 55a	185 ± 20a	105 ± 5a	123 ± 8b	1.9 ± 0.7a	1.9 ± 1.2a
Petals	177 ± 35a	156 ± 20a	275 ± 26a	227 ± 6b	3.4 ± 0.9a	2.7 ± 0.5a
Fuchsia hybrida						
Leaves	266 ± 53a	253 ± 24a	80 ± 6a	140 ± 6b	10.2 ± 1.0a	11.4 ± 0.7a
Petals	168 ± 28a	164 ± 30a	109 ± 14a	108 ± 3a	125 ± 9a	130 ± 9a
Sepals	119 ± 18a	107 ± 20a	19 ± 1a	27 ± 3b	29 ± 6a	36 ± 8a

at this wavelength for all tissues investigated (not shown). On the basis of its absorption spectrum the anthocyanin in rose petals was tentatively identified as a cyanidin derivative. In fuchsia leaves and sepals a different cyanidin was observed, which was identical for both tissues. In fuchsia sepals a pelargonidin derivative was found. The anthocyanin level in rose leaves was too low to provide sufficient spectral information for component identification.

Discussion

The results in this study show that supplemental UV-A irradiation of fuchsia and rose tissues leads to increases in the levels of some antioxidant pigments, especially of flavonoids in leaves (Table 3, Fig. 1) and less so for antioxidants in petals and sepals. These changes did not lead to increased overall antioxidant capacity, but rather enhanced the shielding potential in leaves.

Most studies on effects of exposure to short wavelength irradiation have focused on the more damaging UV-B radiation, given its increase at the earth's surface as a result of stratospheric ozone depletion. In numerous studies effects of UV-B have been reported to result in growth inhibition and increases in antioxidant pigments and other antioxidants (Singh 1996, Ambasht and Agrawal 1998, Lingakumar et al. 1999, Pal and Sengupta 2000). In almost all these studies protection against UV was afforded by the formation of UV-shielding compounds, e.g. flavonoids (Day and Demchik 1996, Cuadra et al. 1997, Burchard et al. 2000, Harborne and Williams 2000, Robakowski and Modzynski 2000). Most of these effects were highly variable among plant species and even among cultivars. In a statistical meta-analysis of plant studies Searles et al. (2001) concluded that in plants only the formation of UV-shielding compounds can be generalized.

In the present study UV-A supplementation had no visible effect on growth parameters such as plant height and dry matter content (Table 1). Hence, the observed increases in the levels of the antioxidants investigated cannot be due to lower water content of the tissues examined. A second general observation is that leaves react more consistently and in a more pronounced way to UV-A than non-photosynthetic tissues, e.g. petals and sepals, with regard to increases in antioxidant compounds. A logical explanation for this higher responsiveness of leaves is the requirement for the protection of photosynthetic pigments, which are particularly sensitive to damaging effects of UV (Clendennen et al. 1996, Goetz et al. 1999).

In leaves L-ascorbate levels increased in fuchsia, but decreased in rose, while low-molecular weight thiols were unaffected by UV-A (Table 1). The variability in response of these antioxidants is consistent with the highly differential effects on their levels upon UV-B exposure reported among various plant species and tissues (Kalbin et al. 1997, Ambasht and Agrawal 1998, Baumbusch et al. 1998, Laakso et al. 2001). This variability is probably related to the differential environmental conditions and genetic potential, in which each plant species and tissue has to optimize its response.

Chlorophylls and carotenoids also were differentially affected by UV-B irradiation among species and tissues (Doehler and Lohmann 1995, Rao et al. 1995, Doehler 1998, Correia et al. 1999, Robakowski and Laitat 1999, Robakowski 1999, Robakowski and Modzynski 2000, Searles et al. 2001). With regard to UV-A exposure, the effects are much more consistent and indicate an enhancing effect on the levels of these pigments as was observed in our study with fuchsia and rose leaves and also in other studies (Doehler and Lohmann 1995, Lingakumar and Kulandaivelu 1998, Jahnke 1999, Altamirano et al. 2000). Chlorophylls, which are involved in photosynthesis but not in protection, increase as a result of supplemental UV-A, which resembles their response to higher levels of white light (Thayer and Björkman 1990). The increase in carotenoid levels, from which only that for antheraxanthin was significant, was not selective for those carotenoids involved in the xanthophyll-cycle (Table 2). This result suggests that this protective mechanism, aimed to safeguard photochemical reaction centres as well as light-collecting antenna complexes of photosystem II, be not selectively induced by UV-A. Such a response is in contrast to observations of increased xanthophyll cycle carotenoid levels upon plant transfer to high intensities of white light (Demming-Adams and Adams III 1996).

Flavonoids are especially suitable in fulfilling a dual role with their high antioxidant capacity and light

absorption of UV-wavelengths. An increase in flavonoids is generally observed upon both UV-A (Wilson et al. 1998, Shiozaki et al. 1999) and UV-B exposure (Wilson et al. 1998, Searles et al. 2001). In accordance with Wilson et al. (1998) we observed a selective, extra increase for some of the kaempferol derivatives in fuchsia leaves upon UV-A exposure (Fig. 1, arrows), as compared to a 2-fold increase in the others and in all quercetin derivatives. (Table 3). In response to UV-B supplementation Olsson et al. (1998) observed a selective increase of quercetin as compared to kaempferol glycosides. This reversed selectivity by UV-B may be related to the much higher antioxidant capacity of quercetin (Cuvelier 1998), which may be required to neutralize the oxidative stress generated by UV-B. The lack of response of anthocyanins to supplemental UV-A (Table 4) suggests that these pigments did not fulfil an additional protective role, or that their constituent levels were sufficient in the species studied. However, other studies have reported enhanced levels of anthocyanin by UV-A supplementation (Dong et al. 1995, Voipio and Autio 1995, Chaturvedi et al. 1998, Hoffman 1999). This may be relevant for improvement of the decorative value of ornamental plants.

Notwithstanding the increases in ascorbate and flavonoids in leaves, the antioxidant capacity, measured as Trolox equivalents, did not increase upon supplemental UV-A, but actually showed a tendency to decrease (Table 4). This result is hard to explain from the increases in antioxidant compounds, although it cannot be excluded that the anticipated higher antioxidant levels are nullified by decreases in other, not measured antioxidant components. In addition, the major proportional increases were observed in quercetin and kaempferol derivatives, quantified in the form of their aglycones. It is difficult to establish how far the quantity of aglycones can be translated into antioxidant capacity of the original flavonoid derivatives.

Since UV-A-induced changes in antioxidant compounds were much more pronounced in leaves as compared to petals and sepals, one might speculate that the responsiveness of leaves is related to the presence of UV-sensitive elements, e.g. the photosynthetic mechanisms mentioned above. Moreover, through the presence of chlorophylls leaves are capable of generating additional reactive oxygen species, such as singlet oxygen and superoxide (Foyer et al. 1994). In our study we observed that higher A_{355}-values of UV-A-exposed leaves were not accompanied by higher values of antioxidant capacity (Table 4). This suggests that leaves primarily respond to UV-A by neutralizing the cause of potential damage, i.e. by shielding, and not by increasing the capacity to scavenge reactive oxygen species, which may be the consequence of supplementary exposure.

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