

## The Photoprotective Role of Epidermal Anthocyanins and Surface Pubescence in Young Leaves of Grapevine (*Vitis vinifera*)

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• **Background and Aims** Depending on cultivar, surfaces of young leaves of *Vitis vinifera* may be glabrous-green ('Soultanina') or transiently have anthocyanins ('Siriki') or pubescence ('Athiri'). A test is made of the hypothesis that anthocyanins and pubescence act as light screens affording a photoprotective advantage to the corresponding leaves, and an assessment is made of the magnitude of their effect.

• **Methods** Measurements were made on young leaves of the three cultivars in spring under field conditions. Photosynthetic gas-exchange and *in vivo* chlorophyll fluorescence were measured. Photosynthetic and photoprotective pigments were analysed by HPLC.

• **Key Results** Compared with glabrous-green leaves, both anthocyanic and pubescent leaves had greater dark-adapted PSII photochemical efficiency and net photosynthesis. In leaves possessing either anthocyanins or pubescence, the ratio of xanthophyll cycle components to total chlorophyll, and mid-day de-epoxidation state of the xanthophyll cycle were considerably smaller, than in glabrous-green leaves. These differences were more evident in pubescent leaves, probably indicating that trichomes were more effective in decreasing light stress than anthocyanins in the epidermis.

• **Conclusions** Light screens, especially in the form of pubescence, decrease the risk of photoinhibition whilst allowing leaves to maintain a smaller content of xanthophyll cycle components and depend less on xanthophyll cycle energy dissipation. This combination of photoprotective features, i.e. decreased photon flux to the photosynthetic apparatus and lower xanthophyll cycle utilization rates may be particularly advantageous under stressful conditions.

**Key words:** Anthocyanins, *Vitis vinifera*, leaf development, photoprotection, photosynthesis, pubescence, xanthophyll cycle.

### INTRODUCTION

Under natural conditions, leaves are exposed to a very large range of light intensities, with photon fluxes (PF), from darkness to full sunlight. Photosynthetic carbon dioxide assimilation in many species is saturated at PF considerably less than full sunlight. Therefore, leaves may absorb excessive light energy, which causes photooxidative damage. Due to such diverse light conditions, the photosynthetic machinery has evolved high efficiency under low light intensities, while having the ability to avoid the effects of large PF, by protection given by physical shade barriers or pigments which prevent light of particular wavelengths reaching the photosynthetic machinery. These characters may be transient or permanent during a leaf's life-time. Trichome layers and leaf surface depositions of various materials, respectively, reflect or absorb visible light (Karabourniotis and Bornman, 1999; Karabourniotis *et al.*, 1999; Steyn *et al.*, 2002; Manetas, 2003). Non-photosynthetic red pigments (predominantly anthocyanins) create a yellow-green light umbrella, distributed within or spatially separated from the cells containing chloroplasts (Lee and Collins, 2001). Several studies suggest a photoprotective role for foliar

anthocyanins (Gould *et al.*, 1995; Smillie and Hetherington, 1999; Gould *et al.*, 2002a; Manetas *et al.*, 2002, 2003; Steyn *et al.*, 2002; Hughes *et al.*, 2005). The photoprotective hypothesis is not, however, confirmed since some experimental results show that the anthocyanic screening is not advantageous for photosynthesis (Burger and Edwards, 1996; Lee *et al.*, 2003).

Light energy reaching the chloroplasts is used to drive photosynthetic electron flow. When the photosynthetic machinery is light saturated, excess radiation may be dissipated by several biochemical mechanisms (Demmig-Adams and Adams, 1992a; Asada, 1999; Ort and Baker, 2002). Among these, the dissipation of excess energy as heat through the xanthophyll cycle is considered to be the most important photoprotective feature of leaves (Demmig-Adams and Adams, 1992a; Flexas and Medrano, 2002). In *Vitis vinifera* leaves, thermal energy dissipation by the xanthophyll cycle accounts for almost all non-photochemical quenching (Chaumont *et al.*, 1995, 1997), dissipating approx. 45–64% of the absorbed light energy under non-stressful environmental conditions, while under stress this may rise to 75–92% (Flexas and Medrano, 2002; Hendrickson *et al.*, 2004). Therefore, the xanthophyll cycle may become inadequate to dissipate absorbed energy when high irradiance is combined with

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TABLE 1. Leaf surface area and leaf thickness of leaves of *Vitis vinifera* cultivars

Leaf position (node)	DAE*	Leaf surface area (cm <sup>2</sup> )			Leaf thickness (µm)		
		'Soulтанina', glabrous-green	'Siriki', anthocyanic	'Athiri', pubescent	'Soulтанina', glabrous-green	'Siriki', anthocyanic	'Athiri', pubescent
2	14	1.3 ± 0.2	0.5 ± 0.2	0.9 ± 0.1	80 ± 1	84 ± 0	76 ± 1
3	21	2.9 ± 0.4	1.7 ± 0.2	2.2 ± 0.2	90 ± 5	98 ± 4	88 ± 1
4	28	6.8 ± 1.3	5.9 ± 0.7	6.2 ± 0.5	85 ± 3	104 ± 3	100 ± 11
5	35	18 ± 2	15 ± 1	13 ± 1	90 ± 5	118 ± 4	99 ± 2
6	42	37 ± 7	44 ± 7	31 ± 3	106 ± 8	121 ± 5	109 ± 2
8	56	130 ± 11	168 ± 22	124 ± 11	116 ± 11 <sup>a</sup>	145 ± 4 <sup>b</sup>	134 ± 7 <sup>ab</sup>
10	70	196 ± 16 <sup>a</sup>	266 ± 23 <sup>b</sup>	207 ± 16 <sup>a</sup>	142 ± 13	188 ± 5	161 ± 17

Values are means of three replicates (± standard error of the mean). Different superscript letters denote statistically significant differences between cultivars ( $P < 5\%$ ).

\* Days after emergence; mean values for all cultivars are shown; actual leaf ages are <10 % different between cultivars.

other unfavourable factors, such as drought. Under these conditions, decreasing the light energy reaching the chloroplast by leaf surface screens should be beneficial. Several *V. vinifera* cultivars possess leaves with dense pubescence. Karabourniotis *et al.* (1999) showed that trichomes reduced penetration of both ultraviolet and blue light into the mesophyll. Anthocyanins in the epidermal cells may also strongly modify leaf optical properties (Neill and Gould, 1999; Gould *et al.*, 2002b). These characters are particularly abundant during the early developmental stages of the leaves. Pubescence on the surface and anthocyanins in the epidermises of grapevine leaves could be related to photoprotection during early development. Transient pubescence or pigmentation in leaves of several plant species (Uphof, 1962; Karabourniotis *et al.*, 1995; Ntefidou and Manetas, 1996; Bisba *et al.*, 1997) is considered a defence against photodamage in developing leaves, as these are more susceptible to photoinhibition compared with grown leaves due to their immature photosynthetic apparatus (Ireland *et al.*, 1985). According, it is hypothesized in the present study, that the surface characteristics of grapevine leaves contribute to photoprotection, so cultivars that lack physical or pigment screens to decrease the PF on the photochemical apparatus should show increased accumulation of photodamage in young leaves. Moreover, the photodamage should diminish in fully grown leaves, because they can endure higher irradiances due either to their fully developed and functional photosynthetic apparatus or the increased photoprotection provided by biochemical mechanisms. A consequence, addressed by this study, is that anthocyanic or pubescent leaves would display less xanthophyll cycle activity (measured by the de-epoxidation state) because less light energy reaches their chloroplasts compared with those in glabrous-green leaves. To address the above questions, photosynthetic and photoprotective parameters were measured in leaves of different ages on field-grown *V. vinifera* plants, differing in pubescence and anthocyanins during spring growth. As far as is known, this is the first field study in which the effects of pubescent and anthocyanins, characteristics thought to contribute to the photoprotection of leaves, on

light interception, absorption and xanthophyll cycle activity are compared in parallel during leaf development.

## MATERIALS AND METHODS

### Plant material and samplings

Experiments were conducted in the vineyard of the Agricultural University of Athens, Greece (37°58'54"N, 23°42'12"E, 35 m a.s.l.). Three grapevine (*Vitis vinifera* L.) cultivars were chosen: 'Soulтанina' having glabrous (i.e. non pubescent)-green (i.e. non-anthocyanic) leaves; 'Siriki' having glabrous leaves with high contents of anthocyanins located in the epidermises when young; and 'Athiri' having non-anthocyanic leaves with dense pubescence when young. At the beginning of February, vines of 'Soulтанina' were cane pruned and vines of 'Athiri' and 'Siriki' were spur pruned. Plants received no irrigation before or during the experiments since soil moisture was adequate for growth. In winter, a full composition fertilizer was applied.

Eight similar 10-year old vines were chosen for each cultivar and three or four shoots per plant, positioned approx. 1 m above soil level, were tagged for samplings and for *in planta* measurements. All selected shoots bore at least ten leaves. Leaves were selected based on their position to the shoot, numbered from the apex. Leaf ages of node positions selected are shown in Table 1. All measurements were made between 13 and 21 May 2003.

### Gas exchange measurements

Measurements of light-saturated net CO<sub>2</sub> assimilation rate and stomatal conductance were made on south-oriented leaves exposed to natural light (PPFD 1500 µmol photons m<sup>-2</sup> s<sup>-1</sup>) and ambient CO<sub>2</sub> concentration (380 µmol mol<sup>-1</sup>) between 14 and 21 May 2003 at 0800–1200 h using a portable open-circuit gas-exchange instrument (LI-6400, Li-COR Inc., Lincoln, NE, USA), equipped with a broad leaf chamber enclosing 6 cm<sup>2</sup> of leaf area. Temperature and relative air humidity inside the chamber were 30 ± 3 °C and 30 ± 2 %, respectively.

Fifteen replicates for each leaf developmental stage for each cultivar were measured (ten readings at steady-state conditions were recorded per replicate).

#### Pigment analysis

Samples were taken before dawn (0530–0700 h) and around noon (1130–1300 h). Three leaf discs (each 71 mm<sup>2</sup>) were collected from five replicate leaves from different plants, immediately frozen in liquid nitrogen and stored at –80 °C until processed. All handling was done at 6 °C under a green light. Discs were powdered in liquid nitrogen and extracted with methanol (2 mL) containing CaCO<sub>3</sub>. Undissolved matter was removed by centrifugation (2570 g, 5 min). Extracts were filtered through an MN Chromafil GF-PET 20/25 membrane filter (Macherey-Nagel, Düren, Germany) and analysed by HPLC according to Darko *et al.* (2000). Pigments (carotenoids and chlorophylls) were analysed with a Zorbax Stablebond SB-C<sub>18</sub> column (5-µm particle size; 250 × 4.6 mm; Agilent Technologies, Palo Alto, CA, USA), connected to an LG-980-02 gradient unit/PU-980 pump (Jasco Corporation, Tokyo, Japan). Pigments were detected at 437 nm using a UV-970 detector. Peaks were assigned to the corresponding pigments by means of chromatographic and spectral characteristics. For the quantification of carotenoids, peak areas were calibrated against lutein (Britton, 1985). Lutein was isolated as a pure standard from fresh leaves by TLC (Harborne, 1998). Depoxidation state of the xanthophyll cycle components (DPS) was expressed as  $(0.5A + Z)/(V + A + Z)$  according to Sobrino *et al.* (2005). For the quantification of chlorophylls, HPLC peak areas of chlorophylls *a* and *b* were calibrated by measuring the same methanolic extracts in a double-beam spectrophotometer (UV-Vis 160A; Shimadzu Co, Tokyo, Japan) according to Lichtenhaler and Welbourn (1983).

For measurements of anthocyanin content (three replicates), leaf discs were powdered in liquid nitrogen and extracted with methanol containing 1% HCl. Solid matter was removed by centrifugation (2570 g, 5 min) and the absorbance was measured at 530 and 657 nm in a double-beam spectrophotometer. The absorbance of anthocyanins was corrected for chlorophyll content and expressed in concentration using the typical molecular extinction coefficient of cyanidin (34.7 AU mM<sup>-1</sup>; Murray and Hackett, 1991).

#### Other measurements

Spectral reflectances of intact leaves (four replicates) were recorded with a diode array spectrometer (Unispec, PPSystems, Haverhill, MA, USA) equipped with a small diameter (2.3 mm) bifurcated fibre optic cable, an internal halogen source and an appropriate leaf clip. A spectralon (reflectance >97%) standard was used as reference and the spectra were dark corrected for stray light with the internal source off. Total leaf surface area was measured using standard image analysis and leaf thickness was

measured under a light microscope using hand-cut cross-sections of fresh leaves (three replicates). Trichome layers were excluded from the measurement of leaf thickness in 'Athiri'. Specific leaf area (SLA) was determined for leaves dried at 80 °C for 48 h and kept in a desiccator for 24 h before weighing. The intrinsic photochemical efficiency of PSII,  $F_v/F_m$ , was measured in the field on 15 replicate leaves from different plants at each age, 4–5 h into the dark period, using a portable chlorophyll fluorescence instrument (FIM 1500, ADC Ltd, UK). Excitation light was adjusted to 1050 µmol photons m<sup>-2</sup> s<sup>-1</sup>.

## RESULTS

Morphological and anatomical variables of the leaves were similar between the three cultivars during leaf expansion (Table 1). However, at full expansion, area per leaf of the anthocyanic cultivar was 36% greater than the glabrous-green cultivar (Table 1). Also, anthocyanic and pubescent leaves were thicker by 32% and 13%, respectively, than the glabrous-green leaves (Table 1). The presence of anthocyanins or pubescence in developing leaves affected their optical properties, as shown by the reflectance spectra of the adaxial and abaxial leaf surfaces of the three cultivars (Fig. 1). Young anthocyanic leaves, especially at the 5th node stage, had reduced light reflectance at approx. 550 nm, characteristic of the presence of anthocyanins (Fig. 1). Young pubescent leaves reflected a large proportion of radiation between 400 and 700 nm, but this decreased at full expansion. Similar results were obtained from both leaf surfaces; however, the abaxial surface of pubescent leaves was more reflective than the adaxial surface. At full leaf expansion, the differences in optical properties between cultivars were diminished (Fig. 1).

As expected, leaf anthocyanin concentration was very large in the anthocyanic cultivar, greatest in leaves of the 4th node but was also high in leaves up to the 6th node (Fig. 2A), in accordance with the reflectance spectra for the corresponding developmental stages (Fig. 1). Anthocyanin concentrations in the leaves of the anthocyanic cultivar were as small as those of the other two varieties at full expansion (Fig. 2A); glabrous-green leaves contained little anthocyanin which was practically absent in very young pubescent leaves (Fig. 2A). The total content of xanthophyll cycle components per unit leaf area (called 'pool' hereafter) (Fig. 2B) increased gradually with leaf age in all cultivars. Contrary to the leaves of the other two cultivars, pubescent leaves showed large fluctuations in the pool size during development (Fig. 2B). However, the pool size of the xanthophyll cycle components was very similar between the three cultivars since there were no statistically significant differences. Non-xanthophyll carotenoids were lower in glabrous-green leaves than in the other two cultivars (Fig. 2C). In all cases the concentration of carotenoids increased gradually with leaf age. Variations in the content of carotenoids in all cultivars were similar between xanthophyll and non-xanthophyll pigments (compare B and C in Fig. 2)

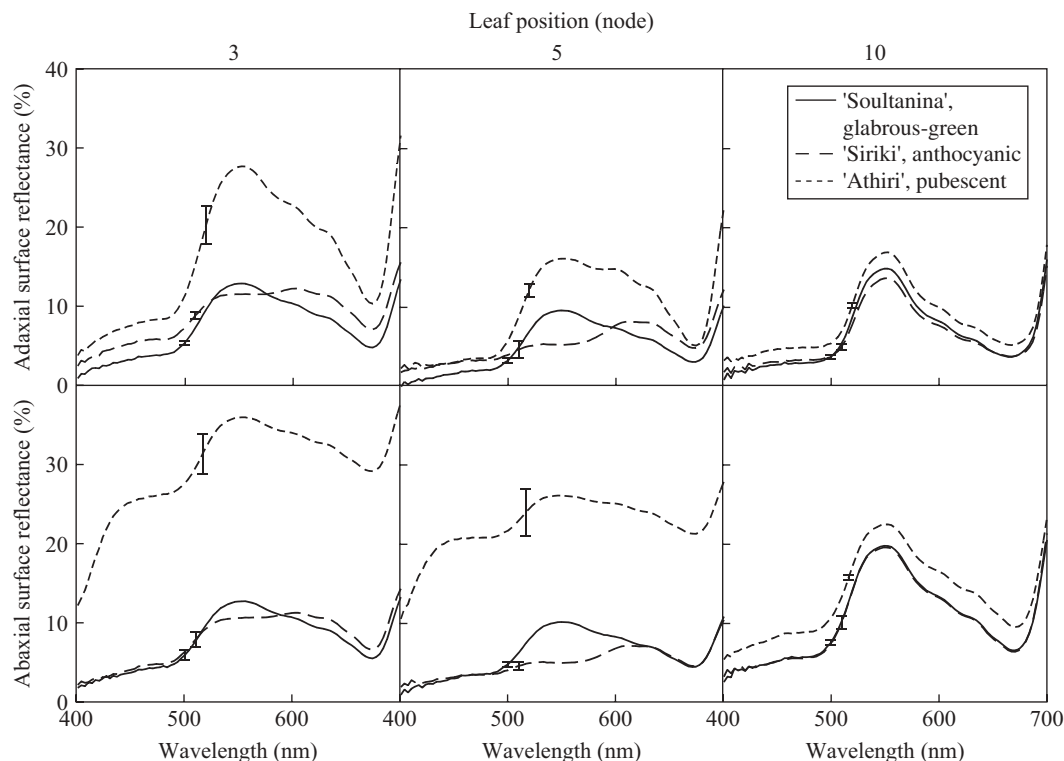


FIG. 1. Reflectance spectra of young leaves of *Vitis vinifera* as a function of wavelength. Data are means of four replicates and error bars indicate s.e. of the mean.

implying coordination in the build-up in the pools of the various components.

Chlorophyll [Chl (*a+b*)] concentrations increased gradually with leaf development. Pubescent leaves had significantly greater Chl (*a+b*) content when very young (3rd to 6th node), whilst older anthocyanic leaves contained greater concentrations (Fig. 3A). In almost all stages, glabrous-green leaves had the smallest Chl (*a+b*) content. The Chl *alb* ratio of pubescent leaves was considerably lower than that of the other two cultivars in early development and increased gradually with age (Fig. 3B). On the other hand, the Chl *alb* ratio gradually decreased with age in anthocyanic and glabrous-green leaves. In fully expanded leaves, all cultivars had similar values (Fig. 3B).

Gas-exchange and chlorophyll fluorescence measurements were only possible from leaves of the 5th node stage when leaf area was large enough. Intrinsic photochemical efficiency of photosystem II (PSII) was significantly different between the three cultivars (Fig. 4A). At all developmental stages pubescent leaves had the highest PSII photochemical efficiency, while glabrous-green leaves had the lowest. Irrespective of cultivar,  $F_v/F_m$  increased with leaf age (Fig. 4A). Net CO<sub>2</sub> assimilation rate was positive from the 6th node stage and above. In almost fully expanded leaves (8th and 10th nodes) the photosynthetic rate was largest in anthocyanic leaves and smallest in glabrous-green leaves (Fig. 4B). Fluctuations in net photosynthetic rate paralleled those of stomatal conductance (Fig. 4C). Glabrous-green leaves

showed the smallest stomatal conductance, irrespective of the developmental stage. With fully expanded (10th node) leaves, however, the values were comparable between the three cultivars (Fig. 4C).

The ratio of xanthophyll cycle components to Chl (*a+b*) (Fig. 5A) was much greater for glabrous-green leaves than the other two cultivars, especially when young. In general, the ratio of anthocyanic leaves was between the other two cultivars (Fig. 5A). Irrespective of cultivar, the depoxidation state (DPS) was higher in young leaves than old (Fig. 5B). Between cultivars, the highest values were in glabrous-green leaves of all ages while pubescent leaves showed the lowest DPS. Pubescent leaves showed a notable increase in DPS values of the 5th node stage (Fig. 5B). It is notable that DPS of anthocyanic and pubescent leaves was identical in expanded leaves (10th node) while that of glabrous-green leaves was far higher (Fig. 5B).

According to Fig. 6A, DPS was negatively correlated with  $F_v/F_m$ . Also, anthocyanin concentration for the leaves of the anthocyanic cultivar and trichome mass per leaf area of the pubescent cultivar (data for trichome mass per area obtained from Karabourniotis *et al.*, 1999) were correlated with DPS (Fig. 6B).

## DISCUSSION

Anthocyanins or pubescence in young grapevine leaves considerably modify visible light penetration. The present results show that light reflectance at approx. 500–630 nm

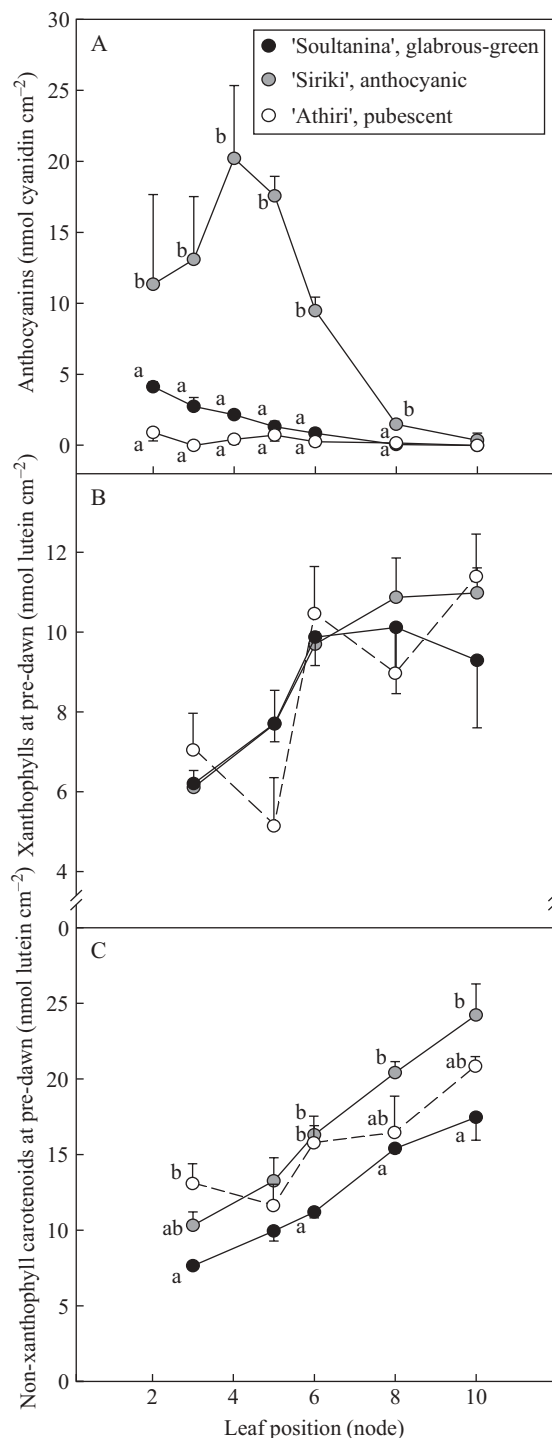


FIG. 2. Anthocyanin concentration (A), concentration of xanthophyll cycle components before dawn (B) and concentration of non-xanthophyll cycle carotenoids before dawn (C) in the leaves of the three grape cultivars in relation to leaf developmental stage. Values are means of three (A) or five (B and C) replicates ( $\pm$  s.e. of the mean). Different letters denote statistically significant differences between cultivars ( $P < 5\%$ ).

decreased due to anthocyanins in the epidermises. Consequently light entering the mesophyll must be poorer in this spectral band compared with glabrous-green leaves since anthocyanic epidermises absorb a significant portion

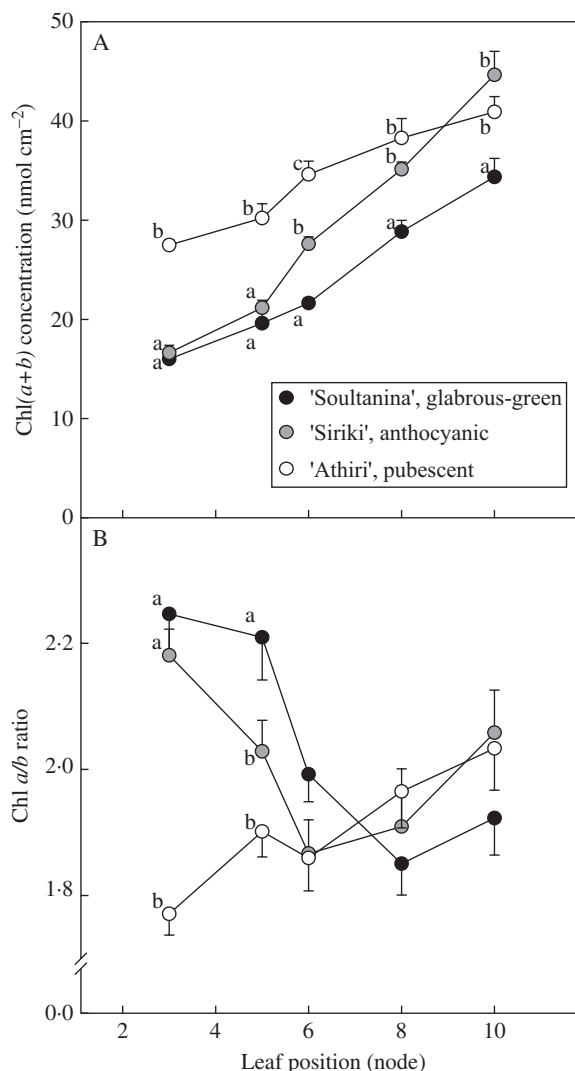


FIG. 3. Concentration of chlorophylls [Chl (a+b)] (A) and Chl a/b ratio (B) in the three grape cultivars in relation to leaf developmental stage. Values are means of five replicates ( $\pm$  s.e. of the mean). Different letters denote statistically significant differences between cultivars ( $P < 5\%$ ).

of green and yellow light (Neill and Gould, 1999). However, the photoprotective significance of the anthocyanins is still questionable. Both laboratory and field experiments have shown contrasting results (Steyn *et al.*, 2002). It has been argued that the spectral properties of foliar anthocyanins are not optimized for protection of the photosynthetic apparatus from visible light since they absorb weakly in the blue waveband while absorption of the red waveband is practically absent (Neill and Gould, 1999). Karabourniotis *et al.* (1999) found no difference in the penetration of blue light between green and anthocyanic (red) grapevine leaves but the optical fibre apparatus used was not suitable for measurements at longer wavebands. On the contrary, the attenuation of ultraviolet-B and blue light was considerable when pubescent leaves were examined (Karabourniotis *et al.*, 1999). Judged from the reflectance spectra, the dense trichome mat acts as a neutral density filter by reflecting

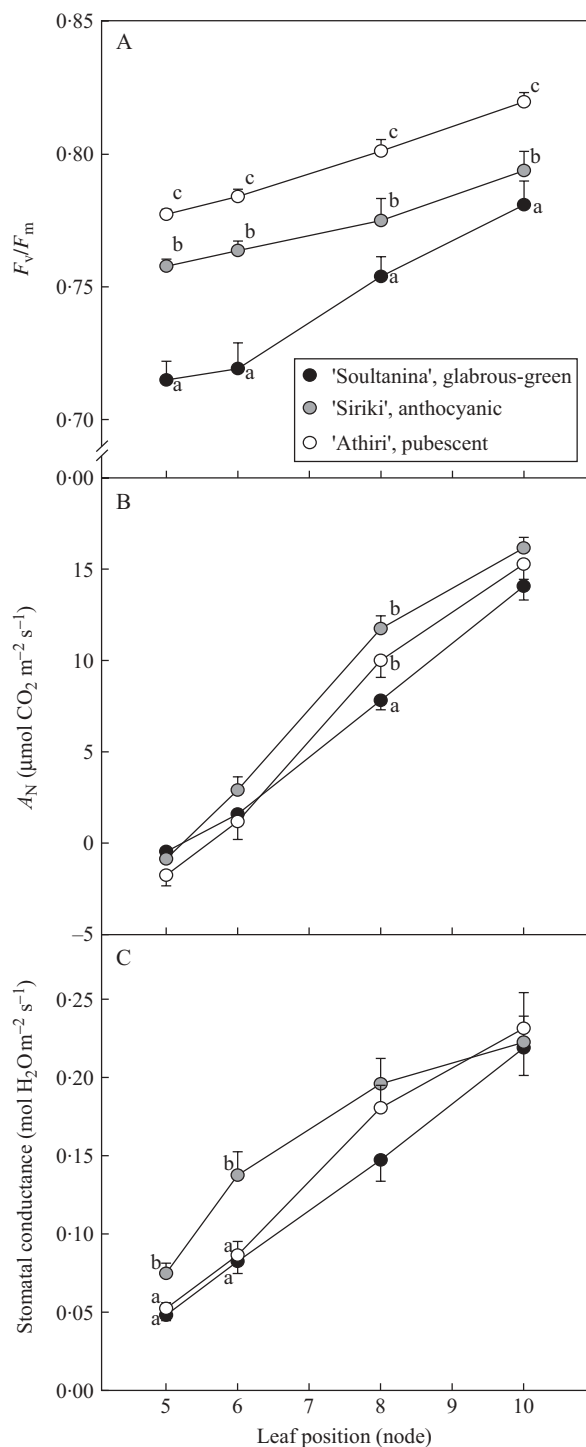


FIG. 4. (A) Ratio of variable ( $F_v$ ) to maximum ( $F_m$ ) chlorophyll fluorescence, (B) net  $\text{CO}_2$  assimilation rate and (C) stomatal conductance in the leaves of the three grape cultivars in relation to leaf developmental stage. Values are means of 15 replicates. Different letters denote statistically significant differences between cultivars ( $P < 5\%$ ).

and scattering light, resulting in considerable shading of the chlorenchyma. According to the present results, leaf reflectance of the abaxial surface of the pubescent leaves was greater than that of the adaxial surface, probably due

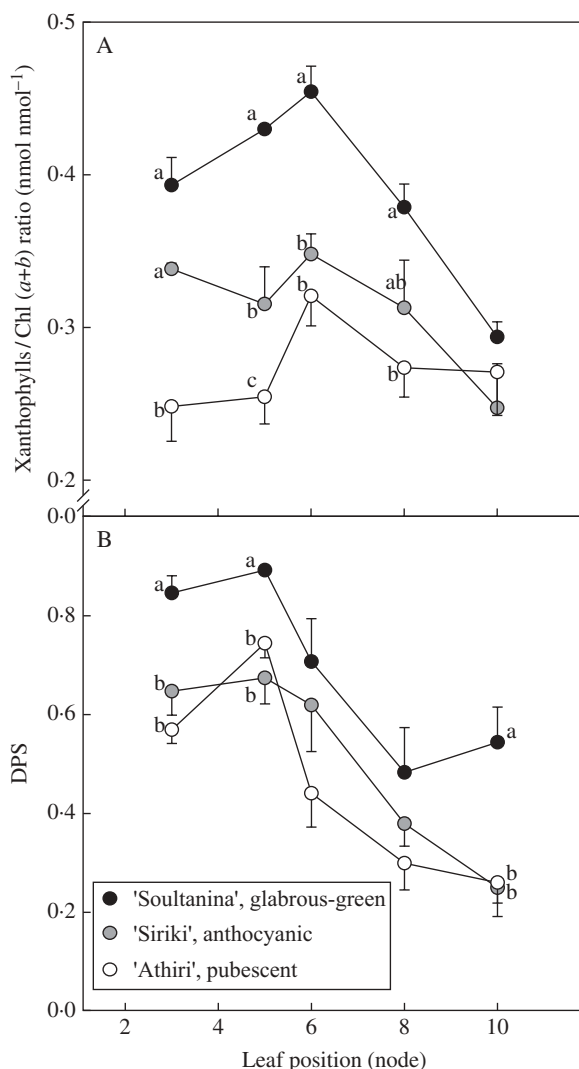


FIG. 5. Xanthophyll components/chlorophylls ratio (A) and de-epoxidation state of the xanthophyll cycle components (DPS) (B) in the three grape cultivars in relation to leaf developmental stage. Values are means of five replicates ( $\pm$  standard error of the mean). Different letters denote statistically significant differences between cultivars ( $P < 5\%$ ).

to denser indumentum (see Karabourniotis *et al.*, 1999). Light filtering of the trichome layer, especially in young leaves, may protect against both ultraviolet-B (Karabourniotis *et al.*, 1992) and visible radiation damage (Lang and Schindler, 1994; Karabourniotis and Bornman, 1999; Karabourniotis *et al.*, 1999). In *V. vinifera*, anthocyanins and pubescence are present in both epidermises during early leaf development (2nd to 4th leaf nodes), but gradually disappear at full expansion, which roughly corresponds to the period when leaves are competent regarding photosynthesis and carbon export, as found in other species (Barker *et al.*, 1997; Choinski and Wise, 1999). The susceptibility of young leaves to photooxidative damage due to light stress has been investigated by several researchers (Krause *et al.*, 1995; Bisba *et al.*, 1997). Irrespective of cultivar, higher xanthophyll cycle DPS and ratio of xanthophyll cycle

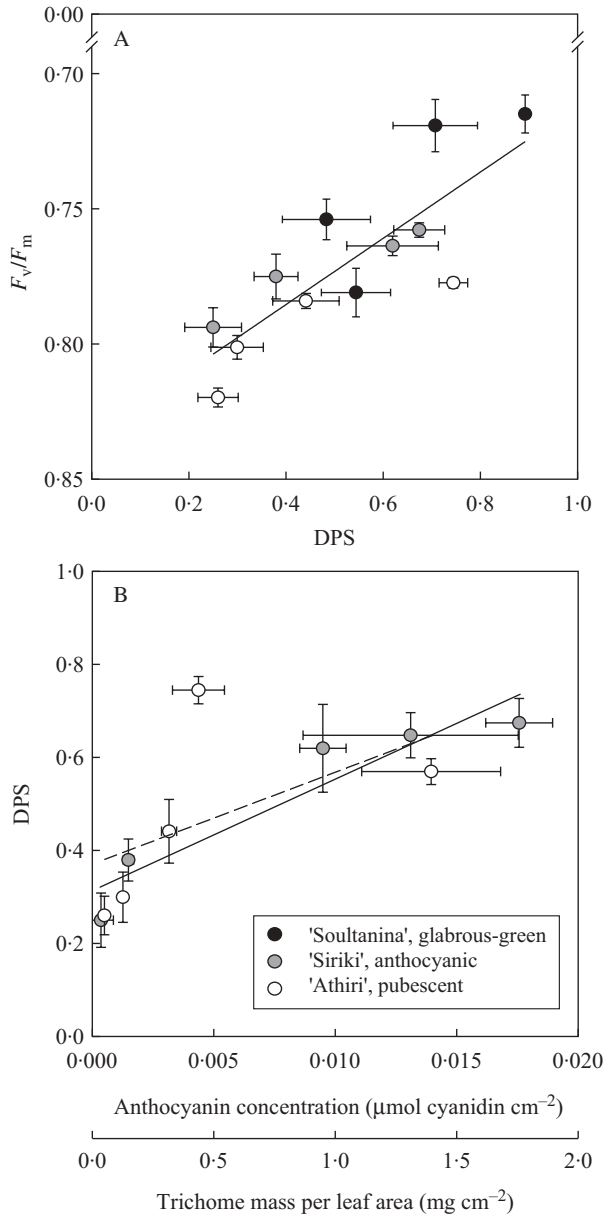


FIG. 6. (A) Linear regression between DPS and  $F_v/F_m$ . Values from all cultivars and all developmental stages are included. Values are means of 15 ( $F_v/F_m$ ) or five (DPS) observations ( $\pm$  standard error of the mean). For the regression,  $F_v/F_m = -0.122 \text{ DPS} + 0.834$ ,  $r^2 = 0.6789$ . (B) Linear regressions between anthocyanin concentration and DPS for leaves of the anthocyanic cultivar (solid line) or between trichome mass per leaf area and DPS for leaves of the pubescent cultivar (dashed line). For each of the two cultivars, values from all developmental stages are included. Values are means of three (anthocyanins) or five (DPS) observations ( $\pm$  standard error of the mean). Values for trichome mass per area were taken from Karabourniotis *et al.* (1999) and are sums of trichomes of abaxial and adaxial leaf surfaces. For the regressions,  $\text{DPS} = 24.0 [\text{anthocyanins}] + 0.313$ ,  $r^2 = 0.885$ ;  $\text{DPS} = 0.199 (\text{trichome mass per area}) + 0.370$ ,  $r^2 = 0.293$ .

components to Chl ( $a+b$ ) were apparent during early development, suggesting that there is greater risk of light stress during these stages.

On the other hand, trichomes (due to either decreasing light energy or limiting gas phase conductance by

decreasing that of the boundary layer) or anthocyanins (due to decreasing PF) could restrict photosynthetic rate. According to Choinsky and Wise (1999), excessive trichome coverage of *Quercus marilandica* young leaves may have limited conductance by increasing the boundary layer. According to the present results, pubescent leaves had stomatal conductances comparable to those of glabrous-green leaves. Grammatikopoulos *et al.* (1994) showed that removal of hairs from olive leaves had no effect on the total resistance to water vapour diffusion. Interestingly, anthocyanic leaves had significantly greater stomatal conductances than leaves of the other two cultivars in early development. Moreover, anthocyanic (similar to the results of Gould *et al.*, 1995) and pubescent leaves had higher net photosynthetic rates at light saturation than glabrous-green leaves.

Young anthocyanic (Gould *et al.*, 1995; Manetas *et al.*, 2003) and young pubescent leaves (Karabourniotis *et al.*, 1999; see also Ehleringer, 1984; Morales *et al.*, 2002) displayed characteristics of shade-adapted leaves, probably due to attenuation of visible light. High concentrations of chlorophylls and low Chl  $a/b$  ratios are indicative of acclimation to low light intensities (Brugnoli *et al.*, 1994; Bertamini and Nedunchezian, 2002). Despite the fact that the concentration of chlorophylls increased with leaf age in all three cultivars, leaves of the pubescent cultivar showed Chl ( $a+b$ ) and Chl  $a/b$  values which can be attributed to acclimation to low light intensity. Concentration of chlorophylls in the anthocyanic leaves increased with leaf age possibly because high anthocyanin concentrations developed in older leaves compared with pubescence (compare Fig. 2B of the present study with figure 1 in Karabourniotis *et al.*, 1999). On the other hand, except at the 5th node stage, the Chl  $a/b$  values of the anthocyanic leaves were not indicative of low light acclimation. Moreover, light-saturated net  $\text{CO}_2$  assimilation (Fig. 3A) and carboxylation efficiency (data not shown) did not coincide with acclimation to low light intensities for either leaf type.

Acclimation of leaves to a particular light regime is reflected in the size of the pool of the xanthophyll cycle components and the xanthophyll cycle interconversions (Demmig-Adams and Adams, 1992b), and this has also been observed in *V. vinifera* (Chaumont *et al.*, 1995, 1997). Additionally, the high ratio of xanthophyll cycle components to chlorophyll indicates acclimation to excess light (Morales *et al.*, 2002). The occurrence of anthocyanins or pubescence is expected to decrease excess light energy and subsequently the need for energy dissipation, resulting in a lower requirement for photoprotection. Indeed, lower values of both the DPS of the xanthophyll cycle and the ratio of xanthophyll cycle components to chlorophylls were observed in anthocyanic and pubescent leaves compared with glabrous-green leaves, suggesting a smaller requirement for energy dissipation in the presence of light screens. Overall, this was more evident in pubescent leaves, probably indicating that dense pubescence was more effective in reducing light stress than were anthocyanic epidermises. In glabrous-green leaves, with no external photoprotection,

internal photoprotection via the xanthophyll cycle was enhanced. This is judged by the high xanthophyll cycle components/chlorophyll ratio and the almost complete de-epoxidation state, especially in the youngest leaves. At late stages of development, the size of the pool of the xanthophyll cycle components was increased in anthocyanic compared with glabrous-green leaves. Anthocyanin degradation and the subsequent increase of the light energy penetrating the mesophyll of the anthocyanic leaves might be related to this increase. The size of the pool of the xanthophyll cycle components in leaves of the pubescent cultivar changed greatly over the course of leaf development. It is noteworthy that this was preceded by a sudden increase in the DPS. The above changes correspond to the presence of trichomes on young pubescent leaves which is followed by >60% loss in later stages (Karabourniotis *et al.*, 1999). When light intensity increases beyond that to which a particular leaf is acclimated, the size of the pool of the xanthophyll cycle components increases after some time (Brugnoli *et al.*, 1994; Demmig-Adams, 1998; Logan *et al.*, 1998), within days, when leaves in low-light are suddenly exposed to high light (Logan *et al.*, 1998; Niinemets *et al.*, 2003; García-Plazaola *et al.*, 2004). Considering all the above, it is proposed that in pubescent leaves, loss of trichomes resulted in exposure to high light intensities within a few days, resulting in light stress which, in turn, increased the rate of synthesis of the xanthophyll cycle carotenoids. It is concluded that the size of the pool of the xanthophyll cycle components, apart from the influence of the developmental stage, responded to the changes in light intensity perceived by each leaf type as a result of the changes in the optical screens.

An inverse correlation between photoinhibition and  $F_v/F_m$  has been documented (Björkman and Demmig, 1987). Since increased DPS values indicate dissipation of excess energy, it is assumed that under conditions of stronger thermal energy dissipation, the photochemical efficiency of PSII should be potentially lower due to higher photoinhibition. The decreased PSII intrinsic photochemical efficiency in young glabrous-green leaves compared with leaves possessing optical screens for visible light, suggests greater photoinactivation of PSII (Björkman and Demmig-Adams, 1994; Flexas *et al.*, 2001). PSII efficiency was closely correlated with DPS (Fig. 6A), suggesting that the de-epoxidation state of the xanthophyll cycle components at noon is related to the intrinsic quantum yield efficiency of PSII. Since a large DPS indicates strong thermal energy dissipation (Demmig-Adams and Adams, 1996), the results show that excessive light may be responsible for photodamage to PSII (Kato *et al.*, 2003). The fact that anthocyanins or trichomes are abundant when DPS values are high (Fig. 6B), suggests that external light screens and large dissipation of excess light energy work in co-operation, depending on the developmental stage of the leaves. Young leaves of *V. vinifera* are susceptible to photoinhibition derived from excess light, most likely caused by the low photosynthetic capacity in early development but ameliorated by anthocyanins or trichomes.

It is concluded that pubescence, as trichome layers (Karabourniotis *et al.*, 1999), and anthocyanins in epidermises of young leaves of *V. vinifera* reduce the PF reaching the photosynthetic apparatus of chlorophyllous cells. These transient light screens offer protection from excessive light to the developing photosystems when the photosynthetic capacity of leaves is poorly developed. As a result, young pubescent and anthocyanic leaves of *V. vinifera* are more efficiently protected against light stress than glabrous-green leaves. Whilst thermal energy dissipation is fully engaged in glabrous-green leaves, pubescent and anthocyanic leaves may sustain a lower xanthophyll cycle activity and maintain better photosynthetic parameters. This situation may prove important in stress-tolerance because the net photodissipative capacity of these leaves may further increase under unfavourable conditions.

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