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Photoprotection in leaves of grapevines: Responses of the xanthophyll cycle to alterations of light intensity

by

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Summary: Under conditions of light intensities exceeding the photosynthetic use carotenoids of the xanthophyll cycle have been shown to be involved in the dissipation of excess energy. When, after a period of darkness, low light-adapted leaves (400 $\mu mol \cdot m^{-2} \cdot s^{-1}$) of cv. Orion vines were suddenly exposed to high light (800 $\mu mol \cdot m^{-2} \cdot s^{-1}$) the zeaxanthin (Z) content of the leaves increased significantly within 3 min at the expense of violaxanthin (V); a steady state was reached after ca. 20 min. On the contrary, when high light-adapted leaves were abruptly exposed to darkness the Z content decreased and the V content increased to a steady state within 2.5 h. In both trials, the intermediate substance of the xanthophyll cycle, antheraxanthin (A), remained almost constant at a relative low level.

In field experiments with cv. Gf.Ga-47-42 an increase of sunlight in the morning was accompanied by increases of A+Z and decreases of V while a decline of sunlight in the afternoon was associated with decreases of A+Z and an increase of V. In laboratory and field experiment the xanthophyll-irradiance relation showed hysteresis. The epoxidation state (EPS, V+0.5A / V+A+Z) decreased in the morning to a minimum at noon and then increased again in the afternoon reflecting a distinct depression of photosynthesis at midday.

Key words: carotenoids, xanthophyll cycle, photoprotection, light, stress, photosynthesis.

Introduction

Leaves of field-grown grapevines exposed to full sunlight experience great diurnal alterations of incident light. Solar energy is used in photosynthesis but may exceed the photosynthetic optimum, in particular if rates of photosynthesis are reduced by factors like water stress or low temperature (CHAUMONT *et al.* 1995; GARCÍA-PLAZAOLA *et al.* 1997; DÜRING 1998). To avoid overexcitation and damage of the photosynthetic reaction centres due to excessive light intensities ('photoinhibition') the photosynthetic systems of sun-adapted leaves have evolved protection mechanisms. One of the major processes to dissipate excess excitation energy is the conversion of light energy into heat ('thermal' or 'nonradiative dissipation'; BJÖRKMAN and ADAMS 1994). In recent years much evidence has been provided that in the thylakoid membranes the carotenoids of the xanthophyll cycle are involved in photoprotection by non-radiative energy dissipation (reviews: DEMMIG-ADAMS and ADAMS 1992, 1996; HORTON *et al.* 1996). The xanthophyll cycle comprises the enzymatic conversion of violaxanthin (V, a di-epoxid) *via* antheraxanthin (A, a mono-epoxid) to zeaxanthin (Z, epoxid-free) under excess light conditions and the reversed reaction when light becomes limiting to photosynthesis (Fig. 1). The causal involvement of the xanthophyll cycle mediating energy dissipation has been demonstrated by the close relationship between non-photochemical energy dissipation (NPQ) and the xanthophyll cycle (BJÖRKMAN and DEMMIG-ADAMS 1994). Moreover, infiltration of an inhibitor of Z formation led to an increased susceptibility to photoinhibition during longer-term exposure to high light (WINTER and KÖNIGER 1989).

In experiments with grapevine sun-adapted leaves have been shown to differ from shade-adapted leaves in their V and Z content (VÁRADI *et al.* 1992) and the xanthophyll pool size (V+A+Z) was found to be distinctly higher in sun-adapted leaves (DÜRING, accepted). CHAUMONT *et al.* (1995) studied the effects of excessive light on photosynthetic and photoprotective processes in vine leaves and demonstrated that the Z content was correlated to the efficiency of photosystem II (Fv/Fm) during photoinhibition and recovery. Just recently higher amounts of Z were reported in skins of grape berries exposed to ambient UV-B compared to berries shielded by UV-B-absorbing material (SCHULTZ *et al.* 1998), and BUREAU *et al.* (1998) report effects of shading on the carotenoid composition in grape berries.

Material and Methods

Laboratory experiments: The effects of high light and darkness on the composition of carotenoids were studied under constant conditions in the laboratory (25/20±1 °C day/night temperature; 12 h light·d^{-1}; 40-45 % rel. humidity). Potted, ungrafted plants of the fungus-resist-

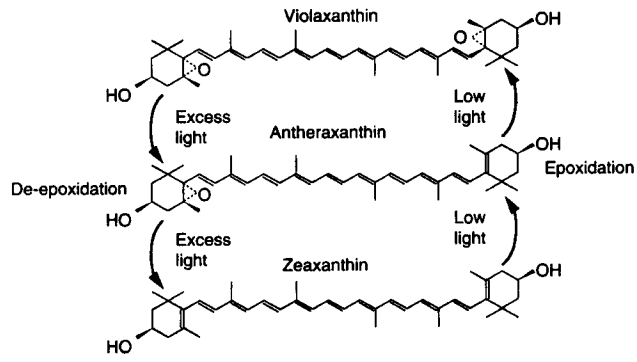

Fig. 1: The xanthophyll cycle as a 'buffer' of excessive light energy.

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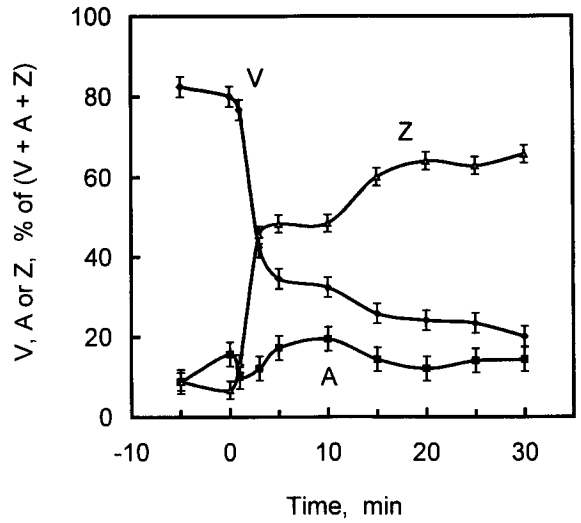

Fig. 2: Responses of the violaxanthin (V), antheraxanthin (A) and zeaxanthin (Z) contents of dark-adapted cv. Orion leaves to high light. Onset of high light at time = 0. Mean values, bars denote confidence limits at the 5 % level.

ant variety Orion (Optima x Villard blanc) were cultivated at 400 µmol quanta·m^{-2}·s^{-1} (Osram HQI-T 400W/DH), determined at the adaxial side of leaves used for experiments. Just before the end of a 12-h-night period a fully expanded but not senescent leaf was wrapped in aluminium foil and then suddenly exposed to high light (800 µmol·m^{-2}·s^{-1}, Osram HQI-T 400W/DH) for 30 min. In a second trial vines were exposed to 800 µmol quanta·m^{-2}·s^{-1} for 3.5 h and then light was switched off. Before and after leaves were exposed to light or darkness, leaf samples were punched out by a cork borer (1.7 cm diameter). Leaf discs were immediately frozen in liquid N_2 and stored at -20 °C until extraction. Experiments were repeated 4 times.

Field experiments: On August 12, 1997, diurnal changes in the carotenoid composition were determined in leaves of 6-year-old field-grown vines of the fungus-resistant variety Gf.Ga-47-42 (Bacchus x Seyval) grafted to Kober 5 BB and pruned to 6-8 buds·m^{-2} with shoots positioned vertically. From 7 a.m. to 7 p.m. every hour 5 leaf discs were punched out from leaves at the top of the canopy; they were immediately frozen in liquid N_2. Synchronously, light intensity was determined by a Quantum-Meter 185-B (Li-Cor, Lincoln, Nebraska, USA). This day turned out to be cloudless, temperatures increased from 20.5 °C at 7 a.m. to 28.8 °C at 2.30 p.m. and declined to 21.8 °C at 7 p.m.

Carotenoid analysis: With slight modifications carotenoids were extracted and analysed according to a method described by THAYER and BJÖRKMAN (1990). Leaf discs were ground with liquid N_2 in a small iced mortar. 0.1 ml iced acetone was added and the slurry transferred into a 1.5 ml Eppendorf tube. Pistil and mortar were washed two times with acetone and the washes were decanted into the Eppendorf tube which was subsequently centrifuged at 0-1 °C (15,000 x *g*, 5 min). The supernatant was decanted and the pellet resuspended with 0.1 ml acetone. After centrifugation (0-1 °C, 15,000 x *g*, 5 min) the supernatant was used immediately for HPLC analysis. Pigments were analysed with a LDC Constametric HPLC system, a Gradient Master and a Waters Ass. Absorbance Detector Mod. 440 and detected by their absorbance at 436 nm. Separation of pigments was performed on a Dupont non-endcapped Zorbax ODS column (4.6 mm x 250 mm, 5 µm particle size). Column temperature was maintained at 33 °C, the flow rate at 1 ml·min^{-1}. The pigments were eluted using 100 % solvent A (acetonitrile:methanol 85:15) for 14.5 min, followed by a linear gradient to 100 % solvent B (methanol:ethyl acetate 68:32) within 2 min. This continued isocratically until the end of the 30 min separation. Purified standards of lutein and zeaxanthin (Hoffmann-La Roche AG, Basel, Switzerland) were available to identify peaks in the chromatograms by their retention time. In addition, retention times were compared to those obtained by THAYER and BJÖRKMAN (1990). Peak size of violaxanthin (V), antheraxanthin (A) and zeaxanthin (Z) was related either to the xanthophyll pool (V+A+Z) or to neoxanthin which remained unaffected by alterations of light during the experiments.

Results and Discussion

Laboratory experiments: First attempts to study the time course of responses of some carotenoids to high light and darkness were performed in the laboratory with low light-adapted vines, cv. Orion. At the end of a 12-h-dark period leaves were suddenly exposed to high light (800 µmol·m^{-2}·s^{-1}; Fig. 2, time = 0). Compared to values obtained at darkness the zeaxanthin (Z) content of the leaves increased rapidly (within 3 min) after light was switched on; thereafter the increase slowed down. The alterations of Z were paralleled by a rapid decrease of the violaxanthin (V) content within 3 min and a slower decrease thereafter. The antheraxanthin (A) content remained almost constant at a low level.

When a high light-adapted leaf was suddenly exposed to darkness (Fig. 3, time = 0) the Z content of the leaf declined slowly to reach a steady state after ca. 2.5 h. In contrast, the V content increased steadily to reach a plateau

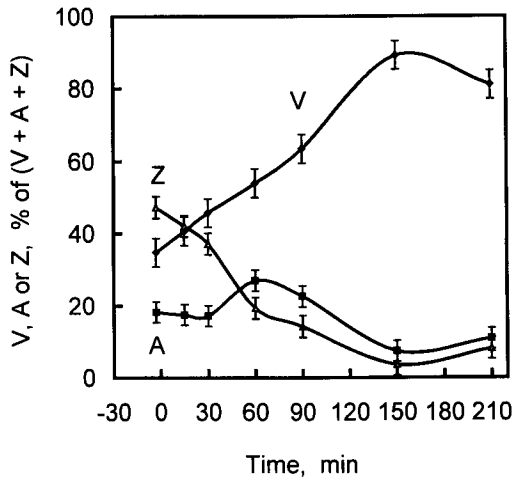

Fig. 3: Responses of the violaxanthin (V), antheraxanthin (A) and zeaxanthin (Z) contents of high light-adapted cv. Orion leaves to darkness. Onset of darkness at time = 0. For details see Fig. 2.

after ca. 2.5 h. The content of lutein, β-carotene and neoxanthin was not significantly affected by light or darkness. The results demonstrate that de-epoxidation of V to Z occurs more rapidly than epoxidation of Z to V. Fig. 1 shows that V is only partly (50-60 %) converted to Z under high light conditions; the intrinsic reason might be that the unconverted V pool is bound to a light harvesting center (LHCII; PFÜNDEL and BILGER 1994; HAVAUX 1998).

Diurnal changes in the field: From 7 a.m. to 7 p.m. the leaf carotenoid composition was determined in leaves of cv. Gf.Ga-47-42 positioned at the top of the canopy.

Fig. 4 shows that increasing photon flux density (PFD) in the morning and decreasing PFD in the afternoon were paralleled by increases and decreases of A+Z while V decreased to a minimum at noon and slowly increased in the afternoon. Compared to the reactions in the morning the decrease of A+Z and the increase of V were delayed in the afternoon when light intensity declined. Thus, the xanthophyll-irradiance relationship shows hysteresis (e.g. diurnal cahnges of Z, Fig. 5) which is in agreement with the results demonstrated in Figs. 2 and 3. A complete equilibrium might be achieved later, possibly during the following night, as has been described for the stomatal conductance - irradiance relationship (NG and JARVIS 1980). Referring to ADAMS and DEMMIG-ADAMS (1992) hysteresis in the Z content between ascending and descending light response curves occurs in species which de-epoxidize a large fraction of the V pool. The amounts of lutein, β-carotene and neoxanthin were shown to be fairly constant during the day (data not shown), confirming results of ADAMS AND DEMMIG-ADAMS (1992).

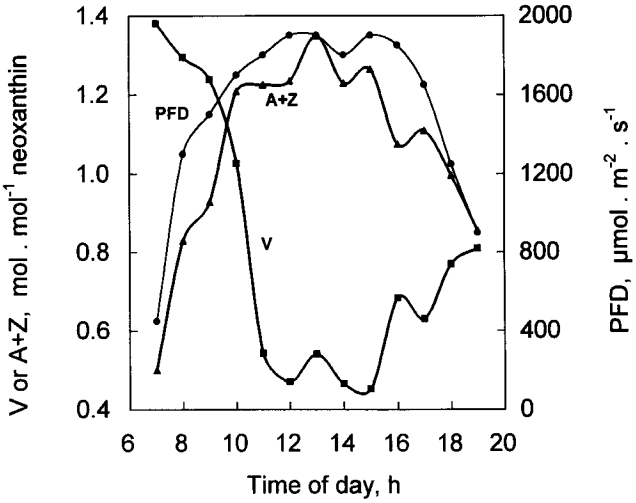

Fig. 4: Diurnal changes of incident light (PFD, photon flux density) and of the violaxanthin (V) and antheraxanthin + zeaxanthin (A+Z) contents in leaves of field-grown vines. Variety: Gf.Ga-47-42. Mean values of 5 replicates.

In Fig. 6 diurnal changes of PFD and the 'epoxidation-state' (EPS, V+0.5A / V+A+Z) are shown. EPS values which are closely related to the apparent quantum yield (or apparent photon efficiency) of photosynthesis (THAYER and BJÖRKMAN 1990) were lowered at peak irradiance by ca. 55 % reflecting a distinct depression of photosynthesis at noon

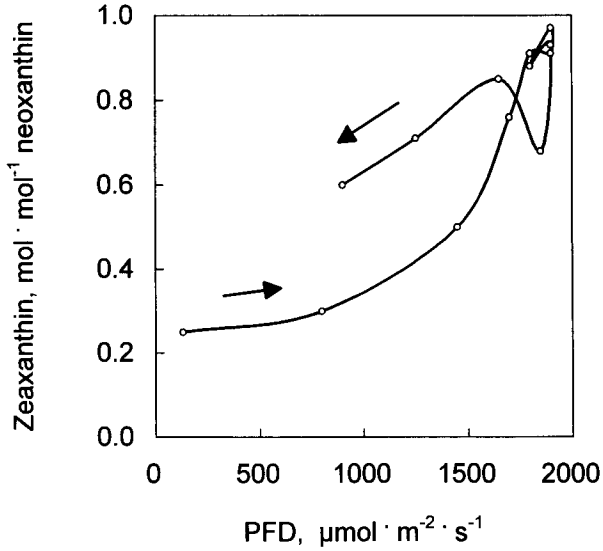

Fig. 5: Changes of the zeaxanthin (Z) content of leaves in response to diurnal changes of PFD (photon flux density). Arrows indicate the course of changes of PFD and Z in the morning and afternoon, respectively. Variety: Gf.Ga-47-42.

and in the early afternoon. This is probably associated with the 'midday depression of photosynthesis' which has been observed in grapevines (CHAVES *et al.* 1987; CORREIA *et al.* 1990; DÜRING 1991) and other species (TENHUNEN *et al.* 1984, RASCHKE and RESEMANN 1986, DEMMIG-ADAMS *et al.* 1989). The degree to which the xanthophyll cycle is de-epoxidized at midday varies between species and depends on the capacity of leaves for photosynthetic electron transport. Compared to the species investigated by ADAMS and DEMMIG-ADAMS (1992) the vine variety Gf.Ga-47-42 used in our trials revealed high Z formation (and low EPS) at noon. Outdoor-grown leaves of this variety are characterized by a slightly pale green colour. E.g., compared to leaves of cv. Regent the chlorophyll a+b and β-carotene content of Gf.Ga-47-42 was reduced by 16 %, the lutein content by 15 % and the neoxanthin content by 12 %. On the other hand, the pool size of the xanthophyll cycle pigments (V+A+Z) was higher in Gf.Ga-47-42 leaves (+24 %) (DÜRING, unpubl.). This composition of pigments of Gf.Ga-47-42 leaves may lead to a better adaptation to high light. Recent results obtained with *Hordeum vulgare* L. have shown that under high light and high tem-

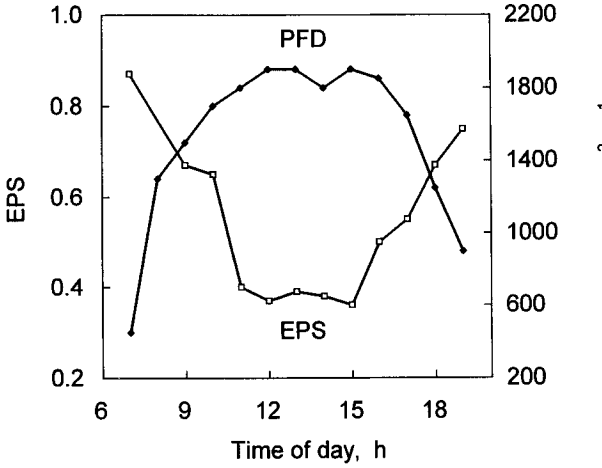

Fig. 6: Diurnal changes of PFD (photon flux density) and EPS (epoxidation state: V+0.5A/V+A+Z). Variety: Gf.Ga-47-42.

perature conditions a low pigment content (chlorophyll and some carotenoids) of leaves was associated with reduced light absorption; in addition, a fast and high conversion of V to Z led to an increased photostability of photosystem II (TARDY *et al.* 1998).

Fig. 3 shows a close relationship between the A+Z content of leaves and PFD during the day. An analoguous relationship was found in isolated guard cells of *Viola faba* leaves and its close correlation to diurnal changes of PFD led to the assumption that Z, besides its role as photoprotector, is a suitable molecular photosensor in guard cells (SRIVASTAVA and ZEIGER 1995).

A series of recent results indicate that under conditions of excessive light and/or high temperature components of the xanthophyll cycle are involved in the stabilization of the lipid phase of the thylakoid membranes as well (review: HAVAUX 1998). As high light and elevated temperatures are often associated this additional, 'structural' role of the xanthophylls stresses the importance of xanthophylls in adaptational processes under harmful environmental conditions. Work is in hand to extend our studies to xanthophylls in berries of grapevine which can be damaged by high light and/or high temperatures.

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