Developmental changes of primary processes of photosynthesis in sun- and shade-adapted berries of two grapevine cultivars

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Summary

Light utilisation and thermal dissipation of field-grown sun- and shade-adapted berries of cvs Kerner (white) and Portugieser (red) were investigated during berry development by determining chlorophyll fluorescence parameters. In stages I and II of berry development for both cultivars the maximum quantum yield of photochemical energy conversion of dark-adapted berries (Fv/Fm) was close to the maximum (0.75-0.80). It decreased significantly in sun- and shade-adapted berries of cv. Kerner two weeks after veraison indicating damage of photosystem II (PSII) during ripening. When at veraison anthocyanins accumulated in the skin of berries chlorophyll fluorescence of cv. Portugieser berries could no longer be determined. In both cultivars the linear electron transport rate at light saturation (ETRmax) increased in stage I and reached maxima in stage II, sun-adapted berries showing higher rates than shade-adapted. After high values in stage I non-photochemical quenching at light saturation (NPQmax) decreased in light- and shade-adapted Kerner berries in stage II indicating a lowering of the thermal dissipation efficiency. The pool size of the xanthophyll cycle pigments diverged significantly in sun- and shade-adapted berries during their development: under clear, warm and dry weather conditions in shade-adapted berries the xanthophyll pool size decreased to low levels, while in sun-adapted berries it increased to maximum values shortly before (cultivar Kerner) or at veraison (cultivar Portugieser) and subsequently declined. Unripe, sun-adapted berries of both cultivars showed a transient decline of the xanthophyll pool size during a rain period suggesting pool size adjustment to changes of ambient conditions in the longer term. It is concluded that unripe, sun-adapted berries are better adapted to high light than shade-adapted berries due to their higher capacity of photosynthetic energy consumption and thermal energy dissipation; at the onset of ripening these photoprotective mechanisms appear to loose importance.

K e y   w o r d s : Grape berry, light adaptation, photosynthesis, chlorophyll fluorescence, electron transport rate, thermal dissipation, xanthophyll cycle.

Introduction

Under natural conditions leaves but also grape berries are exposed to most variable light intensities on a diurnal or seasonal time scale and excess light, possibly associated with other ambient stress factors, has been hypothesized to be responsible for 'sunburn'-like disorders of grape berries (Pearson and Goheen 1988, review: Mohr and Düring 2001).

Determination of primary processes of photosynthesis in leaves of field-grown vines have confirmed that shade- and light-adapted leaves differ in their capacity to utilize light energy in photochemical processes under conditions of increasing light intensity. Moreover it was shown that relative electron transport rate at light saturation (ETRmax) differs between genotypes and that the dissipation of excess energy is associated with the xanthophyll pool size (Ortoidze and Düring 2001); the latter is known to play a decisive role in photoprotection of plants (Björkman and Demmig-Adams 1994; Horton et al. 1996). While most experiments were carried out with stressed leaves (grapevine: Düring 1998; Flexas et al. 1999; Iacono and Sommer 2000) and few with dormant bud and shoot tissue (Ortoidze et al. 1988; Ivanov et al. 1990; Düring et al. 1990), to the best of our knowledge, determination of primary processes of photosynthesis was not reported for fruit. The fact that the outer hypodermis and the mesocarp of grape berries contain chloroplasts (Hardie et al. 1996) encouraged us to determine chlorophyll fluorescence of berries.

The aim of the present study was to elucidate for two grapevine cultivars the capability of grape berries for light utilisation and photoprotection during their development by determining chlorophyll fluorescence parameters and the xanthophyll cycle pigments in the exocarp ('skin') of berries.

Material and Methods

P l a n t   m a t e r i a l : From early July till the end of September 2001 berry growth of field-grown 16-year-old Kerner (Trollinger x Riesling) and Portugieser vines grafted to Kober 5BB was determined weekly or, in stage II of berry development, twice a week. The rows of the experimental vines (vertical espalier) were oriented north-south. The diameter of 20 randomly chosen sun- or shade-adapted berries was determined *in situ* at the berry 'equator' using calipers (resolution: 0.05 mm). 'Sun-adapted' berries were defined as sun-exposed berries at the outer part of the canopy. while 'shade-adapted' berries were exposed to restricted light intensity (<150 µmol m^{-2} s^{-1}) in the inner part of the canopy.

C h l o r o p h y l l   f l u o r e s c e n c e   of berries (cvs Kerner, Portugieser) was determined in the laboratory using a MINI-PAM Fluorescence Meter and WinControl

Software (Walz, Effeltrich, Germany). In the morning berries with part of their pedicel were cut by scissors at the eastern (sun-exposed) part of the canopy, while in the afternoon berries were cut at the western side. Berries were kept in the dark in wet plastic bags for 1 h until the experiments were started. After brief drying by a soft paper tissue berries were fixed under the upper ring of the 'leaf clip holder' of the MINI-PAM system. A saturating light pulse (about 6,000 µmol m^{-2} s^{-1}) was applied to the dark-adapted berries to determine Fv/Fm, the maximum quantum yield of PSII. Light response curves of electron transport rates (ETR) and non-photochemical quenching (NPQ) were derived from yield measurements (Fv'/Fm') at the end of 8 illumination steps (duration of each: 60 s) with light intensities increasing from 0 to 2,350 µmol m^{-2} s^{-1}. Chlorophyll fluorescence parameters were calculated as described in Schreiber et al. (1994) and Ortoidze and Düring (2001). Light response curves obtained from 8-10 berries were averaged. In additional experiments with sun-adapted berries we differentiated between the fully sun-exposed ('front') and the partly sun-exposed ('back') side of single berries. The maximum difference of chlorophyll fluorescence parameters ranged between 6 and 11 %, depending on the developmental stage.

For c a r o t i n o i d   a n a l y s i s berries without petioles (cvs Kerner and Portugieser) were cut from clusters in the field, frozen immediately in liquid N$_2$ and kept at -20 °C until analysis. The number of berries used for carotinoid analysis varied with berry growth from initially 10 to 5. After peripheral thawing the skin of berries, which includes the chlorophyll containing outer hypodermis (Alleweldt et al. 1981; Hardie et al. 1996), was peeled off with a scalpel, frozen in liquid N$_2$ and freeze-dried overnight. After adding N$_2$, skins were ground in a pre-cooled mortar. 100-150 mg of the ground material were transferred to Eppendorf tubes and extracted in acetone (100 %) by centrifugation (64 g, 15 000 rpm) for 10 min at 5 °C. The liquid phase was centrifuged three times and subsequently carotinoids were analysed by HPLC following the procedure proposed by Thayer and Björkman (1990) which was slightly modified (Düring 1999).

Carotinoids of the xanthophyll cycle were related to neoxanthin which was shown to be rather unaffected by light treatments (Björkman and Demmig-Adams 1994; Adams et al. 1999). Analyses were repeated 4 times.

Results and Discussion

B e r r y   g r o w t h : Starting in early July, *i.e.* 13 d after flowering (44 d before veraison), berry growth was determined for cvs Kerner and Portugieser. As shown for cv. Kerner berry growth is divided in two successive sigmoid parts (stages I and III) interrupted by a lag period (stage II) which lasted 14 d. Throughout the experiment the diameter of sun-adapted berries was not significantly different from that of shade-adapted berries for both cultivars (Fig. 1, data for cv. Portugieser were almost identical to cv. Kerner and are not shown).

Q u a n t u m   y i e l d   (F v / F m) : To determine photosynthetic energy conversion field-grown sun- and shade-adapted berries of cvs Kerner and Portugieser, after
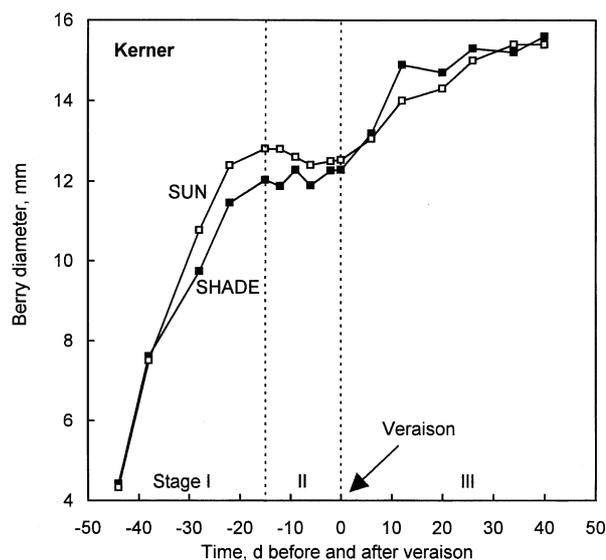

Fig. 1: Growth of sun- and shade-adapted berries (cv. Kerner) and developmental stages I-III.

dark adaptation were exposed to stepwise increasing light intensities.

Fv/Fm, the potential (maximum) quantum yield of PSII, was determined immediately after the dark period when all PSII reaction centres are assumed to be open and thermal dissipation relative to fluorescence emission and photochemistry is minimal (Schreiber 1997). Fv/Fm is an indicator of the maximum efficiency by which excitation energy is absorbed by the reaction centres, a lowering can be ascribed to a damage of PSII (Long et al. 1994; Schreiber 1997).

In sun- and shade-adapted berries of cv. Kerner (Fig. 2 a) Fv/Fm remained close to the maximum (0.75 - 0.80) until about 14 d after veraison; then it declined to about 0.5 (Fig 2a). For cv. Portugieser, Fv/Fm values were also close to the maximum, sun- and shade-adapted berries not being distinctly different (Fig. 3 a). In contrast to the white cultivar Kerner, in the red cv. Portugieser chlorophyll fluorescence could not be determined in stage III due to anthocyanins accumulating in the berry skin.

E l e c t r o n   t r a n s p o r t   r a t e   (E T R) : In berries of cvs Kerner and Portugieser the maximum linear electron transport rate (ETRmax) obtained from light response curves increased in stage I and II, reached a maximum in stage II and, for Kerner, started to decrease before the onset of ripening (Figs 2 b, 3 b). ETRmax of cv. Kerner was low in stage III. For both cultivars in stage I the ETRmax values of sun-adapted berries were higher compared to shade-adapted berries confirming results obtained with leaves of grapevine (Ortoidze and Düring 2001).

There is some evidence that under laboratory conditions ETR and CO$_2$ assimilation can correlate well (Edwards and Baker 1993). Despite the fact that chlorophyll fluorescence emanates from only the top few layers of chloroplast containing cells while gas exchange integrates deeper cell layers as well (Maxwell and Johnson 2000) the present data agree with earlier results of Geisler and Radler (1963), Kriedemann (1968), Frieden et al. (1987) showing that in the first period of rapid berry growth (stage I) part of the absorbed light is utilised in CO$_2$ assimilation which, on a per
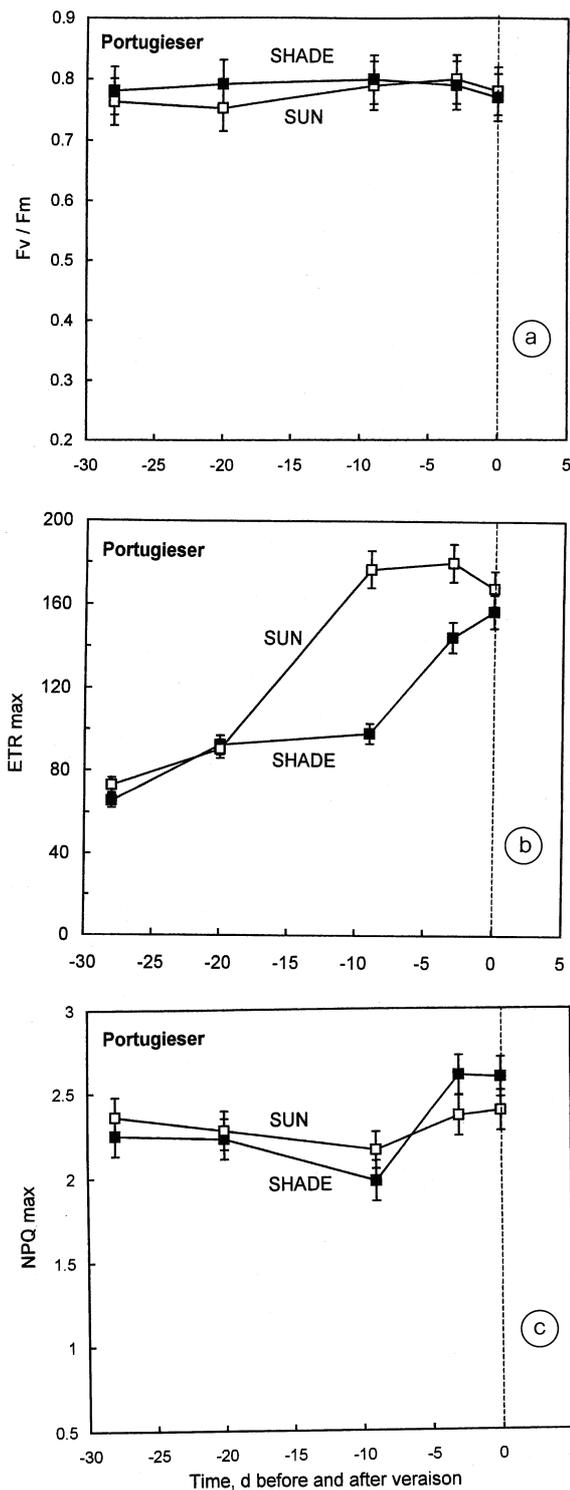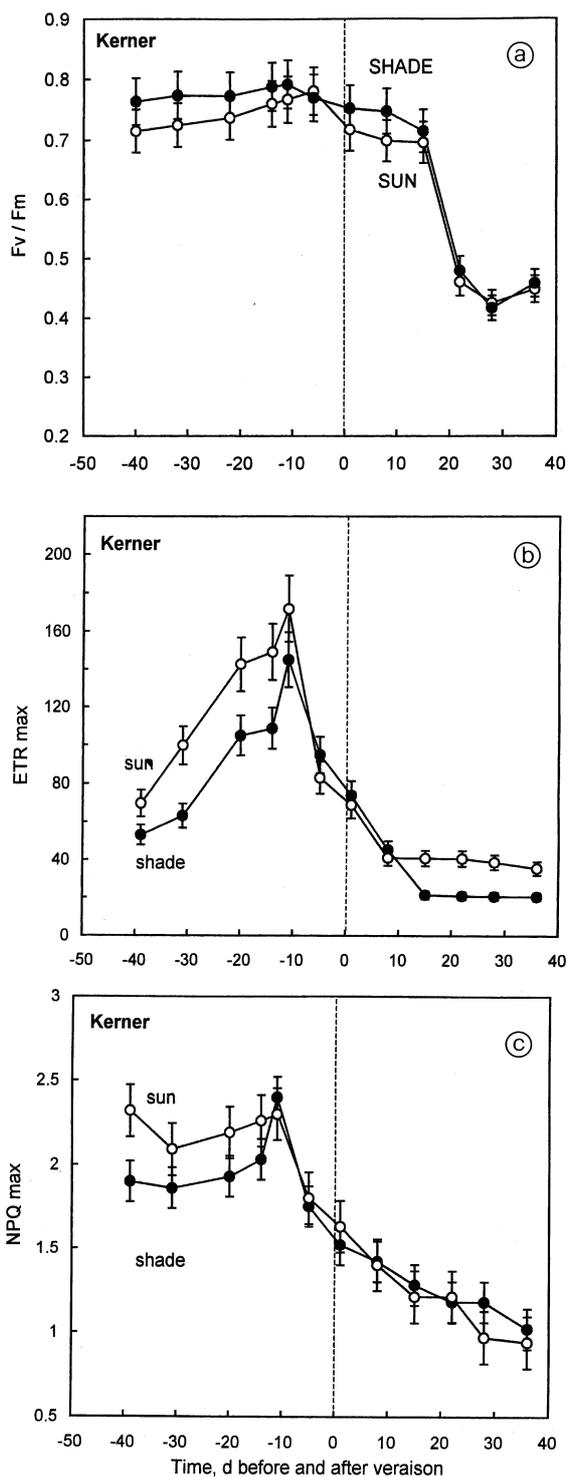
Fig. 2: Chlorophyll fluorescence parameters during the development of sun- and shade-adapted berries of cv. Kerner; dashed line indicates veraison, values are means ±SE. **a)** Maximum quantum yield of dark adapted berries (Fv/Fm); **b)** Linear electron transport rate at light saturation (ETRmax); **c)** Non-photochemical quenching at light saturation (NPQmax).

berry basis, increased until the end of stage I and decreased thereafter. Moreover, findings of HARDIE *et al.* (1996) indicating that the chlorophyll concentration and content of berries after high values before veraison decrease during ripening agree with the above mentioned changes of photosynthetic activity.

Fig. 3: Chlorophyll fluorescence parameters during the development of sun- and shade-adapted berries of cv. Portugieser (**a**, **b**, **c**; details: Fig. 2)

Thermal dissipation of excess energy

Non-photochemical quenching (NPQ) : NPQ denotes that part of non-photochemical quenching which reflects heat dissipation of excitation energy in the antenna system relative to the dark adapted state (BJÖRKMAN and DEMMIG-ADAMS 1994); values are usually in the range

0.5-3.5 at saturating light conditions (MAXWELL and JOHNSON 2000). Maximum NPQ (NPQmax, attained at approximately 2,000 µmol m^{-2} s^{-1}) of sun-adapted berries of cv. Kerner initially varied between 2.0 and 2.5 but started to decline rapidly 5 d before veraison (Fig. 2 c). In stage I NPQmax values of shade-adapted berries of cv. Kerner were somewhat lower compared to sun-adapted berries, but the maximum rate was close to that of sun-adapted berries. In stage II, *i.e.* before veraison, NPQmax started to decline in both, sun- and shade-adapted berries. In stages I and II NPQmax in berries of cv. Portugieser also varied between 2 and 2.5 and slightly increased shortly before veraison; values were not affected by different light regimes (Fig. 3 c).

Xanthophyll pool size: Sun-adapted berries: The process of thermal dissipation protecting plant tissue from damage due to excessive light involves the light-induced formation of the carotenoid zeaxanthin (Z) from violaxanthin (V) and antheraxanthin (A) (BILGER and BJÖRKMAN 1990; YOUNG 1991; DEMMIG-ADAMS and ADAMS 1996; grapevine: DÜRING 1999; ORTOIDZE and DÜRING 2001). It has been shown that these xanthophyll cycle components respond to excessive light both, in the short term (reversible de-epoxidation) and in the longer term (pool size adjustment). The V + A + Z pool size determines the upper limit of thermal dissipation capacity under excess light (BJÖRKMAN and DEMMIG-ADAMS 1994).

In the skin of sun-adapted berries of both cultivars the V + A + Z pool started to increase on July 17 (for cv. Kerner 28 d, for cv. Portugieser 17 d before veraison) and reached maxima on August 9 (for cv. Kerner 11 d before and for cv. Portugieser at veraison) (Fig. 4 a, b). From the coincidence of pool size changes with time rather than berry development we suggested that they were imposed by ambient factors. End of July/early August 15 d of sunny, warm and dry weather coincided with a significant raise of the pool size. This 'pool size adjustment' is further corroborated by the fact that a rain period (August 3 to 8) associated with a lowering of sunshine and temperature led to a transient decline of the V + A + Z pool in sun-adapted berries of both cultivars at the same time but, again, at different developmental stages of berries. HANSON AND SHARKEY (2001) observed that xanthophyll cycle pigments increased in high light but not in high temperature; thus the transient pool size changes in our experiments may be causally related with changes of light intensity during the rain period.

In contrast, the decline of pool size in sun-adapted berries at veraison and thereafter can not be related to changes of weather and is assumed to have developmental reasons.

Comparing maximum pool size of light-adapted Kerner and Portugieser berries the latter cultivar has a distinctly higher limit of thermal dissipation capacity and appears to react less sensitive to the transient low light period.

Shade-adapted berries: At the onset of experiments the V + A + Z pool size in shade-adapted berries of both cultivars was close to that of sun-adapted berries but decreased significantly to low values in stages II and III in both cultivars (Fig. 4 a, b) indicating a distinctly lower capacity of thermal dissipation compared to sun-adapted berries in both cultivars.
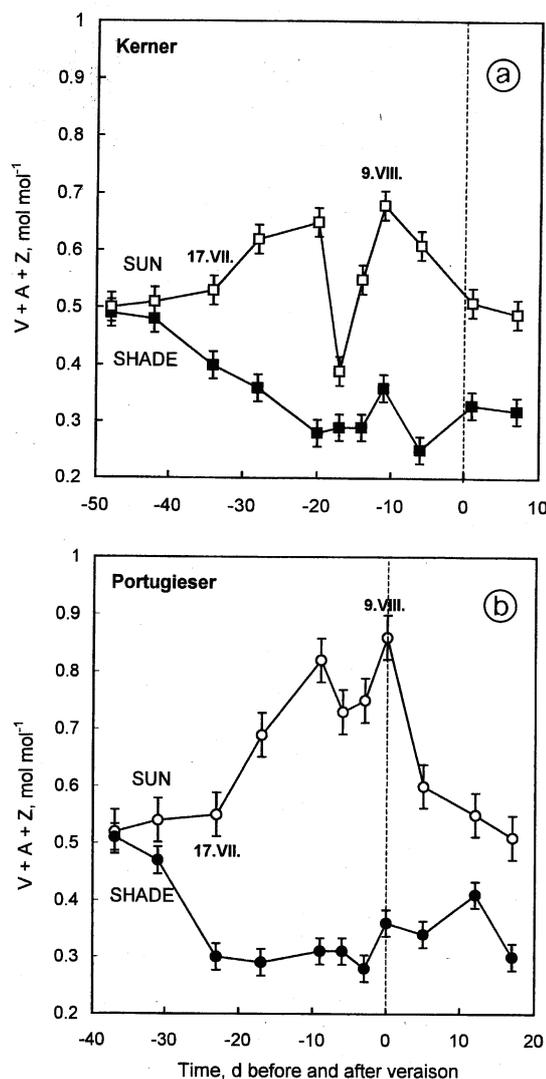

Fig. 4: The xanthophyll pool (violaxanthin + antheraxanthin + zeaxanthin) in the skin of sun- and shade-adapted berries during berry development. **a**) cv. Kerner; **b**) cv. Portugieser.

It has been outlined by MAXWELL AND JOHNSON (2000) that an increase of NPQ may indicate both, processes that protect a plant tissue from excess light or the damage itself. The increase of NPQ in shade-adapted berries presumably can not be ascribed to excess light, since Fv/Fm values in that stage remained high (Figs. 2 a, 3 a), *i.e.* is not indicating photoinhibition. The same is true for the V + A + Z pool size which in shade-adapted berries of both cultivars even slightly decreased.

According to SCHREIBER (1997) shade-adapted plants tend to invest more in the system of antennae to optimise light absorption than in pool size of ETR systems while sun-adapted plants invest more in the ETR system to improve flow rates.

Conclusion

From the present data it can be concluded that before the onset of ripening primary processes of photosynthesis in sun- and shade-adapted berries were not inhibited by

high light (constant high Fv/Fm values). In these stages sun-exposed berries appear to increase their adaptation to high light by raising energy consumption (increasing ETR, high CO_2 assimilation) and high thermal energy dissipation. Shade-adapted berries are expected to be more susceptible to high light exposure, as induced, *e.g.*, by partial defoliation of the cluster zone, which according to our observations can lead to 'sunburn' of berries before veraison.

In contrast, two weeks after veraison (stage III) high light exposure leads to damage of PSII in the berry skin (decline of Fv/Fm). A possible reason is the degeneration of the granal and stromal structure in chloroplasts in this stage of development (HARDIE *et al.* 1996) explaining the lowering of energy consumption (reduced ETR and CO_2 assimilation) and thermal energy dissipation (low V+A+Z pool size and NPQ level).

The reason of the temporal discrepancy between the onset of decline of ETR and NPQ (before veraison) and Fv/Fm (in the course of maturation) is still unclear and needs further investigation; it may be speculated that processes of senescence start with berry growth ceasing in stage II.

On the basis of the present results it will be interesting to study the effects of single ambient stress factors on primary processes of photosynthesis in berries; work is in hand to elucidate responses of berries to high temperature or excessive light with the aim to analyse variety-specific sensitivities of berries to 'sunburn'.

Acknowledgement

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