

Photochemical and non-photochemical responses of glasshouse-grown grape to combined light and water stress

by

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S u m m a r y : Riesling and Müller-Thurgau vines cultivated in a glasshouse at maximal $400 \mu\text{mol quanta m}^{-2}\text{s}^{-1}$ were exposed to water logging and subsequently to decreasing soil moisture contents (SMC) until severe drought stress occurred. In the course of the daily applied light treatments, consisting of stepwise increases of light intensity from 0 to $3200 \mu\text{mol m}^{-2}\text{s}^{-1}$ with intermittent stabilisation phases, the Fv/Fm of dark-adapted and the Fv/F'm ratios of light-adapted leaves were determined by chlorophyll fluorescence measurements. At moderate SMC (21 %) quantum yield of PSII (Y) of Müller-Thurgau leaves decreased when photosynthetic active radiation (PAR) increased beyond $400 \mu\text{mol m}^{-2}\text{s}^{-1}$. Water stress (water logging and drought) led to a more precipitous decrease, indicating an increased sensitivity of Y to the combined stresses. The deviation of the electron transport rates from (theoretical) maximum quantum yield ("excessive PAR") increased with increasing PAR and was higher in water-stressed than in moderately irrigated vines. Non-photochemical quenching started to increase at relatively low PAR ($400 \mu\text{mol m}^{-2}\text{s}^{-1}$) and reached saturation at $1600 \mu\text{mol m}^{-2}\text{s}^{-1}$; values were higher in water-stressed vines than in moderately irrigated vines. Riesling and Müller-Thurgau vines showed photoinhibition of photosynthesis at high PAR; water logging and drought intensified this tendency. The extent of photoinhibition was higher in Müller-Thurgau than in Riesling leaves. Even under most severe stress conditions photoinhibition was transient, suggesting complete overnight repair of PSII in both varieties.

Key words : grapevine, leaf, light, water stress, photosynthesis, photochemistry, photoinhibition, photosystem II, chlorophyll fluorescence.

Introduction

In many parts of the world, in particular in mediterranean or subtropical climates, grapevines are subjected to periods of low rainfall which are often associated with clear days and high solar radiation. Unless irrigated, grapes may suffer from multiple stresses, i.e. drought, high temperature and high light (OSMOND *et al.* 1986, VALLADARES and PEARCY 1997). It has been shown earlier that, in contrast to field-grown Riesling vines, CO₂ assimilation of glasshouse-grown vines exposed to restricted light intensities declined when light intensity increased beyond $800 \mu\text{mol m}^{-2}\text{s}^{-1}$. Since this decline could not be ascribed to stomatal closure we assumed that high light intensity might have inactivated the photochemistry of the photosystem II (PSII) reaction centres (photoinhibition) (DÜRING 1988). Evidence for photoinhibition to occur also in field-grown grape was provided by GAMMON and PEARCY (1990), IACONO and SOMMER (1996) and SCHULTZ (1996); in contrast CHAUMONT *et al.* (1997) demonstrated the absence of photoinhibition in outdoor-grown vines under two different climatic conditions. Moreover, the significance of water stress to enhance photoinhibition is discussed controversially. GAMMON and PEARCY (1990) concluded that the degree of light-dependent decline of PSII fluorescence (Fv/Fm) did not vary with the water status while BJÖRKMAN and POWLES (1984) suggested that water stress predisposes the leaves to photoinhibition. Diverging results may be due to differences in plant species, stress intensity or prehistory of plants, e.g. the degree of light adaptation. The aim of this paper was to study the effects of two combined stresses, flooding or drought and increasing light intensity on the quantum yield of PSII under controlled labo-

ratory conditions to better understand the mechanism(s) leading to non-stomatal limitation of photosynthesis in grapes.

Material and methods

Plant material : In March and April 1997 experiments were carried out with 3-year-old potted, glasshouse-grown Müller-Thurgau and Riesling vines. Maximum light intensity at the apical leaves was $400 \mu\text{mol m}^{-2}\text{s}^{-1}$ on clear days. Plants were transferred to the laboratory and kept at $23/17 \text{ }^\circ\text{C}$ ($\pm 2 \text{ }^\circ\text{C}$) (day/night) and a photoperiod of 14 h. Light was provided by Osram HQI lamps, 400 W; $300\text{--}400 \mu\text{mol m}^{-2}\text{s}^{-1}$ were measured at the leaf blades selected for experiments. Investigations were started one week after acclimation of vines, which were grown in standard soil ("Fruhstorfer Erde P") containing peat (50 %), granulated volcano-clay (35 %) and bark humus (15 %); they were selected for uniform shoot length (ca. 1.50 m).

Soil humidity and leaf water status : Vines were irrigated daily and supplied with mineral nutrients (Hakaphos grün, Compo, Münster, Germany) twice a week before the experiments were started. At the onset of the experiments they were fully irrigated (100 % water capacity = ca. 50 % soil moisture content (SMC)); thereafter irrigation was withheld for the duration of the experiment (12–14 d). The SMC was determined daily, before and after the light treatments, by a TDR-Trase System (Santa Barbara, California, USA). The leaf water potential was determined at the end of the experiments by the pressure chamber method (SCHOLANDER *et al.* 1965).

Chlorophyll fluorescence: Chlorophyll fluorescence was measured at the adaxial part of fully expanded but not senescent leaves with a PAM chlorophyll fluorometer (H. Walz, Effeltrich, Germany). According to SCHREIBER *et al.* (1994) the minimum (F_0) and maximum fluorescence (F_m) following a saturating light pulse (0.7 s, $3500 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) were used to determine the variable fluorescence (F_v)

$$F_v = F_m - F_0 \quad (1)$$

The F_v/F_m ratio of dark-adapted leaves is a measure of the potential (maximum) quantum yield of PSII, the F_v/F'_m ratio of light-adapted leaves is an indicator of the actual quantum yield of PSII. The relative electron transport rate (ETR) can be derived from quantum yield of PSII (Y), photosynthetic active radiation (PAR) and a constant ($c=0.42$) (SCHREIBER 1997):

$$\text{ETR} = Y \cdot \text{PAR} \cdot c \quad (2)$$

Non-photochemical fluorescence quenching (NPQ) was calculated according to the Stern-Volmer equation (BJÖRKMAN and DEMMIG-ADAMS 1994):

$$\text{NPQ} = F_m/F'_m - 1 \quad (3)$$

Light treatments: Each experiment started and ended by darkening a leaf with aluminium foil for 30 min. All treatments/measurements were performed at the same site of a leaf: determination of F_v/F_m (A in Fig. 1), exposure to $20 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (Osram L 18 W/77 Fluora) for 20 min, exposure to 400, 800, 1600 and $3200 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (20 min, fiber illuminator, Type KL 1500, Schott, Mainz, Germany) and determination of F_v/F'_m by three saturating light pulses, separated by 30 s, at the end of each light treatment. Between each light treatment the leaf was exposed to $20 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (stabilisation phases, according to MAURY *et al.* 1996). After light treatments and leaf darkening F_v/F_m (B) was determined. The relative rate of photo-inhibition (%) was calculated by relating F_v/F_m (B) to the initial F_v/F_m (A) (SCHREIBER 1997). Experiments were repeated 4 times, results in Fig. 3 and 4 were calculated from average values in Fig. 2; results in Fig. 5 were obtained from two consecutive experiments.

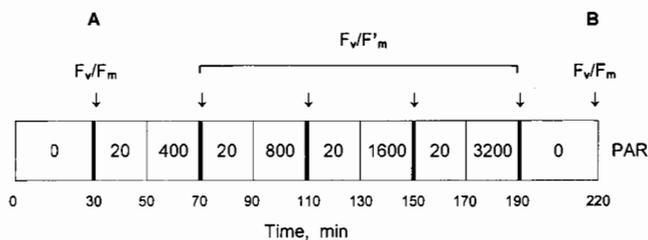


Fig. 1: Experimental scheme of light treatments starting with a dark period (30 min) followed by periods of increasing light intensity with stabilisation phases inbetween (PAR, $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) and a final dark period. F_v/F_m - quantum yield of PSII of dark-adapted leaves, F_v/F'_m - quantum yield of PSII of light-adapted leaves.

Results

Photochemical and non-photochemical reactions: During their development under glasshouse and laboratory conditions the vines used in our experiments were kept under restricted light conditions (maximum light intensity (PAR) ca. 1/5 of full sunlight). Experi-

ments were started with fully irrigated vines. By withholding irrigation soil moisture content (SMC) decreased slowly to 5.5 % (predawn leaf water potential = -0.60 MPa). Fig. 2 illustrates the relative quantum yield of PSII (Y) at various PARs and high, medium and low SMCs. Irrespective of SMC the initial Y -values of dark-adapted leaves were always close to 0.80 (=100 %) indicating fully open PSII centres and complete overnight recovery from previous high light stress during the experiment (SCHREIBER 1997). At medium SMC (21.1 %) Y started to decrease at $\text{PAR} > 400 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. At high and low SMC increasing PAR led to a precipitous lowering of Y compared to medium SMC. Fig. 3 shows the relative electron transport rate (ETR) in leaves at the three different SMCs as a function of PAR. At high and low SMC

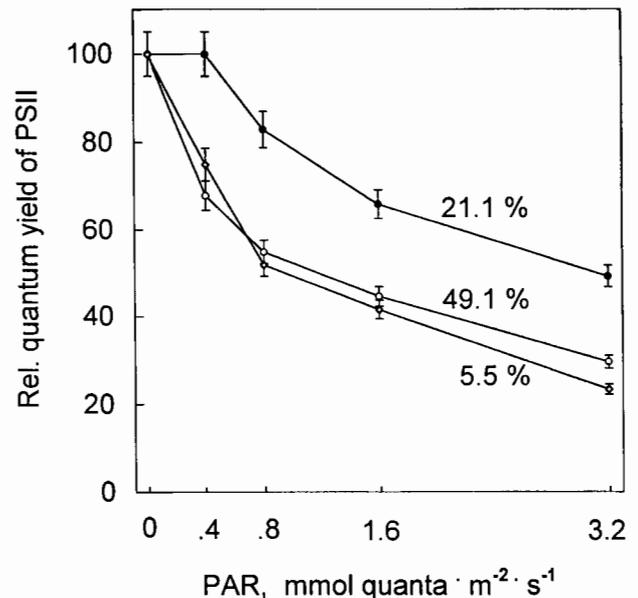


Fig. 2: Quantum yield of PSII (F_v/F'_m) of Müller-Thurgau leaves exposed to increasing light intensities at three different soil moisture contents (%). Bars denote 95 % confidence limits.

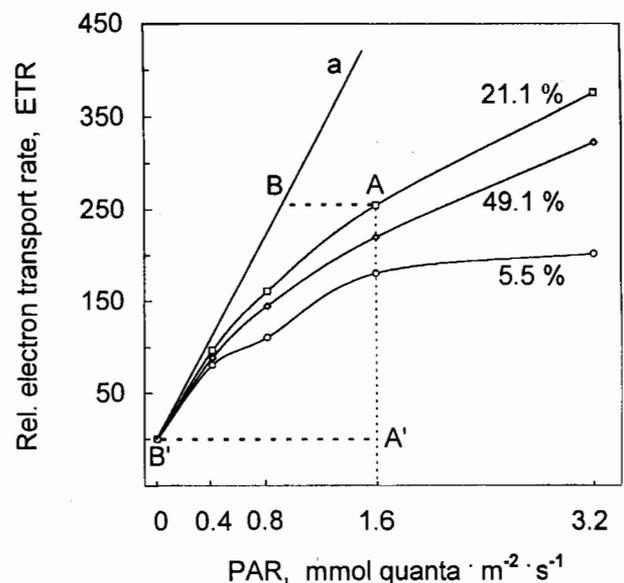


Fig. 3: Relative electron transport rate (ETR) as a function of PAR in leaves of Müller-Thurgau at medium (21.1 %), high (49.1 %) and low (5.5 %) soil moisture content. Line "a" is the (theoretical) optimum quantum yield line, 'excessive PAR', e.g. at medium soil moisture content, can be calculated by relating A - B to A' - B' (for details: Material and methods).

ETRs were reduced compared to medium SMC; e.g. at $1600 \mu\text{mol m}^{-2} \text{s}^{-1}$ ETR was reduced by 14 % (high SMC) and 29 % (low SMC). At this light intensity 'excessive PAR' (SCHREIBER *et al.* 1994), calculated from experimental light response curves and the maximum quantum yield line "a" in Fig. 3, was highest at 5.5 % SMC ($960 \mu\text{mol m}^{-2} \text{s}^{-1}$) followed by 49.1 % SMC ($816 \mu\text{mol m}^{-2} \text{s}^{-1}$) while at 21.1 % SMC "excessive PAR" was $640 \mu\text{mol m}^{-2} \text{s}^{-1}$. It is interesting to note that even under favorable soil moisture conditions the capacity of photosynthetic electron transport is limited to the light intensity the vines were adapted to before the onset of experiments. Responses of non-photochemical quenching (NPQ) to increasing PAR at the three soil moisture levels are shown in Fig. 4. Even at low PAR drought and flooding led to distinctly higher NPQ values compared to medium SMC. Independent of SMC NPQ saturation was observed at $\text{PAR} > 1600 \mu\text{mol m}^{-2} \text{s}^{-1}$ indicating that the maximum capacity was reached and that leaves were unable to further counteract the increase of excess excitation energy (BJÖRKMAN and DEMMIG-ADAMS 1994).

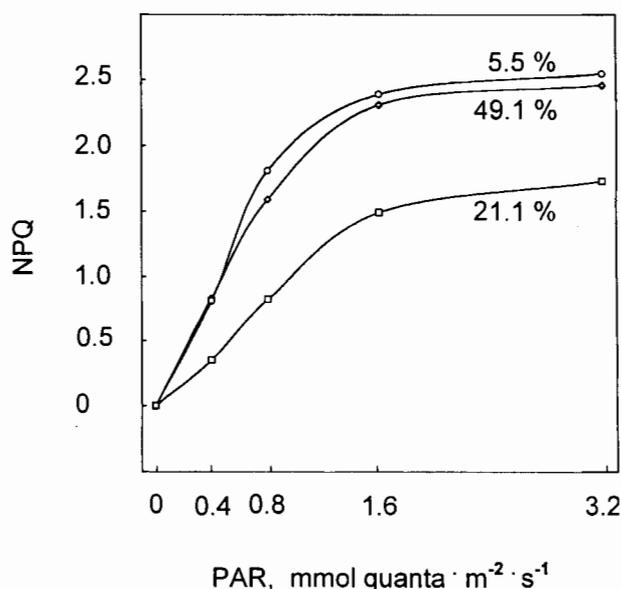


Fig. 4: Non-photochemical quenching (NPQ) related to PAR at 5.5, 49.1 and 21.1 % soil moisture content. Variety: Müller-Thurgau.

Evidence for photoinhibition: According to POWLES (1984), DEMMIG-ADAMS and ADAMS III (1992), LONG *et al.* (1994) and TREBST (1994) light intensities exceeding the photosynthetic demand can cause a slowly reversible retardation of photosynthesis, independent of any developmental change, due to a damage of PSII, the so-called photoinhibition of photosynthesis. SCHREIBER (1997) proposed to determine photoinhibition by the rate of recovery of Fv/Fm in a dark period after high light treatment. Fig. 5 shows the relative quantum yield of PSII (B/A ratio, Fig. 1) of Riesling and Müller-Thurgau leaves which were exposed daily to the light treatment during the decline of SMC from ca. 50 % to ca. 4 % corresponding to a decrease of the predawn leaf water potential from zero to -0.7 MPa. Obviously under flooding (SMC >40 %) and drought conditions (SMC <15 %) the relative quantum yield of PSII (Y) was reduced as compared to the medium range of SMC. Except for extreme drought (SMC <5 %) Y values of Riesling were

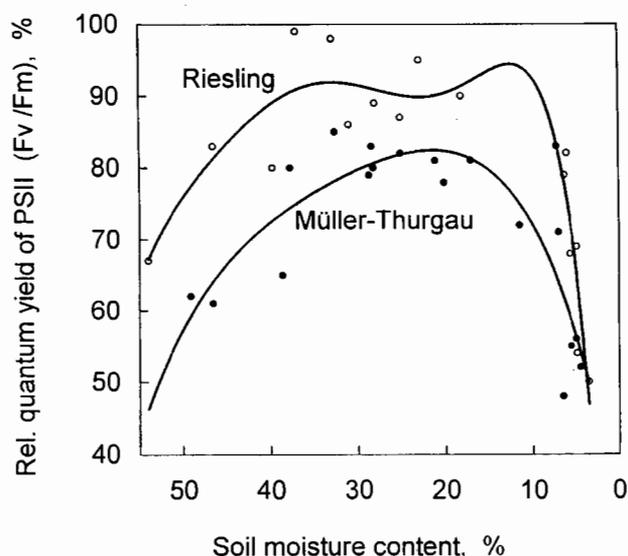


Fig. 5: Light- and water stress-induced photoinhibition of photosynthesis of Riesling and Müller-Thurgau vines. The relative quantum yield of PSII was calculated by relating Fv/Fm after the light treatment (B) to Fv/Fm before the light treatment (A) (for details: Fig. 1). The correlation coefficient (r^2) for Riesling is 0.86, for Müller-Thurgau 0.69.

always higher than those of Müller-Thurgau. It is interesting to note that even under favorable SMC (ca. 20-35 %) most Y values of Riesling and Müller-Thurgau were depressed. This indicates that the PSII function is partly inactivated even at favorable SMC due to excessive light, leaves of Müller-Thurgau being more severely damaged than those of Riesling. In no case reductions of Y were observed the following day at the end of the first dark period, indicating a complete overnight repair of PSII.

Discussion

In this paper we investigated responses of the photosynthetic apparatus of grape leaves to the absorption of increasing levels of visible light. Since we used vines adapted to restricted light ($\text{PAR} < 400 \mu\text{mol m}^{-2} \text{s}^{-1}$) the range of PAR which was photosynthetically utilised, was relatively small, in unstressed vines $800 \mu\text{mol m}^{-2} \text{s}^{-1}$ leading already to a reduction of quantum yield of PSII and electron transport; water-stressed vines were shown to be still more sensitive. The small range of light utilisation by photosynthesis is also demonstrated by calculating NPQ which increased already at relatively low light intensities reaching saturation at $1600 \mu\text{mol m}^{-2} \text{s}^{-1}$. These observations confirm that "low light leaves" have a limited capacity of photosynthetic electron transport and energy conversion but also a limited capacity for photoprotective responses such as energy dissipation. In contrast, sun-adapted, outdoor-grown grapes had shown no depression of photosynthesis at increasing PAR even when exposed to $2250 \mu\text{mol m}^{-2} \text{s}^{-1}$ (DÜRING 1988). An exposure of our glasshouse-grown vines to light intensities exceeding maximum solar radiation caused "photoinhibition" according to the definition of SCHREIBER (1997). This transient limitation of quantum yield of PSII was modified by

two factors, soil water and genotype. Obviously high light combined with soil water stress (water logging or drought) had an additive effect, i.e. both stresses depressed quantum yield of PSII more than high light treatments at moderate soil water conditions. The transient character of quantum yield limitation is demonstrated by the complete overnight repair of PSII, indicating that the "PSII repair cycle" (migration of light-damaged PSII centres out of the granal thylakoids and migration of undamaged PSII centres into the stromal lamellae) might have been involved (for details: DEMMIG-ADAMS and ADAMS III 1992, ARO *et al.* 1993, LONG *et al.* 1994). The variety-specific reactions of photoinhibition may be associated with leaf structure since Riesling leaves showing less photoinhibition are thicker and resemble more to a sun-adapted leaf type than leaves of Müller-Thurgau. Moreover, Riesling leaf tissue has been shown to be more drought resistant due to its higher elasticity compared to Müller-Thurgau (DÜRING 1986) which might have contributed to mitigate the stress response in Riesling leaves in the present experiments as well. Light-adapted, field-grown vines were shown recently not to be affected by photoinhibition (CHAUMONT *et al.* 1997); however, from the present results it can be deduced that photosynthesis of shade-adapted leaves in the inner part of a canopy is reduced by high solar radiation, e.g. after summer pruning or by sunflecks (KRIEDEMANN *et al.* 1973).

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