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FUNCTIONAL PLANT BIOLOGY

Continuing Australian Journal of Plant Physiology

FPB

VOLUME 29, 2002
© CSIRO 2002

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Effects of drought on light-energy dissipation mechanisms in high-light-acclimated, field-grown grapevines

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This paper originates from a presentation at the Light Stress satellite meeting of the 12th International Photosynthesis Congress, held at Heron Island, Queensland, Australia, August 2001

Abstract. The response of several light-energy dissipation mechanisms to water shortage was analysed in a 10-year study in field-grown, high-light-acclimated grapevines, and compared with those of greenhouse-grown, low-light-acclimated grapevines. Dissipation mechanisms, except leaf photochemistry, differ among cultivars and acclimate to the prevailing light conditions during growth. However, no additional acclimation to drought was observed. The dependence of the dissipation responses on stomatal conductance suggests that low CO₂ availability in the chloroplasts during drought triggers variations in the energy dissipation pattern. In irrigated grapevines under high light, more than 50% of total absorbed energy is thermally dissipated. There is evidence that implicates the xanthophyll cycle as the main thermal dissipation processes. CO₂ assimilation is the most important photochemical pathway of dissipation in irrigated plants, but is replaced by photorespiration when CO₂ assimilation declines under mild drought. Under moderate to severe drought, both photosynthesis and photorespiration decline, and thermal dissipation increases to account for up to 90% of total dissipation. Involvement of other processes in light dissipation is minimal in grapevines. Even in severely-stressed leaves, the incidence of photoinhibition is very low, indicating that safe dissipation of absorbed energy is very effective in grapevines.

Introduction

A combination of high irradiance, high temperature and water stress (i.e. drought) is common during summer under Mediterranean and other semi-arid conditions. Therefore, drought is a multiple stress, involving the interaction of light, temperature and water stress. Any stress leads to immediate plant responses, which are usually characterized by detrimental decreases in rates of physiological processes like photosynthesis. Grapevine (*Vitis vinifera* L.) is a species well-adapted to drought, since it is traditionally a non-irrigated crop that develops most of its phenological cycle during summer in drought-prone areas (Mullins *et al.* 1998). Even so, water stress limits photosynthesis and decreases the light required to saturate photosynthesis in grapevines (Flexas *et al.* 1998; Escalona *et al.* 1999). Therefore, as drought progresses, light is in excess of that

which can be used in photosynthesis, which may increase susceptibility to light stress and photoinhibition (Osmond *et al.* 1999). Several studies have shown that grapevines become photoinhibited upon exposure to a combination of high light and other environmental stresses, like heat (Gamon and Pearcy 1990a) or chilling (Chaumont *et al.* 1995).

Most of these studies used low-light-grown potted plants, in which stress conditions were applied rapidly. However, under field conditions, these stresses are imposed gradually and persist for a long period (e.g. days or weeks), which is likely to cause differences in acclimation. Acclimation is the morphological and physiological homeostatic adjustment by plants to compensate for stress-induced decline in performance (Lambers *et al.* 1998). This adjustment occurs through changes in activity or synthesis of new biochemical constit-

Abbreviations used: A, antheraxanthin; A_N , rate of net CO₂ assimilation; A_G , A_G^* , rate of gross CO₂ assimilation accounting for dark respiration and respiration plus photorespiration, respectively; DEPS, de-epoxidation state of the xanthophyll cycle; ETR, electron transport rate; F_v/F_m , quantum efficiency of PSII in dark-adapted leaves; g , light-saturated maximum stomatal conductance; LWD, leaf water deficit; NPQ, non-photochemical quenching of chlorophyll fluorescence; R_D , R_L , rate of respiration in the dark and light, respectively; RWC, leaf relative water content; V, violaxanthin; Z, zeaxanthin; Ψ , leaf water potential.

uents such as enzymes, which require changes in expression of certain genes. Although the concept of plant hardiness to drought (i.e. acclimation to drought induced by controlled pre-exposure to drought conditions) and some of its physiological bases have been known for a long time (Henckel 1964), the underlying genetics are still poorly understood (Shinozaki and Yamaguchi-Shinozaki 1998). A similar situation occurs with respect to light stress, and to the combination of light and drought stresses. Only recently have gene expression and biochemical changes (i.e. the molecular basis for acclimation) in response to environmental stress attracted attention, and only a few genes whose regulation is modified by light stress or drought have been identified (Osmond *et al.* 1999).

At present, there are limitations on the performance of gene expression studies in the field, where plants are more likely to acclimate to prevailing conditions. However, acclimation to a given stress condition can be examined by comparing the response of different genotypes to the same environment (Schultz 1998) or by comparing the response of a given genotype to different environments (Chaumont *et al.* 1997). For instance, comparing field-grown plants with plants grown under controlled environments may be a good method for acclimation studies.

The aim of the present work is to summarize our knowledge of light energy dissipation in grapevines under drought. For this purpose we compiled the results of a 10-year study of two cultivars, with contrasting response to drought growing under field conditions, in which dissipation mechanisms under combined high light and drought were examined. To gain some insight into plant acclimation processes, we focussed on both comparisons between several cultivars and comparisons between field and greenhouse experiments.

Materials and methods

Plant material and processes analysed

Results from a 10-year study in field-grown grapevines were re-examined. The study was undertaken in Mallorca, Spain, under Mediterranean climate, with plants 12 years of age at the beginning of the experiment. Two cultivars were used: Tempranillo, a common Spanish cultivar, and Manto Negro, a Mallorcan cultivar reputed to be more drought tolerant. Cultural practices were as described previously (Flexas *et al.* 1998; Escalona *et al.* 1999), and two treatments were established: moderate irrigation, accounting for 30% of the weekly measured evapotranspiration, and water stress (i.e. rain-fed plants). Environmental conditions during the experiments (June to September) were characterized by high light intensities, day temperatures usually above 30°C, and potential evapotranspiration of about 150 L m⁻² per month. Rainfall was almost null, and intra- and inter-annual climatic variations have already been described (Flexas *et al.* 1998; Escalona *et al.* 1999). Additional experiments were performed simultaneously with these and other grapevine cultivars growing in pots, under both field and glasshouse conditions (Flexas *et al.* 1999a, b, 2000, 2002a; Bota *et al.* 2001). Comparison of results obtained in field-grown plants with those of greenhouse-grown plants, which grow under lower light

intensities, may reveal some insights into plant acclimation to high light and water stress.

The effects of drought on several energy dissipation processes were analysed. Processes analysed are summarized in Fig. 1, which is a simplification of a scheme by Niyogi (1999) in which all the known leaf energy dissipation processes were listed. In particular, the effects of drought on chlorophyll content, leaf photochemistry, CO₂ assimilation, photorespiration, Mehler reaction, xanthophyll-dependent thermal dissipation, and photoinactivation of PSII were analysed.

Plant water status and stress determination

Pre-dawn leaf water potential (Ψ) was determined to be the best indicator of grapevine water status (Flexas *et al.* 1998, 1999a; Escalona *et al.* 1999). In some of the studies, relative leaf water content (RWC) and leaf water deficit (LWD) were also determined (Flexas *et al.* 1999a, b, 2000). However, as recently discussed (Flexas and Medrano 2002; Flexas *et al.* 2002a; Medrano *et al.* 2002), the response of

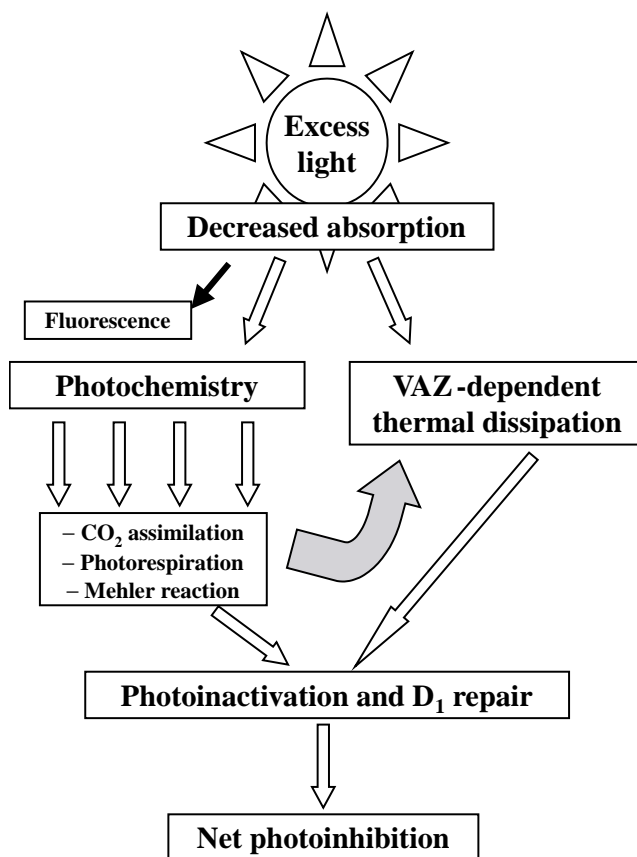


Fig. 1. Different dissipation mechanisms analysed (modified from Niyogi 1999). Excess light can be avoided by decreasing its absorption by the leaf. When absorbed light is excessive, a negligible part is dissipated by chlorophyll fluorescence, and most of the energy is used to drive photochemistry or is dissipated as heat in the antenna via a VAZ-dependent mechanism. Photochemical energy can be used for CO₂ assimilation, photorespiration, the Mehler reaction, and other processes (not shown). All these mechanisms may be insufficient for harmless dissipation of all absorbed energy, and some PSII units eventually become photoinactivated. The D₁ repair mechanism replaces photoinactivated PSII units with functional units. Whenever the rate of repair is lower than the rate of photoinactivation, net photoinhibition occurs.

photosynthesis to Ψ and/or RWC in field-grown grapevines, as well as in other C_3 species, strongly depends on the conditions during plant growth and measurements. Moreover, grapevine is an isohydric species, in which similar Ψ and RWC are reached at midday by both irrigated and drought-stressed plants. Nevertheless, when light-saturated maximum stomatal conductance (g) was used as the reference parameter reflecting drought intensity, a common response pattern was observed, much less dependent on species and conditions. Many photosynthetic parameters (e.g. electron transport rate, carboxylation efficiency, intrinsic water-use efficiency, respiration rate in the light) were also more strongly correlated with g than with water status itself (Flexas and Medrano 2002; Flexas *et al.* 2002a; Medrano *et al.* 2002). Therefore, in the present study, g will be used as the more general parameter indicative of drought intensity.

Chlorophyll fluorescence and gas exchange measurements

Chlorophyll fluorescence parameters were measured on attached leaves under natural saturating light conditions, and used to analyse the effects of drought on leaf photochemistry, thermal dissipation and photoinactivation. A portable pulse amplitude modulation fluorometer (PAM-2000; Walz, Effeltrich, Germany) was used in field experiments. Measuring light of about $0.5 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ was set at a frequency of 600 Hz to determine, at pre-dawn (06:00 local time), the background fluorescence signal (F_0), maximum fluorescence (F_m), and maximum quantum efficiency of PSII [$F_v/F_m = (F_m - F_0)/F_m$]. The same measuring light was used to measure the steady-state fluorescence signal (F_s) under sunlight, except that its frequency was increased to 20 kHz. To obtain the steady-state maximum fluorescence yield (F_m'), saturation pulses of about $10000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ and 0.8 s duration were applied. PSII photochemical efficiency (F/F_m' ; Genty *et al.* 1989) was then calculated as:

$$\Delta F/F_m' = (F_m' - F_s)/F_m'$$

and used for calculation of the linear electron transport rate (ETR) according to Krall and Edwards (1992):

$$\text{ETR} = \Delta F/F_m' \times \text{PPFD} \times 0.5 \times 0.84,$$

where PPFD is the photosynthetic photon flux density incident on the leaf, 0.5 is a factor that assumes equal distribution of energy between the two photosystems, and 0.84 is the assumed leaf absorptance (see Flexas *et al.* 2002b for discussion of these constants). Non-photochemical quenching of fluorescence (NPQ), which is proportional to the rate constant of thermal energy dissipation (Björkman and Demmig-Adams 1994), was calculated as:

$$\text{NPQ} = (F_m - F_m')/F_m'$$

Fluorometers other than PAM-2000 were used in some experiments. These included a remote sensing Fi-PAM prototype developed at Orsay (Flexas *et al.* 2000), a PAM-101 (Walz) modified to measure at distance (Ounis *et al.* 2001), and a plant efficiency analyser (Hansatech, King's Lynn, Norfolk, UK) (Flexas *et al.* 1999b, 2001).

Gas exchange measurements, including net CO_2 assimilation (A_N) and g , were always performed using a portable infra-red gas analyser (Li-6400; Li-Cor Inc., Lincoln, NE, USA). Rates of respiration in the dark (R_D) and in the light (R_L), as well as light- and CO_2 -response curves, were measured in some of the reviewed works (Escalona *et al.* 1999; Flexas *et al.* 1999b, 2002a, b).

Estimation of photorespiration and Mehler reaction rates

In one of the revised studies (Flexas *et al.* 1999b), direct estimation of O_2 uptake rates was obtained by measuring $^{18}\text{O}_2$ exchange with a closed cuvette coupled to a mass spectrometer (MM6; VG, Winsford, UK). A component of net O_2 uptake that was CO_2 -dependent was ascribed to photorespiration, while a component apparently CO_2 -independent was ascribed to the Mehler reaction.

In the studies performed under field conditions (Flexas *et al.* 1999a, 2002a), photorespiration and Mehler reaction rates were estimated by combining gas exchange and chlorophyll fluorescence measurements. The ratio ETR/A_N has previously been used as an indicator of electron transport to acceptors other than CO_2 , of which O_2 is thought to be the most important (Krall and Edwards 1992; Flexas *et al.* 1998, 1999a, b). However, this ratio must be strongly influenced by respiration, particularly under conditions of low A (Krall and Edwards 1992) as occurs under drought. To account for respiration rates we calculated the ratio of ETR to gross CO_2 assimilation (ETR/A_G), in which A_G is the sum of A_N and R_D . Any increase of ETR/A_G may be indicative of increased electron transport to processes other than photosynthesis, of which electron transport to O_2 is most likely. However, this ratio does not allow us to separate photorespiration from the Mehler reaction. Flexas *et al.* (2002b) described a rough method to separate the two processes, based on an independent estimation of photorespiration rate. This was obtained by extrapolating photosynthesis to null internal CO_2 concentration during CO_2 -response curves. An estimate of R_L (i.e. the sum of mitochondrial respiration and photorespiration) was thus obtained, and added to A_N to obtain a more precise estimation of gross photosynthesis (A_G^*). ETR/A_G^* includes electron use by photorespiration, so that any increase of this ratio can be attributed to an increased rate of the Mehler reaction or other electron-consuming processes. Once the rate of the Mehler reaction is discounted from total ETR, the rate of photorespiration can be calculated according to Epron *et al.* (1995).

Pigment analysis

Pigment analyses, including chlorophylls and xanthophylls, were performed in three years of the study (1995, 1997 and 1998). Immediately after chlorophyll fluorescence measurements, similar leaves of the same plants, showing the same orientation as those used for fluorescence measurements, were punched and *in situ* submersed in liquid nitrogen. In 1995 and 1998 samples were taken only at midday, whereas in 1997 samples were taken six times during the diurnal time-course, both in south-east- and north-west-facing leaves.

Four leaves from different plants were sampled at each time for each treatment and cultivar, except in 1998 (six samples). Pigments were extracted by grinding in a mortar with acetone (1 mL solvent cm^{-2} leaf tissue) in the presence of sodium ascorbate. Pigments (neoxanthin, taraxanthin, lutein, violaxanthin, antheraxanthin, zeaxanthin, chlorophyll *a*, chlorophyll *b*, α -carotene and β -carotene) were quantified by HPLC following the method of Abadía and Abadía (1993). The de-epoxidation state of the xanthophyll cycle (DEPS) was calculated as $(Z + 0.5A)/(V + A + Z)$, where Z is zeaxanthin, A is antheraxanthin, and V is violaxanthin.

The chlorophyll content of leaf discs was determined spectrophotometrically according to Porra *et al.* (1989) in one of the studies (Flexas *et al.* 2001), while it was estimated with a portable meter (SPAD-502; Soil-Plant Analysis Development Section, Minolta Camera Co., Osaka, Japan) in another (Flexas *et al.* 2000).

Determination of functional PSII units

The relationship between F_v/F_m and the number of functional PSII reaction centres was determined in grapevines by Flexas *et al.* (2001). The number of functional PSII reaction centres was determined according to Chow *et al.* (1989, 1991) using a leaf-disc O_2 electrode system (Hansatech). Following initial dark equilibration of the leaf disc for about 10 min, repetitive single-turnover xenon flashes [10 Hz, $2.5 \mu\text{s}$ full width at half peak intensity (type FX200; E. G. & G Electro Optics, Salem, OR, USA)] of saturating intensity were applied for 4 min, followed by 4 min of darkness. Any limitation of electron transport by PSI was avoided via use of background far-red light (Chow *et al.* 1991).

Results and discussion

Chlorophyll content and light harvesting

It has been reported that, in grapevines, drought decreases light absorption by leaves by changing their orientation (Gamon and Pearcy 1989; Flexas *et al.* 1998). However, it seems that this is simply due to leaf wilting and, consequently, may not be part of an acclimatory response to drought. Nevertheless, changes in leaf position due to wilting may strongly reduce light absorption through the course of a day, therefore reducing the impact of excess light in droughted leaves. It may also help in saving water in these leaves.

Another mechanism to decrease total light absorption in leaves is to adjust antenna size and/or chlorophyll content per unit area. Flexas *et al.* (2001) showed in grapevines that antenna size (number of chlorophyll molecules per PSII unit) was quite constant, but chlorophyll content clearly acclimated to prevailing light conditions during growth (the higher the maximum irradiance during growth, the higher the chlorophyll content). This acclimation to light is also observed when comparing different studies performed under different light conditions. For instance, leaves of Fernao Pires, Pinot Noir, Manto Negro and Tempranillo plants grown in the field during summer in Lisbon, Portugal, or Versailles, France (Chaumont *et al.* 1997), Alentejo, Portugal (Maroco *et al.* 2002), or Mallorca (present data), had typical chlorophyll contents of 0.25–0.45 g m⁻². Leaves of field-grown *Vitis riparia* sampled in spring in Canberra, Australia, had only 0.20 g m⁻², and leaves of Chardonnay grown in a greenhouse only 0.16 g m⁻². However, young potted plants of Cabernet Sauvignon acclimated to a relatively low light regime in a greenhouse in Orsay, France (Flexas *et al.* 2000) presented chlorophyll contents comparable to those of field-grown plants. In addition, no difference in chlorophyll content was observed between south-east- and north-west-facing leaves of field-grown plants (Figs 2A, B, respectively). This result might be expected, since similar total daily irradiance was observed on both sides of the canopy, although maximum incident light intensity was achieved at midday in south-east-facing leaves, and at mid-afternoon in north-west-facing leaves (not shown).

When high light was accompanied by water stress in field-grown plants in Mallorca, no further adjustment in chlorophyll content was observed (Figs 2A, B). Moreover, similar chlorophyll contents were observed in different cultivars, and from June to the end of August (not shown). Chaumont *et al.* (1997) have also reported similar leaf chlorophyll content in irrigated and non-irrigated plants. By contrast, Maroco *et al.* (2002) found a significant reduction of total chlorophyll content in drought-stressed Tempranillo plants grown in the field. Also, during the experiment conducted in a greenhouse in Orsay with Cabernet Sauvignon plants (Flexas *et al.* 2000), leaf chlorophyll

content clearly decreased during a 15-d drought cycle, showing remarkable correlation with LWD (Fig. 3). In summary, it seems that chlorophyll content (but not antenna size) generally acclimates to light in grapevines, but further acclimation to drought seems to depend on some unknown additional factors. However, there is no evidence that the decrease of chlorophyll content eventually observed in leaves of drought-stressed plants results in decreased leaf absorptance. Indeed, Schultz (1996a) observed a narrow range of leaf absorptance in grapevines during a summer cycle, except for very young and very old leaves.

Leaf photochemistry and thermal dissipation

The maximum capacity for ETR in grapevines is similar in many cultivars, ranging from 130 to 160 $\mu\text{mol e}^- \text{m}^{-2} \text{s}^{-1}$ (Chaumont *et al.* 1994; Flexas *et al.* 1998; Bota *et al.* 2001). Moreover, grapevines grown in a greenhouse and acclimated to moderate light regimes reach light-saturated ETR similar to, or even slightly higher than, that of field-grown plants acclimated to high light (Düring 1998; Flexas *et al.* 1999a, b, 2000, 2002a, b). In contrast, glasshouse-grown vines reach maximum NPQ values of 2.5–3.5 (Düring 1998; Flexas *et al.* 1999b, 2000, 2002a), whereas field-grown plants reach values as high as 6 or greater (Flexas *et al.* 1999a, 2002b).

With respect to the effects of drought on leaf photochemistry in grapevines, early studies yielded some conflicting results (Flexas *et al.* 1998, 1999a). In field-grown Manto Negro and Tempranillo, ETR was occasionally found to be lower in non-irrigated than in irrigated plants (Flexas *et al.* 1998), but no correlation was found between ETR and Ψ . In contrast, in Tempranillo plants grown in large pots and subjected to a 20-d drought cycle, a highly significant negative correlation between ETR and Ψ was observed (Flexas *et al.* 1999a). However, when plotting both sets of data against g , a single hyperbolic relationship was obtained (Flexas *et al.* 2002b; Medrano *et al.* 2002). Moreover, a very similar relationship was obtained when plotting together data from 22 different cultivars grown in small pots (Bota *et al.* 2001), in which drought was rapidly imposed within 1 week. In addition, even when water stress was imposed within 1 h by cutting the leaf petiole in air, close correspondence between ETR and g was observed (Flexas *et al.* 2002a). A similar direct correspondence between ETR and g is observed when carefully analysing data from Schultz (1998) in which two cultivars (Syrah and Grenache) were compared under field conditions. Although Grenache maintains higher Ψ during a drought cycle than Syrah, both present an identical pattern of ETR and g decline. Consequently, and unlike the relationship between ETR and Ψ , the relationship between ETR and g can be generalized among cultivars and conditions. Down-regulation of leaf photochemistry and increased NPQ in response to drought have also been described by Düring (1998) in low-light-acclimated grapevines.

Now it is clear that, as drought induces decreases in g from a maximum down to about $0.15 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$, ETR remains largely unaffected. When stomata close, a further downregulation of ETR occurs, which is compensated for by increased thermal dissipation (NPQ). These findings strongly suggest that drought-induced downregulation of ETR and increased thermal dissipation may respond directly

to low CO_2 availability in the chloroplast due to stomata closure, therefore being independent of the rate of drought imposition and acclimation to drought.

The following sections analyse the physiological mechanisms that drive photochemistry and thermal dissipation under progressive drought.

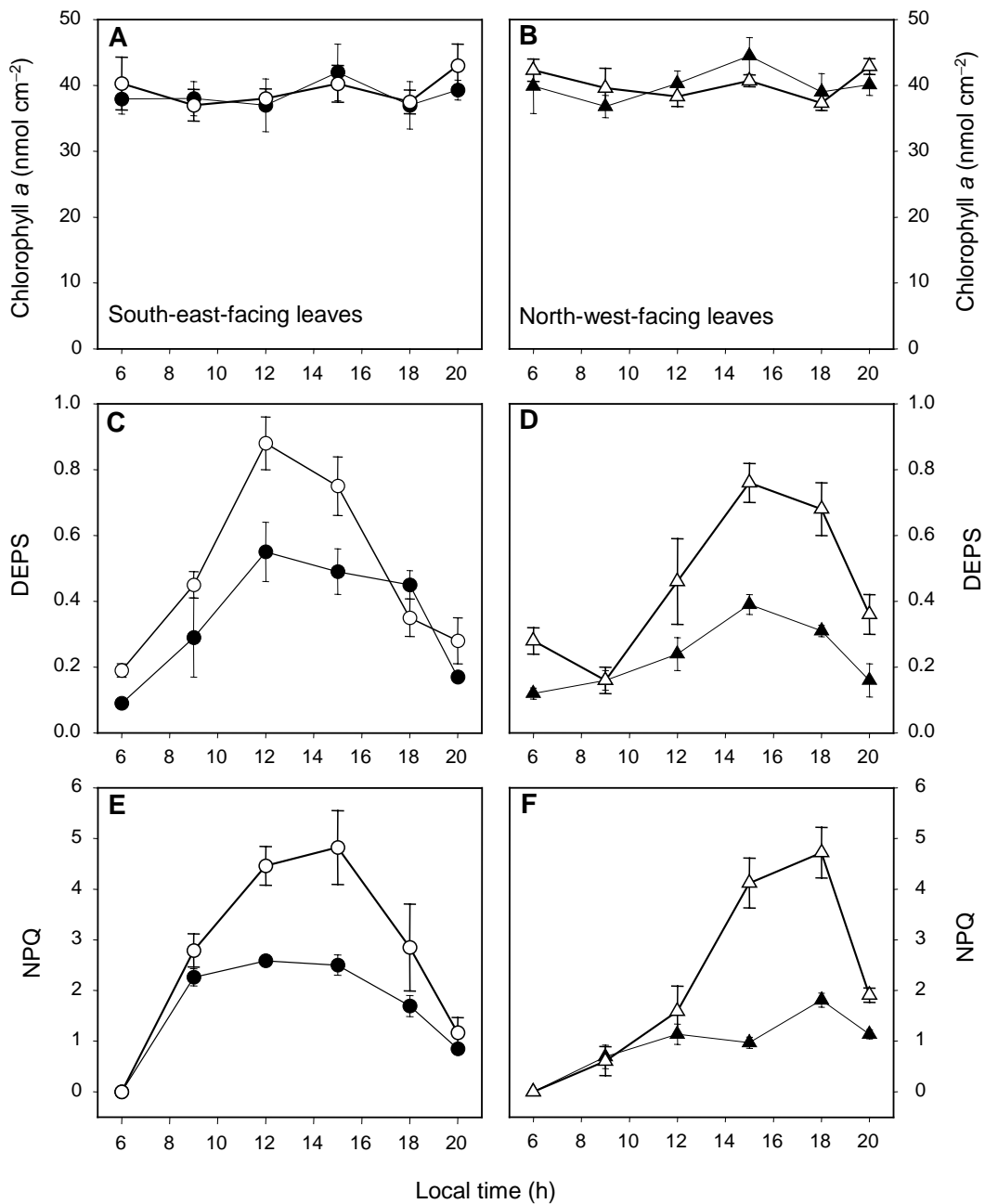


Fig. 2. Diurnal variations of chlorophyll *a* content in south-east (*A*) and north-west- (*B*) facing leaves of field-grown Manto Negro (data correspond to August 1997). Diurnal variations of the de-epoxidation state of the xanthophyll cycle (DEPS; *C*, *D*) and non-photochemical quenching of chlorophyll fluorescence (NPQ; *E*, *F*) are also shown for the same leaves. Data are means \pm s.e. of four (chlorophyll and DEPS) and six (NPQ) replicates for both irrigated (closed symbols) and drought-stressed (open symbols) plants.

CO₂ fixation, photorespiration and the Mehler reaction

Most of the ETR is used to drive three processes: CO₂ fixation, photorespiration and the Mehler reaction (Osmond and Grace 1995). Since CO₂ is highly diluted in the current atmosphere and an immediate response of plants to drought is stomatal closure, CO₂ availability in the chloroplasts becomes progressively more limiting for photosynthesis as drought intensifies. O₂, by contrast, is at a high enough atmospheric concentration to diffuse into leaves even when stomata are tightly closed. This led to a proposal that electron transport to O₂ is an effective way to dissipate excess energy in drought-stressed leaves (Powles and Osmond 1978; Osmond *et al.* 1980).

In grapevines in particular, A_N has frequently been shown to decrease in parallel to g as drought progresses (e.g. Escalona *et al.* 1999; Flexas *et al.* 2002b). Similarly to ETR, the relationship between A_N and g is largely independent of the cultivar and/or the velocity and conditions of drought imposition (Flexas *et al.* 2002b). However, and in contrast to ETR, no clear plateau is observed, even at relatively high g . Therefore, when g drops down to 0.15 mol H₂O m⁻² s⁻¹, ETR remains unaffected while A_N progressively drops from about 16 to 9 μmol CO₂ m⁻² s⁻¹. At lower g , both A_N and ETR decline, but ETR/ A_N and ETR/ A_G increase progressively along the entire gradient of g , which strongly suggests increased electron transport to O₂ (Flexas *et al.* 2002b). However, ETR/ A_G^* remained almost constant along the gradient of g , except for a certain increase (although with data strongly scattered) at very low g (< 0.03 mol H₂O m⁻² s⁻¹). These results suggest that the Mehler reaction may play only a minor role in electron consumption in grapevines, both under

well-watered conditions and drought. Flexas *et al.* (1999b) have suggested an increased rate of the Mehler reaction in drought-stressed grapevines, based on direct observations of ¹⁸O₂ uptake at 2.5% CO₂. However, it has recently been suggested that these observations could be due to drought-induced increases of mesophyll resistance, making an atmospheric concentration of 2.5% CO₂ insufficient to block photorespiration (Flexas *et al.* 2002b). Even if the rates of O₂ uptake observed by Flexas *et al.* (1999b) were due to the Mehler reaction, they were observed in severely-stressed plants (very low g), and could account for ETR as low as 20 μmol e⁻ m⁻² s⁻¹. This is in agreement with the slight increase of ETR/ A_G^* at very low g observed by Flexas *et al.* (2002b) in field-grown plants.

Thus, it seems that most of the ETR is devoted to CO₂ assimilation plus photorespiration in grapevines. An integrated photosynthesis–photorespiration model (Takeba and Kozaki 1998) predicts that recycling of O₂ and CO₂ may be able to sustain as much as 75% of the maximum ETR at CO₂ compensation point (i.e. when no net CO₂ uptake takes place, which occurs when g is almost zero in drought-stressed plants). During drought cycles in potted grapevines, both under field conditions (Flexas *et al.* 1999a) and in a greenhouse (Flexas *et al.* 2000), g and A_N were reduced to zero about 15 d after withholding water. In the field experiments, when measured after prolonged exposure to saturating light, ETR was strongly reduced (Flexas *et al.* 1999a). In contrast, ETR was maintained at 75% of control values when measured at 200 μmol photons m⁻² s⁻¹ during the experiment in the greenhouse at Orsay (Fig. 4). This comparison suggests that photorespiration

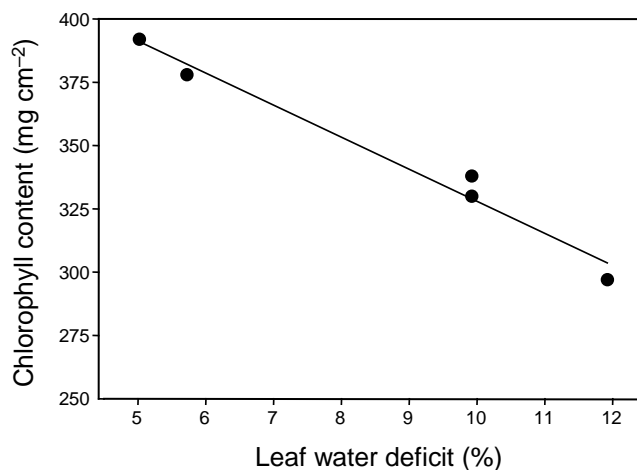


Fig. 3. Dependence of total chlorophyll content on leaf water deficit ($r^2=0.97$) during a 15-d drought cycle in an experiment performed with a potted Cabernet Sauvignon plant growing in a greenhouse at Orsay, France, with a high frequency of low-light days, as described in Flexas *et al.* (2000).

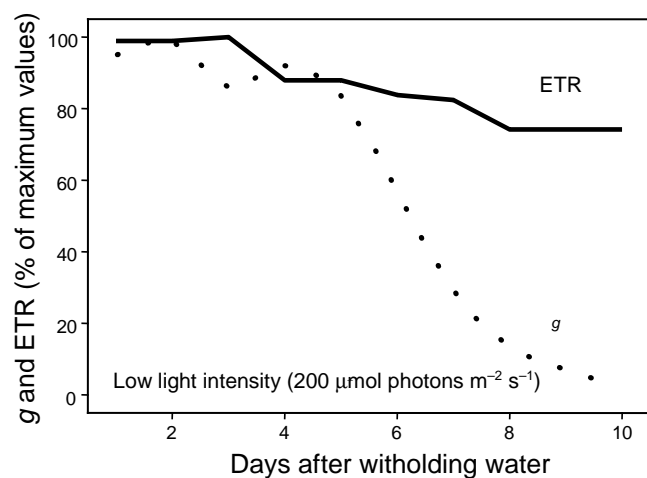


Fig. 4. Progression of ETR and g during a drought cycle in a potted Cabernet Sauvignon plant grown at relatively low light (conditions described in Flexas *et al.* 2000). Maintenance of 75% of maximum ETR under drought-induced complete stomatal closure is observed. Presumably, photorespiration and CO₂-recycling are responsible for high ETR under these conditions.

plus recycling may drive substantial electron transport during drought, and may be enough to photoprotect PSII at low to moderate light. At high light intensity, photorespiration alone may not be sufficient for photoprotection, and other photoprotective mechanisms may be enhanced in order to reduce ETR. Differences in Rubisco activation at different light intensities could also account for different ETR reductions.

Xanthophyll-dependent thermal dissipation

It has been claimed that xanthophyll-dependent thermal dissipation is the most effective mechanism contributing to NPQ in high-light-acclimated plants (Demmig-Adams and Adams 1992; Niyogi 1999). This mechanism depends on the development of a trans-thylakoid ΔpH produced by electron transport, and on de-epoxidation of the xanthophyll (VAZ) pool from the most epoxidized form (V) to the non-epoxidized (Z).

Both total accumulation of VAZ and other carotenoids, and maximum DEPS capacity depend on the cultivars and their acclimation to light. Chaumont *et al.* (1995, 1997), for instance, have shown that the total amount of carotenoids was 33% less in non-acclimated leaves of cuttings maintained in a growth chamber relative to field-grown plants, and 20% less in Fernao Pires relative to Pinot Noir. The maximum DEPS observed was about 85% in field-grown Pinot Noir, 75% in field-grown Fernao Pires, and only 60% in non-acclimated cuttings of Pinot Noir. In contrast, Düring (1999) found similar DEPS (60–70%) in plants acclimated to either low light in the laboratory or high light in the field.

To see if drought induced further acclimation of pigment composition, field-grown Manto Negro and Tempranillo under irrigation and drought, were analysed for 3 years at three sampling times per year. Total VAZ pool and concentration of other carotenoid pigments analysed (α - and β -carotene, lutein, taraxanthin and neoxanthin) were different between years (Table 1). For instance, total pigment accumulation was lower in 1995, a year with an especially mild summer (see Flexas *et al.* 1998 for details). These differences may reflect acclimation to prevailing conditions during initial plant growth (i.e. spring). However, pigment composition was similar between seasons. The only difference observed between cultivars was in their carotenoid composition, with a slightly higher VAZ pool in Manto Negro than in Tempranillo (Table 1) at the expense of β -carotene (not shown). Interestingly, drought did not significantly affect the total pool of chlorophyll, VAZ, or total carotenoids (Table 1). Therefore, carotenoid composition does not seem to acclimate to drought in field-grown grapevines, in contrast to other Mediterranean species (Demmig *et al.* 1988; García-Plazaola *et al.* 1997). Figure 5 shows an example of the typical carotenoid composition for these plants.

In field-grown Manto Negro and Tempranillo plants, close correspondence was observed between NPQ and DEPS over diurnal time-courses throughout the experiment. Figure 2 shows a typical diurnal time-course of NPQ and DEPS. Both were higher in water-stressed than in irrigated plants, and closely followed the diurnal time-course of irradiance. Plotting all measurements together (3 years, three different sampling times per year, and two leaf orientations and six samples per day in 1997) gave a significant linear correlation between DEPS and NPQ, but different for each cultivar (Fig. 6). Although points with NPQ greater than 6 strongly deviate from the relationships, this could be due to the low accuracy of NPQ determinations when F_m' is extremely low. This strong correspondence suggests that a large part of NPQ is related to DEPS in grapevines. Among the inter-cultivar differences in the relationships, the data showed (i) a larger scattering in Tempranillo than in Manto Negro, (ii) a higher maximum DEPS in Manto Negro, and (iii) a higher maximum DEPS pre-dawn (i.e. NPQ = 0) DEPS in Manto Negro.

The large scattering in Tempranillo was caused by points corresponding to non-irrigated plants. Indeed, if the regression was fitted only through data from irrigated plants, the correlation increased ($r^2=0.86$). In contrast, if only water-stressed data were taken into account, the regression was non-significant. In Manto Negro, however, both irrigated and water-stressed data fitted the same relationship. These results suggest that there is some non-DEPS-related component of NPQ in water-stressed Tempranillo plants, but not in Manto Negro. This would be consistent with previous observations of pre-dawn F_v/F_m (Flexas *et al.* 1998; Flexas 2000). Although both cultivars presented similarly reduced values of F_v/F_m under certain particularly severe drought conditions, Tempranillo showed reduced F_m and increased

Table 1. Effects of year, sampling time, cultivar and treatment on total chlorophyll content, total VAZ pool and total carotenoid content

Average values are shown for each factor, with other factors pooled. Different letters indicate significant differences at $P<0.05$ (Duncan test)

	Total chlorophyll (nmol cm ⁻²)	Total carotenoids (nmol cm ⁻²)	VAZ (nmol cm ⁻²)
1995	38.5 a	13.1 a	3.8 a
1997	55.8 b	16.5 c	5.0 b
1998	39.9 a	14.4 b	4.4 b
June	46.0 a	14.8 a	4.3 a
July	46.4 a	15.2 a	4.7 a
August	41.8 a	14.7 a	4.3 a
Tempranillo	46.5 a	14.8 a	4.1 a
Manto Negro	43.0 a	14.6 a	4.8 b
Irrigated	44.4 a	14.5 a	4.3 a
Drought stressed	45.1 a	14.8 a	4.6 a

F_o , whereas Manto Negro presented reductions of both F_m and F_o . According to Osmond and Grace (1995), increased F_o may be indicative of photodamage, while decreased F_o may indicate sustained photoprotection. Sustained photoprotection at pre-dawn is characterized by sustained DEPS in cold-stressed plants (Adams and Demmig-Adams 1995; Gilmore and Ball 2000) as well as in water-stressed plants (Demmig *et al.* 1988; Sánchez-Rodríguez *et al.* 1997; Martínez-Ferri *et al.* 2000). In both cultivars, pre-dawn DEPS was higher in water-stressed than in irrigated plants. However, maximum pre-dawn DEPS was less than 0.15 in Tempranillo and as high as 0.30 in Manto Negro, which is consistent with the previous suggestions based on F_o . The fact that the maximum observed DEPS was only about 0.7

in Tempranillo and 0.9 in Manto Negro would also be indicative of a greater xanthophyll-dependent photoprotective capacity in Manto Negro than in Tempranillo, at least under drought. This is in agreement with the reputation of Manto Negro as a more drought-resistant variety (Escalona *et al.* 1999).

PSII photoinactivation and repair

When the conjunct operation of all the dissipation mechanisms described above fails to safely dissipate all the energy absorbed by the leaf, the probability of PSII photoinactivation increases (Niyogi 1999). Alternatively, photoinactivation may be viewed as a probabilistic event that is a function of total photon exposure (Anderson *et al.* 1998),

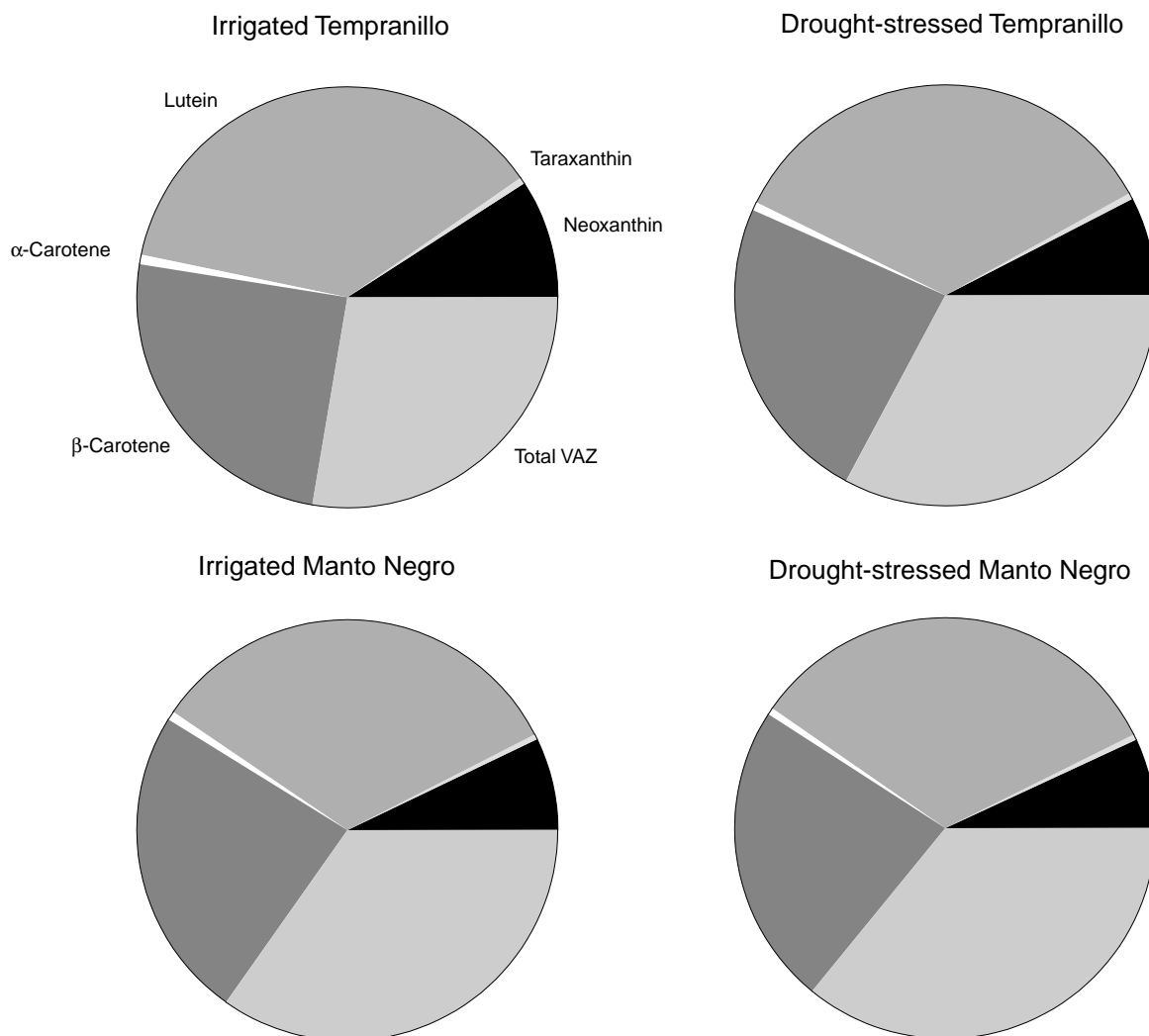


Fig. 5. Typical pigment composition of field-grown Tempranillo and Manto Negro plants under irrigation and drought (shades depicting different pigments are indicated for irrigated Tempranillo). Data are for July 1999. Total pigment concentrations were 16.1 nmol cm⁻² in irrigated Tempranillo, 14.2 nmol cm⁻² in drought-stressed Tempranillo, 14.8 nmol cm⁻² in irrigated Manto Negro, and 13.7 nmol cm⁻² in drought-stressed Manto Negro (differences were non-significant). Differences between cultivars were VAZ being slightly higher in Manto Negro than in Tempranillo, at the expense of β-carotene (see Table 1). No differences were observed between irrigated and drought-stressed plants (see Table 1).

but with a quantum yield that is lower as the plant acclimates to high light (Flexas *et al.* 2001). Photoinactivation of PSII includes loss of function of the D_1 protein, and a D_1 repair mechanism operates in the chloroplasts, which tends to maintain a high number of functional PSIIs (Melis 1999).

Traditionally, and especially in field studies, the balance between photoinactivation and repair has been analysed by measuring the quantum yield of PSII (F_v/F_m). F_v/F_m is about 0.8 in healthy leaves, and may decrease under conditions of PSII photoinactivation (Björkman and Demmig-Adams 1994). Field-grown, high-light-acclimated grapevines only show a slight decline in F_v/F_m (down to 0.7) after high photon exposures at midday, regardless of water

availability (Gamon and Pearcy 1989; Iacono and Sommer 1996; Schultz 1996b; Chaumont *et al.* 1997). Even the low-light-acclimated, severely-stressed plants grown in greenhouses showed high F_v/F_m (> 0.5 ; Quick *et al.* 1992; Flexas *et al.* 2000). At pre-dawn (i.e. after a whole night of relaxation of photoinactivation), F_v/F_m in field-grown grapevines was usually close to 0.8, and declined to 0.74 only under conditions of extremely severe drought where diurnal photosynthesis was almost zero (Flexas *et al.* 1998, 2002b). In low-light-acclimated, drought-stressed grapevines, pre-dawn F_v/F_m declined to 0.6, although at least part of this decline was attributable to oversaturation by laser pulses given by the fluorometer used, as already discussed (Flexas *et al.* 2000). Thus, under normal conditions, F_v/F_m in grapevines remains higher than 0.5–0.6. Lower values can only be achieved under combined high light and extremely low (Chaumont *et al.* 1995) or high temperatures (Gamon and Pearcy 1990a, b; Schultz 1996b).

Flexas *et al.* (2001) found a curvilinear relationship between F_v/F_m and the actual number of functional PSII units, so that the loss of 40–50% of functional PSII will result in only slightly reduced values of F_v/F_m (0.6–0.7). Comparing these results with F_v/F_m values measured in field- and greenhouse-grown grapevines, it can be inferred that up to 40–50% of PSII centres may be photoinactivated during a typical sunny day in field-grown grapevines. Interestingly, even if a reduction of 40% of total functional PSII occurs, this would not affect the maximum photosynthetic rate of leaves, since photosynthetic capacity is not limited by active PSII concentration until about 50% of PSII centres are lost (Lee *et al.* 1999).

Flexas *et al.* (2001) also observed that, at very high photon exposures in non-lincomycin-treated sun and shade leaves of *V. riparia*, a steady state was achieved in which 50% of PSIIs remained functional regardless of increasing photon exposure. Since lincomycin (an inhibitor of the D_1 repair mechanism) impeded attainment of such a steady state, it was concluded that the D_1 repair mechanism was able to maintain the balance between functional and non-functional PSIIs at 50%, in both sun and shade leaves. The main function of these inactivated centres would be photoprotection of the remaining centres, since inactive centres are capable of non-radiative energy dissipation (Lee *et al.* 2001). The similarity of these results with those observed in field- and greenhouse-grown grapevines suggests that the D_1 repair mechanism is well constituted in this species, irrespective of light acclimation and drought. In fact, Chaumont *et al.* (1995), studying low-light-acclimated vines subjected to a 15-h treatment of high light and low temperatures, found that the amount of D_1 was maintained at between 80 and 100% of the maximum throughout the experiment.

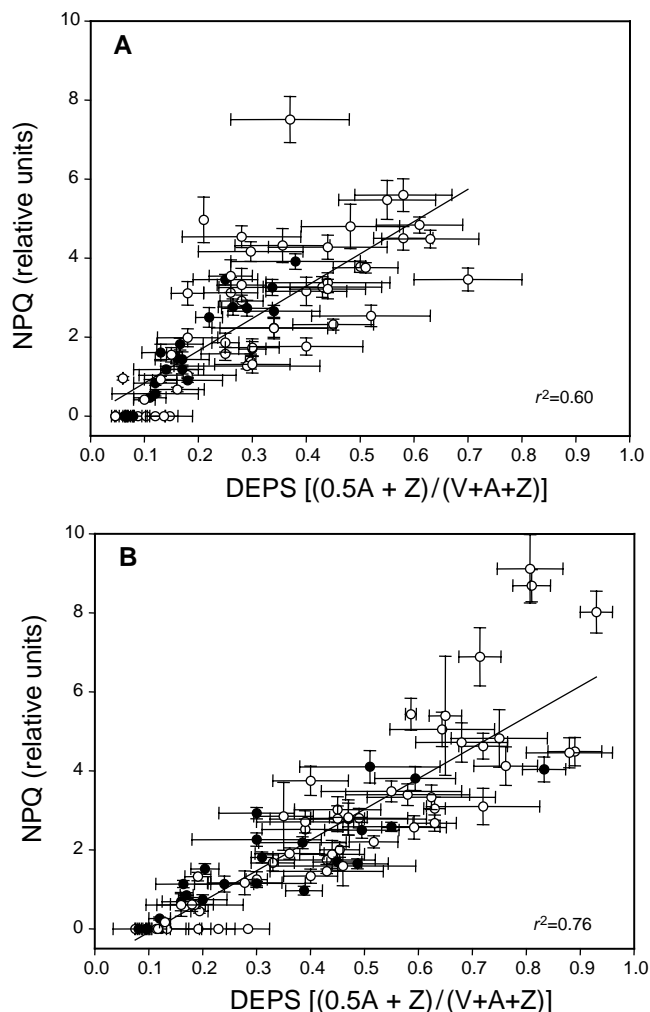


Fig. 6. Relationship between the de-epoxidation state of the xanthophyll cycle (DEPS) and non-photochemical quenching (NPQ) in field-grown Tempranillo (A) and Manto Negro (B) plants. All measured data are plotted together (3 years and three sampling times per year) for both irrigated (closed symbols) and drought-stressed (open symbols) plants. Data are means \pm s.e. of four (DEPS) and six (NPQ) replicates. Regression coefficients (r^2) of single linear regressions using all data together are shown.

Concluding remarks

We analysed different processes in relation to the balance between light absorption and dissipation by leaves in a combination of studies on field-grown grapevines over 10 years. These results were discussed in relation to their possible involvement in plant acclimation to high light and/or water stress.

Chlorophyll content and the capacity for xanthophyll-dependent thermal dissipation clearly acclimate to prevailing light conditions during leaf development. These are also the only metabolic aspects that differ between the two cultivars studied, photoprotective capacity being more effective in Manto Negro, a cultivar reputed to be more drought tolerant, than Tempranillo. The capacity for leaf photochemistry, in contrast, seems to be constitutive of the species and largely independent of light acclimation and cultivar.

Drought-induced variations in these mechanisms are similar irrespective of the velocity of drought imposition, which suggests no further acclimation of these mechanisms in response to drought. Thus, acclimation to excess light alone seems to determine the extent to which each of these mechanisms would be responsive to drought. Drought-induced stomatal closure (i.e. low CO₂ availability in the chloroplasts), rather than decreased RWC in leaves, seems to be the factor that triggers the response of energy dissipation mechanisms to drought. This response is characterized by an increase of photorespiration under mild drought, and large increases of thermal dissipation under moderate to severe drought.

Acknowledgments

We wish to thank all people who participated in the reviewed experiments. Financial support from the Spanish ministry (CICYT) projects ALI91-1122-C03-03, AGF94-0687, AGF97-1180 and 1FD97-1276 is acknowledged. We are indebted to Dr Miquel Ribas-Carbó for helpful comments during preparation of the manuscript.

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Manuscript received 4 February 2002, accepted 14 May 2002