

Steady-state chlorophyll fluorescence (Fs) measurements as a tool to follow variations of net CO₂ assimilation and stomatal conductance during water-stress in C₃ plants

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Water stress experiments were performed with grapevines (*Vitis vinifera* L.) and other C₃ plants in the field, in potted plants in the laboratory, and with detached leaves. It was found that, in all cases, the ratio of steady state chlorophyll fluorescence (Fs) normalized to dark-adapted intrinsic fluorescence (Fo) inversely correlated with non-photochemical quenching (NPQ). Also, at high irradiance, the ratio Fs/Fo was positively correlated with CO₂ assimilation in air, with

electron transport rate calculated from fluorescence, and with stomatal conductance, but no clear correlation was observed with qP. The significance of these relationships is discussed. The ratio Fs/Fo, measured with a portable instrument (PAM-2000) or with a remote sensing FIPAM system, provides a good method for the early detection of water stress, and may become a useful guide to irrigation requirements.

Introduction

Water availability is one of the most important limitations to photosynthesis and plant productivity (Boyer 1982; Tezara et al. 1999). The capacity to improve crop yield under drought conditions through genetically and/or agronomically increasing the efficiency of water use by plants and/or drought tolerance is, at present, limited (Boyer 1996). The primary means to improve crop yield under drought is to increase the efficiency of water delivery and the timing of water application (Boyer 1996). Thus, proper monitoring of plant water stress is essential for the development of appropriate, sustainable, irrigation programs for crop

production in semiarid areas (Sellers et al. 1992, Peñuelas and Filella 1998). In natural vegetation it also has relevance to fire prevention (Peñuelas and Filella 1998).

Light absorbed by leaves in the antenna complexes that is in excess of that used to drive photosynthesis has to be dissipated to avoid excess excitation energy within PSII. Plants have evolved a number of energy dissipation mechanisms. Thus, the excited singlet state of chlorophyll (Chl) is subjected to a number of competing de-excitation reactions, including photochemical trapping, energy transfer, radiationless de-

Abbreviations – A, Net CO₂ assimilation rate; Chl, chlorophyll; DTT, dithiothreitol; ETR, electron transport rate; Fo and Fo', initial fluorescence yields in dark-acclimated and light-acclimated leaves, respectively; Fs, fluorescence yield at steady state; Fs/Fo, Fs normalized to Fo, which is the fluorescence yield when all PSII centres are open; Fm and Fm', maximum fluorescence yield in dark-acclimated and light-acclimated leaves, respectively; Fv/Fm, efficiency of excitation capture by open PSII in dark-adapted leaves; g, stomatal conductance; NPQ, non-photochemical quenching of Chl fluorescence; PPF, photosynthetic photon flux density; PSII, photosystem II; qP, photochemical quenching of Chl fluorescence.

excitation and fluorescence emission (Krause and Weis 1991, Demmig-Adams and Adams 1992, Björkman and Demmig-Adams 1994, Horton et al. 1996). Any change in one of these pathways will result in corresponding changes of fluorescence yield (Krause and Weiss 1991, Schreiber et al. 1998).

Efforts have been devoted to finding non-invasive parameters for remote sensing of plant photosynthesis and/or water status. Changes in canopy reflectance at different wavelengths (Gamon et al. 1990, Sellers et al. 1992, Peñuelas and Filella 1998), though useful for detection of other stresses, have failed to follow water stress associated changes at the whole plant level (Gamon et al. 1990, Peñuelas and Filella 1998). Chl fluorescence parameters (Schmuck et al. 1992, Cornic 1994, Cerovic et al. 1996, Flexas et al. 1998, 1999a) are most commonly used. These permit evaluation of parameters that correlate with net CO₂ assimilation (A), such as the quantum yield of PSII (Genty et al. 1989), which in turn gives estimates of the rate of linear electron transport (ETR), provided the light absorbed by the leaf is known (Krall and Edwards 1992). A good correlation between the ETR and A has been found for C₄ plants (Edwards and Baker 1993). For C₃ plants chlorophyll fluorescence measurements are less representative of net photosynthesis unless they are combined with other approaches, such as stomatal conductance modelling (He and Edwards 1996). The main reason for this is that photorespiration competes with CO₂ assimilation for electron use, an effect which is even more enhanced under water stress (Cornic and Briantais 1991, Krall and Edwards 1992, Flexas et al. 1998, 1999a).

However, it has been reported recently that steady-state Chl fluorescence (Fs) is a water stress sensitive parameter for C₃, C₄ and CAM plants (Cerovic et al. 1996, Flexas et al. 1999a, 2000). The diurnal time course of Fs tracks that of irradiance in well-irrigated plants, whereas water stress induces a diurnal pattern of Fs that mirrors that of irradiance (Cerovic et al. 1996, Flexas et al. 1999a, 2000). Measurements of Fs do not require high-energy sources, such as lasers, and can be measured remotely using either active or passive methods (Moya et al. 1998, Flexas et al. 2000, Ounis et al. 2001).

In the present study we investigated the response of Fs to irradiance in water stressed grapevines. It was clearly shown that, as water stress increases, there is a progressive decline of both the maximum Fs value achieved and the value of PPFD at which this maximum is reached. Evidence is presented that changes in Fs are governed by a rapidly reversible component of non-photochemical quenching of Chl fluorescence (NPQ). A direct correlation between a simple fluorescence parameter (Fs) and important gas-exchange parameters (A and g) is demonstrated in C₃ plants. The use of these findings for the remote detection of water stress and irrigation scheduling is discussed.

Materials and methods

Experiments in controlled environment

Plant material and treatments

Two-year-old grapevines (*Vitis vinifera* L. cv. Tempranillo), grown in 70 l containers were used. The plants were grown outdoors in Mallorca (Spain) during the summer months as already described (Flexas et al. 1999a) and irrigated daily to maintain the soil at field capacity. Water stress was induced slowly by reducing irrigation amounts. Measurements were made at various intervals during the drought cycle to obtain a gradient of water stress situations. Leaf water potential was measured using a pressure chamber, as described (Flexas et al. 1999a). Similar experiments were performed on vines (*Vitis vinifera* L. cv. Cabernet Sauvignon) in Orsay (France).

Light response curves of gas-exchange and Chl fluorescence at ambient and high CO₂ concentrations

Before initiating the measurements, a plant was taken from outdoors during the morning hours (between 9 and 10AM, local time) on cloudless days, and placed inside a darkened growth chamber for 30 min. The chamber was maintained at 25°C and 50% relative humidity. A leaf was placed inside the gas-exchange chamber of a portable photosynthesis apparatus (LI-6400, LI-Cor Inc., Lincoln, NE, USA) using the LI-Cor PAM-2000 adapter chamber, and coupled to a portable modulated fluorometer (PAM-2000, Walz, Effeltrich). This allowed simultaneous measurements of gas-exchange and Chl fluorescence. Light response curves started with measurements of dark respiration rates and dark-adapted Chl fluorescence. The leaf was illuminated at 50 µmol m⁻²s⁻¹ PPFD with a 250-W slide projector and allowed to acclimate to the new light regime for 5–10 min until steady-state gas-exchange and Chl fluorescence values were recorded. The irradiances used for the light response curve were 0, 50, 100, 200, 300, 450, 600, 750 and 1250 µmol m⁻²s⁻¹ PPFD. Leaf heating was avoided by maintaining the leaf temperature at 25°C with the fan system provided with the LI-6400. Several times, after reaching the highest irradiance, an inverse light response curve (decreasing steeply the irradiance) was followed to ensure that the procedure did not induce any time-dependent change on the rates. Light response curves were also conducted on the same leaf measured, as described above, in an atmosphere of high CO₂ (1500 µmol mol⁻¹ air) in N₂ (O₂ depleted air).

Gas exchange and Chl fluorescence parameters were calculated as described (Flexas et al. 1999a). The Chl fluorescence parameters measured were maximum photochemical efficiency (Fv/Fm) of dark-adapted samples, rate of linear electron transport (ETR), Stern-Volmer non-photochemical quenching (NPQ) and Fs. The values of steady-state Chl fluorescence (Fs) were normalized to dark adapted basal rates (Fo) to take into account any difference between plants due to different

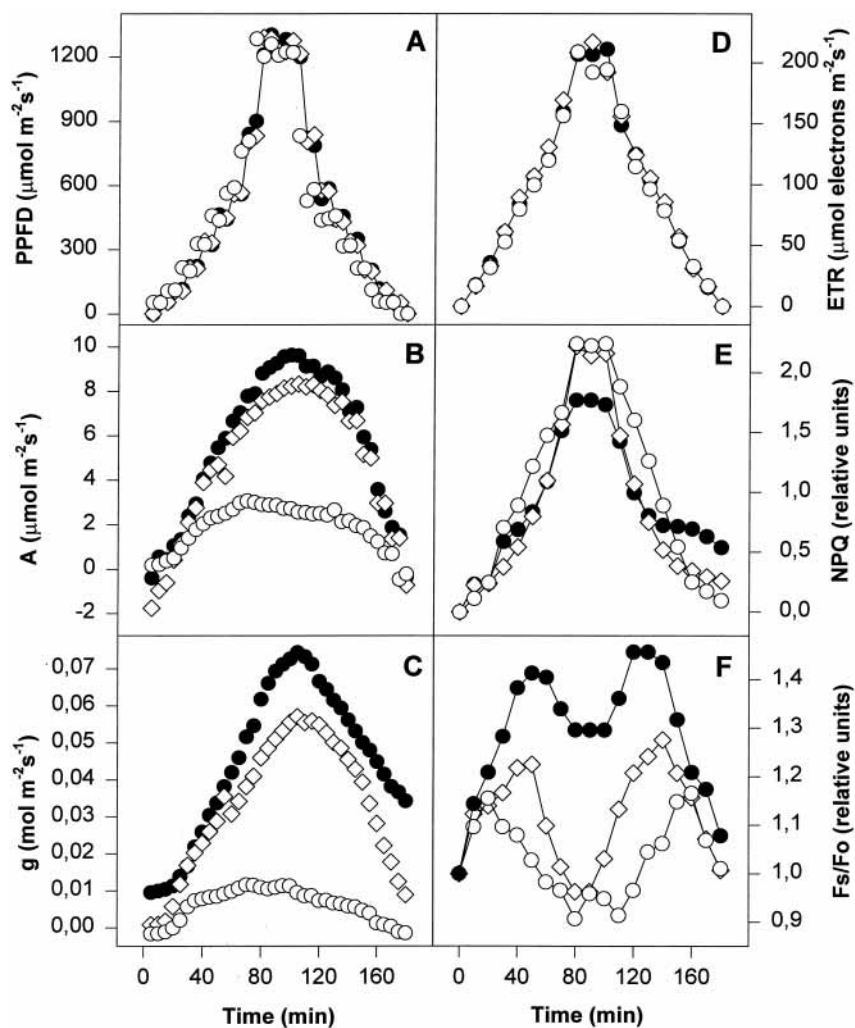


Fig. 1. (A) Variation of photosynthetic photon flux density (PPFD) incident on leaf surface during a typical experiment. (B-F) The response of grape leaves net CO₂ assimilation (A), leaf conductance (g), rate of linear electron transport (ETR), Stern-Volmer non-photochemical quenching of Chl fluorescence (NPQ) and steady-state Chl fluorescence (F_s) normalized to the basal, dark-adapted, fluorescence level (F_o). The three response curves correspond to vines with a predawn leaf water potential (Ψ_{PD}) of -0.3 MPa (●), a Ψ_{PD} of -0.4 MPa (◇) and a Ψ_{PD} of -1.4 MPa (○).

leaf structure, Chl concentrations, etc. F_o' was not directly determined, but this parameter and the photochemical quenching of chlorophyll fluorescence (qP) were estimated from the following relationship (Oxborough and Baker 1997):

$$F_o' = F_o / [(F_v/F_m) + (F_o/F_m)']$$

The experiments performed at Orsay, included Chl fluorescence parameters measured at a distance of 1 m, through the transparent window of the Li-6400 chamber, using the FIPAM already described (Flexas et al. 2000). Some leaves were infiltrated with DTT through the petioles as described by Bilger and Björkman (1994), in order to inhibit xanthophyll deoxidation.

Experiments under field conditions

Plant material and treatments

Twenty-year-old grapevines (cultivars Tempranillo and Manto Negro) growing outdoors in a commercial vineyard (Herederos de Ribas S.A.) in Mallorca, under typi-

cal Mediterranean summer conditions were examined. The plants were trained as already described (Flexas et al. 1998), with five rows (70 plants each) being irrigated twice a week, and the other five rows being maintained without irrigation during the whole summer.

The study was extended to other C₃ species, including field-grown *Solanum melongena* L. plants (herbaceous crop), as well as 5-year-old plants of *Quercus ilex* L. (evergreen sclerophyll tree), *Pistacia terebinthus* L. (deciduous sclerophyll shrub) and *Celtis australis* L. (deciduous mesophyte tree) growing outdoors in 70 l pots. These plants were irrigated daily until the onset of the experiment. Then irrigation was stopped and measurements were taken every 2 days during 3 weeks, during which water deficit progressively developed.

Gas-exchange and chlorophyll fluorescence measurements

Simultaneous measurements of gas-exchange (Li-6400) and Chl fluorescence (PAM-2000) were made under conditions of saturating light, as described (Flexas et al. 1998). Measurements were performed in field-grown grapevines during the summers of 1999 and 2000, three

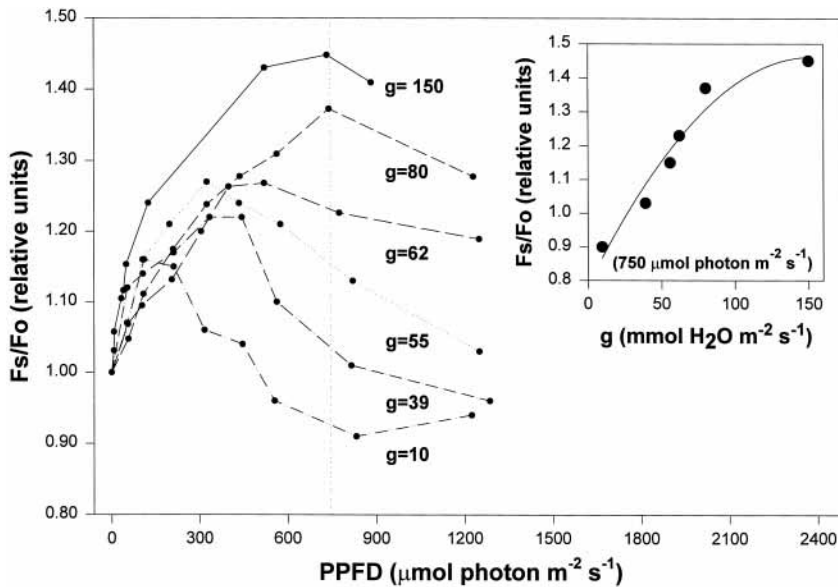


Fig. 2. Light response curves of F_s/F_o at six different gradients of water stress, determined by their g (in $\text{mmol m}^{-2} \text{s}^{-1}$) at $750 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD. Insert: F_s/F_o vs. g at $750 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD (the vertical line in the main figure).

times each summer, three times per day (at 9, 12 and 15 h, local time) on fully expanded, south-orientated leaves. The presented values are averages of 6 different measurements on 6 different plants of each variety, treatment and sampling time. The other species were measured only at midday during 3 weeks in August-September 2000. The values presented are single measurements of different leaves and plants.

Results

Water stress induces stomatal closure and changes in the light response curve of steady-state Chl fluorescence (F_s), which are reversed by high CO_2 and DTT

The dark-adapted F_v/F_m value for all leaves examined was between 0.80 and 0.83 (data not shown), and although F_o remained quite constant during the experiments, small variations were used to normalize F_s values. A typical time course of the response of grapevine leaves to increasing and decreasing irradiance (Fig. 1A), with constant values of predawn leaf water potential (Ψ_{PD}). A mild water stress only slightly reduced A and g , whereas a more severe stress induced drastic reductions of both parameters (Fig. 1B,C). For all three water status conditions ETR did not vary (Fig. 1D). However, differences were observed in NPQ between the well-watered plant and the two water-stressed plants (Fig. 1E). Interestingly, at high irradiance, changes in NPQ were almost inversely matched by changes in F_s (Fig. 1F).

Figure 2 shows the light response of normalized F_s in potted grapevines for six different degrees of water stress, defined by their leaf conductance at $750 \mu\text{mol photons m}^{-2} \text{s}^{-1}$. Increasing water stress diminished the maximum F_s/F_o ratio, as well as the irradiance at which the maximum ratio was observed. When the F_s/F_o value

at a given irradiance ($750 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) was plotted against the corresponding g , a positive curvilinear relationship was observed between the two parameters (Fig. 2, insert).

When irrigated or severely stressed leaves of potted grapevines (similar to those of Fig. 1) were exposed to high CO_2 concentrations ($1500 \mu\text{mol (mol air)}^{-1}$) in the absence of O_2 , a total or partial reversion of water stress effects on A and NPQ was observed (Fig. 3A,B). As expected from Fig. 1, the decrease in F_s/F_o was almost totally suppressed by the treatment (Fig. 3C).

The effects of water stress on F_s were confirmed during a short transient in water stress using the Fi-PAM equipment (Flexas et al. 2000). The Fi-PAM uses a measuring spot larger than that of the PAM-2000, so the leaf areas used for gas-exchange and Chl fluorescence measurements were about the same. Water stress was imposed by cutting the petiole of leaves in air, while maintaining constant the leaf temperature (25°C) and illumination (in this case, $500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$). This procedure caused the well-known Iwanoff effect of an initial stomatal opening, followed by a progressive decline in conductance (Heber et al. 1986). Interestingly, the time course of F_s closely followed that of g (Fig. 4), and the inserts demonstrate a good correlation between F_s and both g and NPQ .

The involvement of the xanthophyll cycle in the observed changes of NPQ and F_s/F_o under water stress, was examined in leaves infiltrated with DTT, a well-known inhibitor of xanthophyll de-epoxidation (Bilger and Björkman 1994). Figure 5 shows that F_s/F_o of a stressed leaf increased when PPFD was increased from 0 to $200 \mu\text{mol m}^{-2} \text{s}^{-1}$, and decreased at 500 and $900 \mu\text{mol photons m}^{-2} \text{s}^{-1}$. The same leaf was cut under water and allowed to absorb DTT through the petiole for 3 h at $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD, until it reached a tissue concentration of about 3 mM (Bilger and Björkman 1994). The quenching of F_s relaxed even at low light, and the F_s/F_o

values increased markedly. Although a part of the reversion could be due to water stress removal by the water infiltrated, F_s/F_o reached a value of 2.3, whereas the maximum value observed even under irrigation was only approximately 1.4 (see Figs 2, 6 and 7). Moreover, at the highest irradiance level, NPQ was only 0.5 after DTT treatment, while it was 1.8 before DTT was added to the leaf (not shown). In addition, the Photochemical Reflectance Index (PRI) was simultaneously measured during this experiment, as described (Moya et al. 1998, Peñuelas and Filella 1998). Infiltrating DTT resulted in suppression of the PRI induction kinetics after each irradiance change, and resulted in increased steady-state PRI at high irradiance from -8×10^{-3} before DTT to 4×10^{-3} after DTT (S. Evain, J. Flexas and I. Moya, unpublished results). PRI is indicative of the ΔpH component of NPQ (Gamon et al. 1990), thus further supporting for the in-

volvement of a zeaxanthin-dependent quenching in the decrease of F_s/F_o .

The general relationship between F_s , NPQ and gas-exchange parameters in steady-state conditions

A positive curvilinear relationship was observed between F_s/F_o and both A and g in a larger number of measurements with potted grapevines under controlled conditions (25°C and $750 \mu\text{mol photons m}^{-2}\text{s}^{-1}$) in the laboratory (Fig. 6A,B). A negative, highly significant correlation was found between F_s/F_o and NPQ (Fig. 6C). As observed in Fig. 1, ETR was maintained constant under these conditions.

In field-grown grapevines and four other established potted C_3 plants grown outdoors F_s/F_o was also well correlated to A , g and NPQ (Fig. 7A-C), in spite of the large variations in leaf temperature (from 28.5 to 39.3°C) and incident PPFD (from 800 to $1250 \mu\text{mol m}^{-2}\text{s}^{-1}$). In contrast to the controlled environment, ETR decreased with water stress under field-conditions. Under these conditions, a good relationship was also observed between ETR and F_s/F_o (Fig. 7D). The best-fit regression between F_s/F_o and the other parameters was positive and linear (A and ETR), hyperbolic (g) or negative and exponential (NPQ). The strongest relationship was observed between NPQ and F_s/F_o ($r^2 = 0.81$).

As expected from the relationship between NPQ and F_s/F_o (Osmond 1994, Demmig-Adams et al. 1996), a close linear relationship was also observed between F_s/F_o and F_o'/F_o (not shown). In contrast, the correlation between F_s/F_o and qP was less clear (Fig. 8).

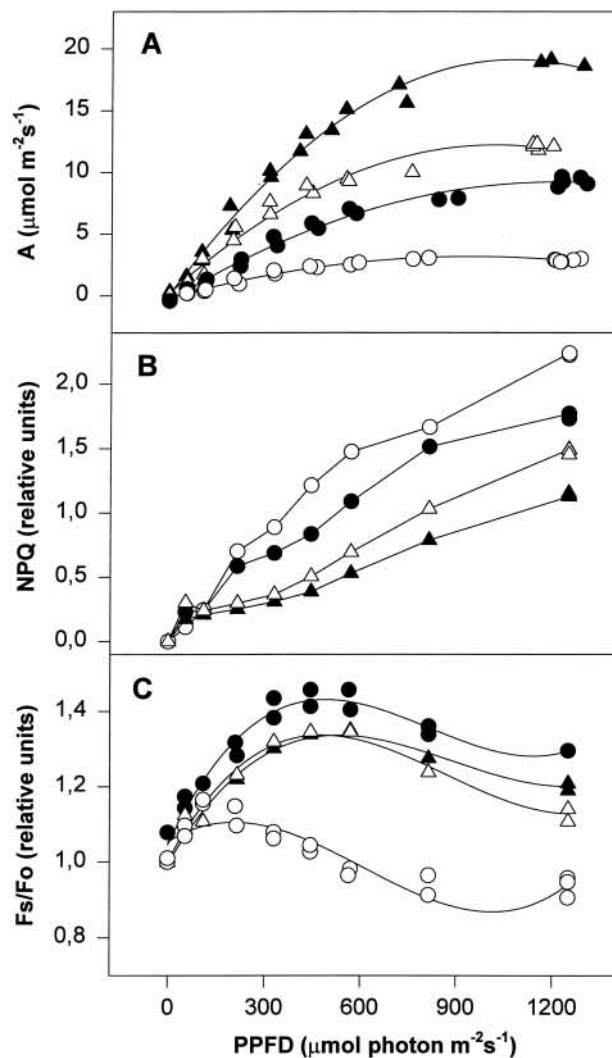


Fig. 3. Light response curves of net CO_2 assimilation (A), NPQ (B) and F_s/F_o (C). The control (\bullet) and severe drought (\circ) examples given on Fig. 1 are re-plotted. The effects of replacing normal air by high ($1500 \mu\text{mol CO}_2 (\text{mol air})^{-1}$) CO_2 in the absence of O_2 are shown for control (\blacktriangle) and water stressed (\triangle) leaves.

Discussion

The relationship between F_s/F_o and photosynthesis

This study confirms the well-known combined effects of water stress and high irradiance on leaf photosynthesis and NPQ observed in field-grown grapevines (Flexas et al. 1998, 1999a,b, Escalona et al. 1999), and other C_3 plants (Cornic 1994, Lawlor 1995). However, we draw attention to the fact that these parameters all correlate well with simple measurements of steady-state chlorophyll fluorescence (F_s). Because F_s can be monitored directly, without the imposition of saturating flashes using remote sensing systems (Moya et al. 1998, Flexas et al. 2000, Ounis et al. 2001), it is particularly useful for long-term monitoring of water stress.

Steady-state fluorescence yield (F_s) is a function of the competition between photochemical and non-photochemical de-excitation of the energy absorbed by the light harvesting complexes (Schreiber et al. 1998). Under normal conditions, electron transport and CO_2 fixation determine this balance at any given irradiance. This balance protects the photochemical apparatus from excessive over-reduction (Krause and Weis 1991, Melis 1999). Safe dissipation of excess energy may continue through electron transport processes, even when, for any reason, net

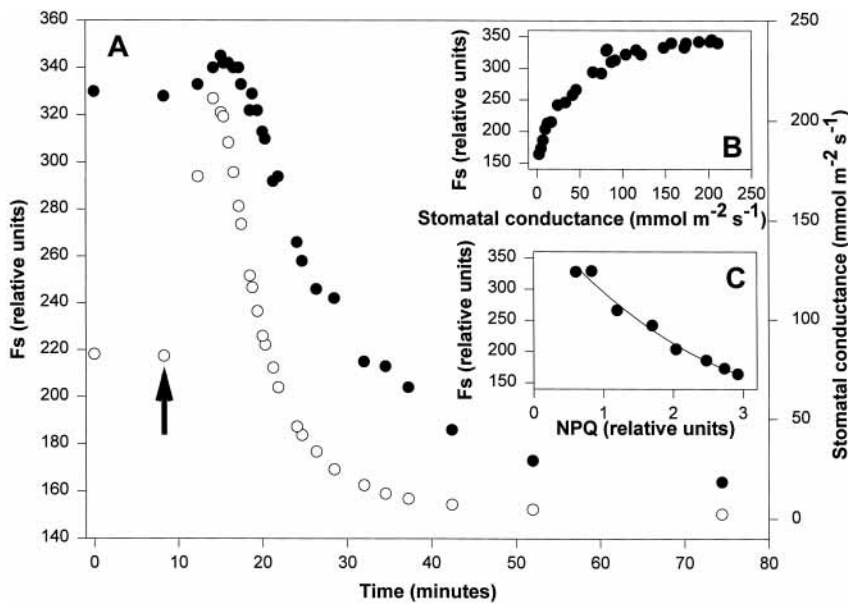


Fig. 4. Evolution with time of Fs (●) and g (○) after cutting a leaf in air (indicated by an arrow) at constant PPFD ($500 \mu\text{mol m}^{-2} \text{s}^{-1}$) and temperature (25°C). Fs values were not normalized as they were measured on the same leaf. The inserts show the relationship between Fs and g and NPQ through the entire time course.

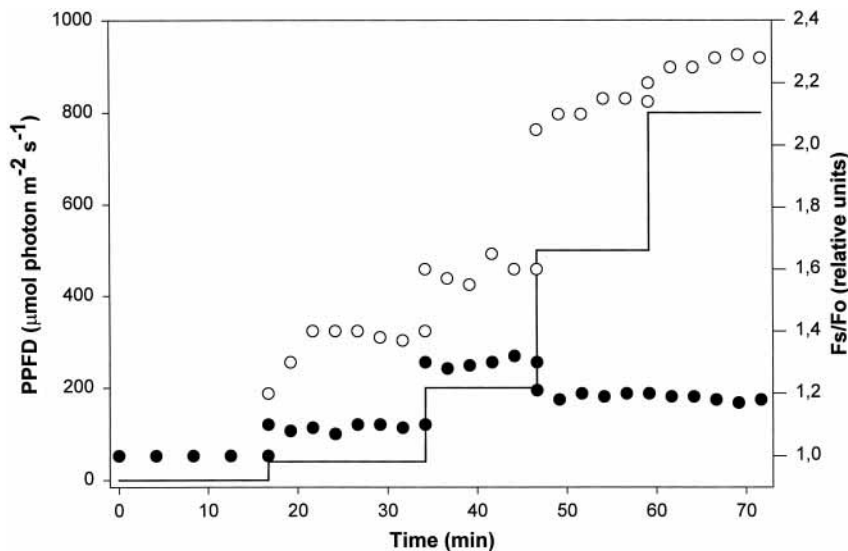


Fig. 5. Time variations of PPFD (line) and Fs (circles) during additional experiments performed at Orsay. Water stressed leaf (●) and the same leaf after being treated with DTT (○).

CO₂ assimilation is reduced. These processes include photorespiration (Wu et al. 1991), the Mehler-ascorbate pathway (Asada 1999) and cyclic electron flow around PSII (Canaani and Havaux 1990) or PSI (Katona et al. 1992). Photoprotection of PSII can also occur through non-photochemical mechanisms, the most important of which is qE-type quenching (Demmig-Adams and Adams 1992, Björkman and Demmig-Adams 1994, Niyogi 1999). This mechanism is induced by high trans-thylakoid ΔpH and is related to de-epoxidation of the xanthophyll cycle (Horton et al. 1996, Gilmore 1997).

Our experiments show that measurement of steady-state Chl fluorescence, the simple parameter Fs normalized to dark-adapted Fo, is an excellent indicator of declining stomatal conductance, CO₂ assimilation, and generation of NPQ during water stress. Effectively,

this parameter consolidates the cascade of consequences that follow from stomatal closure in response to water stress.

Under water stress in our experiments, stomata close in proportion to the degree of stress, progressively limiting CO₂ availability in the chloroplast. CO₂ assimilation is reduced and the CO₂/O₂ ratio drops (Cornic 1994, Lawlor 1995, Flexas et al. 1998, Escalona et al. 1999). As a consequence, photorespiration increases. Increased O₂ uptake under reduced CO₂ assimilation (induced by drought or chilling) has been recently demonstrated in grapevines (Flexas et al. 1999b), and has been ascribed to either oxygenase activity or electron transport to O₂ through a Mehler-type reaction. The Mehler reaction, linked to the ascorbate pathway, uses electrons without consuming

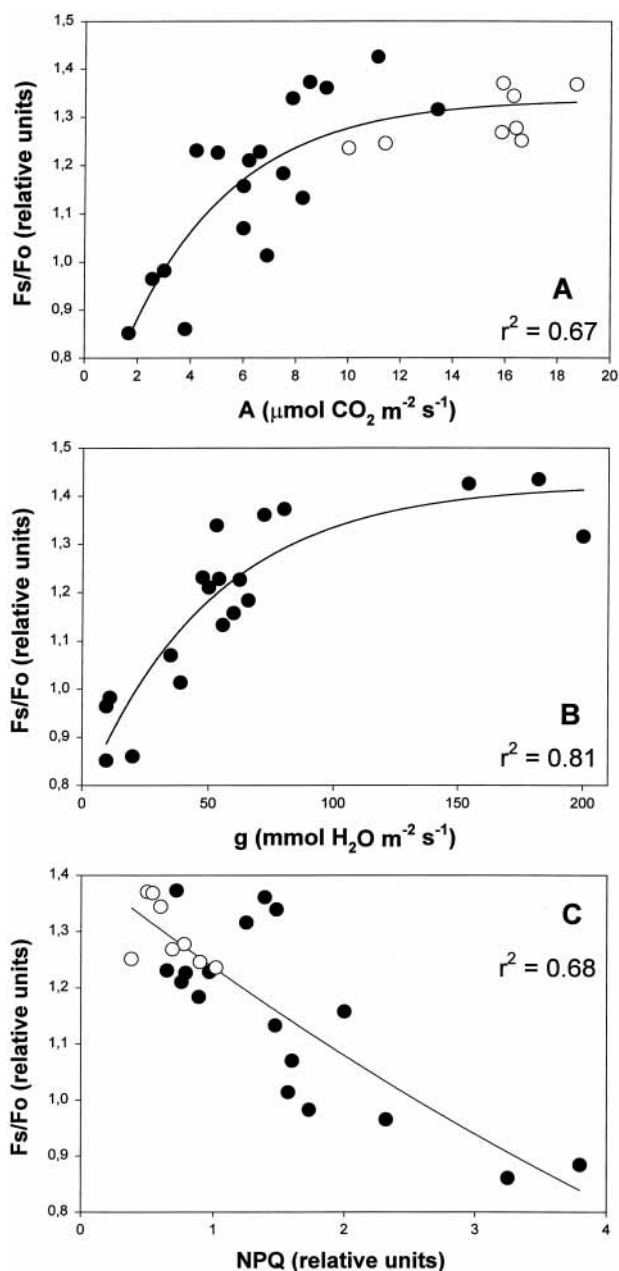


Fig. 6. Relationship between F_v/F_o and several photosynthetic parameters for values measured at $750 \mu\text{mol m}^{-2} \text{ s}^{-1}$ PPFD from all the experiments performed. (A) Relationship between F_v/F_o and A . (B) Relationship between F_v/F_o and g (values at high CO_2 have been removed due to the direct interaction of CO_2 with stomatal opening, which invalidates the correlation). (C) Relationship between F_v/F_o and NPQ. Filled circles are from leaves measured at ambient CO_2 , and empty circles from leaves measured at $1500 \mu\text{mol CO}_2$ (mol air^{-1}). Values at high CO_2 have been removed from the relationship with g . Figures show also hyperbolic fits with their determination coefficient (r^2).

ATP (Asada 1999), thus increasing trans-thylakoid ΔpH (Schreiber and Neubauer 1990). Alternatively, ΔpH may also increase if chloroplast ATPase is directly impaired under water stress (Tezara et al. 1999). The increase in trans-thylakoid ΔpH mediates xantho-

phyll de-epoxidation, leading to increased qE quenching of Chl fluorescence (Gilmore 1997, Niyogi 1999). Thermal dissipation in the antenna becomes progressively more competitive with excitation trapping by the PSII reaction centre, promoting the dissipation as heat. Under these conditions, chlorophyll fluorescence becomes a less competitive process, leading to lowered F_s .

Intriguing questions related to the underlying mechanism are raised by the above correlation: are these changes mediated by increased NPQ or do they reflect variations in the reduction state of Q_A ? The inverse correlation between F_s and irradiance under water stress has already been related to a strong increase in non-photochemical quenching (Cerovic et al. 1996). In our experiments, the strong correlation observed between F_s/F_o and NPQ suggests that F_s/F_o level is most directly responsive to NPQ. The principal determinant of NPQ (F_m') can never be less than F_s , so that a correlation could emerge from the arithmetic alone. However, the strong reversion of F_s/F_o decline by DTT, a well-known inhibitor of xanthophyll de-epoxidation, suggests the involvement of the xanthophyll cycle in NPQ. A strong correlation between NPQ and the de-epoxidation state of the xanthophyll cycle was indeed found in field-grown grapevines (Chaumont et al. 1997, Flexas et al. 2000). The Photochemical Reflectance Index, an indicator of zeaxanthin-dependent NPQ (Gamon et al. 1990), was also correlated with F_s/F_o during light transitions (S. Evain, J. Flexas and I. Moya, unpublished results). Furthermore, the effects of water stress on F_s/F_o were completely reversed by high CO_2 , suggesting the involvement of a rapid-relaxing non-photochemical quenching. Thus, the evidence available favours the direct dependence of F_s/F_o on NPQ. We frequently observed that F_s/F_o drops below 1.0 at very high NPQ, consistent with the presence of F_o quenching. This is not surprising because such a quenching is routinely observed when light-dark transients are followed (Björkman and Demmig-Adams 1994, Demmig-Adams et al. 1996). In contrast, although there was a certain tendency of increasing F_s/F_o with increasing qP, the correlation between these two parameters was less clear than any other, thus supporting further evidence for a direct control of F_s/F_o by NPQ.

F_s/F_o as a tool for remote sensing of water stress and irrigation scheduling

The relationship observed between F_s and g may provide a method for detection of water stress. However, several apparent limitations must be solved before the method becomes applicable. These limitations are: (a) the correlation is sometimes weak and dependent on the experimental conditions (b) the correlation is strongly dependent on irradiance, and (c) the correlation is curvilinear, implying that F_s/F_o is a rather insensible indicator of

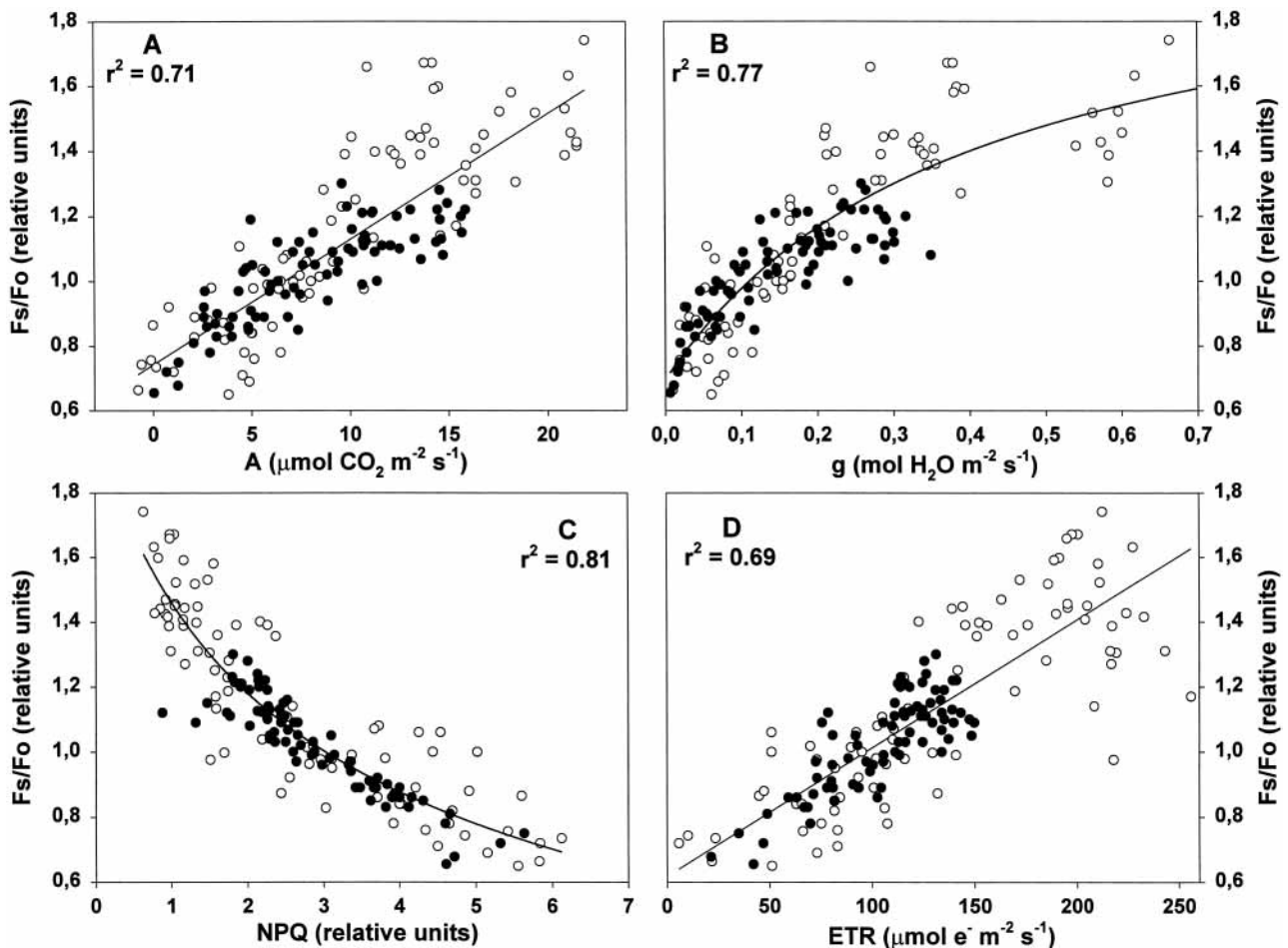


Fig. 7. Relationship between F_s/F_o and several photosynthetic parameters measured at saturating light and atmospheric CO_2 concentration under field conditions. Data from 5 different species: (●) *Vitis vinifera*, and (○) *Quercus ilex*, *Celtis australis*, *Solanum melongena* and *Pistacia terebinthus*, plotted together. (A) Relationship between F_s/F_o and A . The best fit was a linear regression, $F_s/F_o = 0.038 A + 0.74$, $r^2 = 0.71$. (B) Relationship between F_s/F_o and g . The best fit was an hyperbolic regression, $F_s/F_o = 0.69 + 1.40 g / (0.399 + g)$, $r^2 = 0.77$. (C) Relationship between F_s/F_o and NPQ. The curve of best fit was an exponential decay, $F_s/F_o = 0.33 + 0.52e^{-0.99\text{NPQ}} + 0.59e^{-0.19\text{NPQ}} + 0.54e^{-0.18\text{NPQ}}$, $r^2 = 0.81$. (D) Relationship between F_s/F_o and ETR. The best fit was a linear regression, $F_s/F_o = 0.0039 \text{ ETR} + 0.62$, $r^2 = 0.69$.

the initial stomatal closure at the early stages of water stress.

The weakness of the correlation was dependent on the experimental conditions. Clearly, the scattering is maximal in Fig. 7, where several species are included, and data obtained from a somewhat wide range of irradiance (between 800 and 1300 $\mu\text{mol m}^{-2}\text{s}^{-1}$). The highly significant correlation obtained under these conditions serves to reinforce the idea of a cause-effect reason for the relationship, but the large scattering present suggests, however, that the precise relationship should be different for each species and, possibly, growing conditions. The establishment of such a precise correlation should be a prerequisite for its use as a tool for remote sensing of water stress. When a single species is analysed (i.e. grapevines), the data dispersion is lower (see Fig. 6 and filled symbols in Fig. 7), but still high. Although some variability between individuals may be considered, the main reason for data dispersion may be due to sam-

pling methodology. When using the PAM-2000 and the Li-6400, fluorescence measurements are performed over a much smaller leaf area than gas-exchange measurements. This should be the main cause for the above-mentioned scattering, especially since it is known that leaf photosynthesis presents a non-homogeneous distribution over a leaf blade during water stress (Osmond et al. 1999). Using a FIPAM, or the new Laser-PAM recently described (Ounis et al. 2001), may solve this problem, since a bigger area is used for fluorescence measurements which completely covers the transparent window of Li-6400 (i.e. the same leaf area is used for gas exchange and fluorescence measurements). As seen in Fig. 4, the use of these instruments almost completely eliminates scattering. Similar examples have been published elsewhere (Ounis et al. 2001). Preliminary experiments using even larger areas of measurement (including several leaves at time) have yielded promising results (I. Moya, unpublished results). Therefore, the use of these

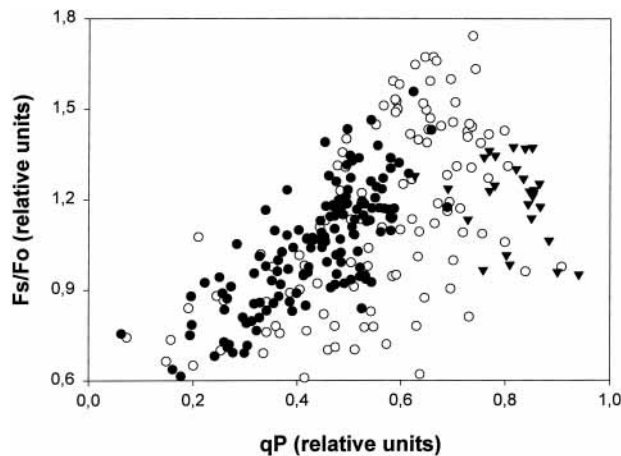


Fig. 8. Relationship between F_s/F_o and qP at saturating light and atmospheric CO_2 concentration. Data from: (▼) potted *Vitis vinifera* analysed in the laboratory (●) field-grown *Vitis vinifera*, and (○) *Quercus ilex*, *Celtis australis*, *Solanum melongena* and *Pistacia terebinthus*, plotted together.

instruments would provide, in addition to the possibility of remote sensing, a way to improve the correlation between F_s/F_o and gas exchange.

The dependence of the relationship on irradiance conditions also imposes a limitation for its use. However, under typical drought conditions, irradiance intensity is above photosynthesis saturation about half of the day, during the midday. Since this is the period when photosynthesis is more clearly affected by drought (Flexas et al. 1999a), it would be the proper time for F_s analysis. With the remote sensing fluorimeters (FIPAM and Laser-PAM), the incident irradiance over the leaf is recorded simultaneously with fluorescence determination and on exactly the same leaf area (Flexas et al. 2000, Ounis et al. 2001). This allows the capture of rapid variations (within seconds) of F_s which occur in response to sudden changes of incident light (Flexas et al. 2000), and this may help to eliminate the difficulties raised by the irradiance dependence of the relationship with the exception, perhaps, of cloudy days.

Finally, the insensitiveness of F_s/F_o to early changes of g would not be a major problem for its use in irrigation scheduling, at least in grapevines. As reported (Flexas et al. 1998, Escalona et al. 1999), these show a very low intrinsic water use efficiency at high g (i.e. little improvement of CO_2 assimilation at the expense of high increases of water loss). Moreover, it has been shown that, in early water stress situations when g -values are still maintained above $100 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$, photosynthesis is mainly limited by stomatal closure. In contrast, for more prolonged water stress when g drops further, non-stomatal limitations become dominant (Escalona et al. 1999, J. Flexas 2000, Thesis, University of the Balearic Islands, Mallorca, Spain). In grapevines, F_s/F_o declines steeply when non-stomatal limitations appear (i.e. when g drops below $100\text{--}150 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$), therefore it may be a sensitive indicator for

this range of water stress. Under moderate water deficit conditions, i.e. when photosynthesis is mainly limited by stomatal conductance, a complete recovery of the maximum A occurred just one night after irrigation (Flexas et al. 1999a). However, if g drops to values as low as $50 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$, photosynthesis did not reverse after irrigation (Quick et al. 1992). Thus, proper monitoring of F_s would be a useful tool for deciding when irrigation must be applied so that plants can be maintained at a limit between water stress and luxurious water consumption, and thus making a rational use of irrigation water. This method is especially useful, as it is not dependent on the measurement of fluorescence during saturating flashes, even for remote sensing (Moya et al. 1998, Flexas et al. 2000, Ounis et al. 2001).

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