Regulation of Photosynthesis of C₃ Plants in Response to Progressive Drought: Stomatal Conductance as a Reference Parameter

H. MEDRANO¹, J. M. ESCALONA¹, J. BOTA¹, J. GULÍAS¹ and J. FLEXAS¹,*

¹Laboratori de Fisiologia Vegetal, Departament de Biologia, Universitat de les Illes Balears – Instituto Mediterráneo de Estudios Avanzados (UIB-IMEDEA), Carretera de Valldemossa Km 7-5, 07071 Palma de Mallorca, Balears, Spain

Received: 1 May 2001 Returned for revision: 2 August 2001 Accepted: 7 September 2001

We review the photosynthetic responses to drought in field-grown grapevines and other species. As in other plant species, the relationship between photosynthesis and leaf water potential and/or relative water content in field-grown grapevines depends on conditions during plant growth and measurements. However, when light-saturated stomatal conductance was used as the reference parameter to reflect drought intensity, a common response pattern was observed that was much less dependent on the species and conditions. Many photosynthetic parameters (e.g. electron transport rate, carboxylation efficiency, intrinsic water-use efficiency, respiration rate in the light, etc.) were also more strongly correlated with stomatal conductance than with water status itself. Moreover, steady-state chlorophyll fluorescence also showed a high dependency on stomatal conductance. This is discussed in terms of an integrated down-regulation of the whole photosynthetic process by CO₂ availability in the mesophyll. A study with six Mediterranean shrubs revealed that, in spite of some marked interspecific differences, all followed the same pattern of dependence of photosynthetic processes on stomatal conductance, and this pattern was quite similar to that of grapevines. Further analysis of the available literature suggests that the above-mentioned pattern is general for C₃ plants. Even though the patterns described do not necessarily imply a cause and effect relationship, they can help our understanding of the apparent contradictions concerning stomatal vs. non-stomatal limitations to photosynthesis under drought. The significance of these findings for the improvement of water-use efficiency of crops is discussed.

Key words: Vitis vinifera L., grapevine, Mediterranean sclerophylls, C₃ plants, photosynthesis, stomatal conductance, photochemistry, carboxylation, drought, gas exchange, chlorophyll fluorescence.

INTRODUCTION

The debate as to whether drought mainly limits photosynthesis through stomatal closure or through metabolic impairment has been running since the earliest reports on the effects of drought on photosynthesis (Jones, 1973; Boyer, 1976; Quick et al., 1992; Lawlor and Uprety, 1993; Cornic, 1994; Lawlor, 1995; Tezara et al., 1999; Cornic, 2000; Flexas and Medrano, 2002a, b). During the last decade, stomatal closure was generally accepted to be the main determinant for decreased photosynthesis under mild to moderate drought (Sharkey, 1990; Chaves, 1991; Ort et al., 1994; Cornic and Massacci, 1996). Previously described non-stomatal effects were mostly attributed to the presence of non-homogeneous stomatal closure during drought (Downton et al., 1988; Terashima et al., 1988). However, evidence has been accumulating that shows that photophosphorylation (Havaux et al., 1987; Meyer and de Kouchkovsky, 1992), RuBP regeneration (Giménez et al., 1992; Gunasekera and Berkowitz, 1993) and Rubisco activity (Castrillo and Calcagno, 1989; Medrano et al., 1997) are impaired under drought. More recently, Lawlor and co-workers (Tezara et al., 1999) pointed out that impaired photophosphorylation and ATP synthesis was the main factor limiting photosynthesis in sunflower, even under mild drought. Thus, the old controversy has surfaced again (Cornic, 2000; Flexas and Medrano, 2002a, b), and was discussed at the SEB Meeting in Canterbury, UK, in April 2001 (Cornic and Fresneau, 2002; Lawlor, 2002; Tang et al., 2002).

Comparing results from different authors is complex due to interspecific differences in the response of stomatal conductance and photosynthesis to leaf water potential and/or relative water content, the parameters most often used to assess the degree of drought (Lawlor, 1995; Cornic and Massacci, 1996). It is clear that stomata close progressively as drought progresses, followed by parallel decreases of net photosynthesis. However, stomatal conductance is not controlled by soil water availability alone, but by a complex interaction of factors internal and external to the leaf.

It is certainly recognized that leaf water status interacts with stomatal conductance and transpiration and, under water stress, a good correlation is often observed between leaf water potential and stomatal conductance. However, the precise relationship is dependent, among other factors, on the species studied, the drought history of the individuals studied, the size of pots in which the plants are rooted or the environmental conditions during drought (Schulze and Hall, 1982; Tardieu and Simonneau, 1998; Flexas et al., 1999a; Tyree, 1999). Even within a given species, comparing results from different studies may be difficult. For instance, we have observed that the photosynthetic response to pre-dawn leaf water potential differs among grapevines, and
depends on conditions during plant growth and measurements, as well as on the cultivar examined (Flexas et al., 1998, 1999a, b; Escalona et al., 1999; Bota et al., 2001).

Moreover, stomata often close in response to drought before any change in leaf water potential and/or leaf water content is detectable (Gollan et al., 1985; Socías et al., 1997). It is now well established that there is a drought-induced root-to-leaf signalling, promoted by soil drying and reaching the leaves through the transpiration stream, which induces closure of stomata. This chemical signal has been shown to be abscisic acid (ABA), which is synthesized in the roots in response to soil drying (Davies and Zhang, 1991). However, its role is not simple, and a direct correlation between xylem ABA content and stomatal conductance has been shown only in some cases (Correia et al., 1995; Socías et al., 1997). Leaf water potential (Tardieu and Davies, 1992; Socías et al., 1997; Tardieu and Simmoneau, 1998), plant nutritional status (Schurr et al., 1992), xylem sap pH (Davies, 2002), farnesyltransferase activity (Pei et al., 1998) and other factors seem to modulate stomatal sensitivity to ABA. Xylem hydraulic conductivity, which is sometimes decreased under drought, has been shown to modulate stomatal closure directly (Salleo et al., 2000; Hubbard et al., 2001). Finally, stomata also close as leaf-to-air vapour pressure deficit (VPD) increases (Raschke, 1979; Dai et al., 1992; Oren et al., 1999), irrespective of soil water availability.

In summary, this complex regulation of stomatal conductance is related to important differences among species and genotypes in the response of stomata to leaf water potential, relative water content, ABA and other parameters, making it difficult to define a pattern of photosynthetic responses to drought. An interesting case is represented by species like grapevine that show isohydric behaviour (Choné et al., 2001). These species can show substantial photosynthetic limitations without any detectable change in their leaf water potential or relative water content (Tardieu and Simmoneau, 1998), thus raising questions as to the suitability of these parameters as a basis for comparison when studying the effects of drought on photosynthesis.

Nevertheless, it must be emphasized that a high degree of co-regulation of stomatal conductance (gs) and photosynthesis is usually found (Wong et al., 1979; Farquhar et al., 2001). Since gs is responsive to all the external (soil water availability, VPD) and internal (ABA, xylem conductivity, leaf water status) factors related to drought, it represents a more integrative basis for the overall effects of drought than leaf water potential and relative water content. Therefore, in searching for a common pattern of photosynthetic response to drought, we have used gs as an integrative parameter reflecting the water stress experienced by the plant. However, stomatal movements are very dynamic due to complex regulation by multiple factors. For this reason, mid-morning, light-saturated stomatal conductance (which is usually correlated with the average daily mean conductance) was taken as a representative value of gs. This was preferred to midday gs because, as drought becomes progressively more intense, the daily peak conductance drops and is displaced from around midday towards the early morning hours (Vadell et al., 1995; Flexas et al., 1999a).

The present report reviews a series of studies of the response of grapevines and other species to progressive drought. In these studies we relate every photosynthetic parameter (measured at steady state and light saturation) to the maximum light-saturated stomatal conductance observed for that plant at the moment of measuring.

**RELATING THE ELECTRON TRANSPORT RATE TO LIGHT-SATURATED STOMATAL CONDUCTANCE GENERALIZES ITS RESPONSES TO DROUGHT IN GRAPEVINES**

Early studies of chlorophyll fluorescence in irrigated and non-irrigated grapevines growing in the field during summer (Flexas et al., 1998) showed that permanent photoinhibition, as determined by pre-dawn photochemical efficiency (Fv/Fm), was rare even under severe drought. The rate of light-saturated electron transport (ETR), measured at midday, sometimes decreased in non-irrigated plants, but decreased to a lesser extent than net CO2 assimilation (A). This was understood as indicative of a relative increase in photorespiration, which has been known to occur under drought since the early studies by Lawlor and co-workers (Lawlor and Fock, 1975, 1977a, b; Lawlor, 1976a, b; Lawlor and Pearman, 1981) and is now well accepted (Wingler et al., 1999, 2000). We have recently demonstrated that O2 uptake increases significantly in water-stressed grapevines, presumably due mainly to photorespiration and only due in minor part to an increase in the Mehler reaction (Flexas et al., 1999b, 2002a). At the time of the first study (Flexas et al., 1998), we assumed that photorespiration might be an important photoprotective mechanism in field-grown grapevines, as suggested for other species (Heber et al., 1996; Kozaki and Takeba, 1996), since ETR remained relatively high even under severe stress. Moreover, although there was a certain tendency for ETR to decrease with decreasing pre-dawn leaf water potential (Ψ), a non-significant relationship was observed between these two parameters (Fig. 1A). These results contrasted with the highly significant linear relationship that was observed recently between ETR and Ψ in 2-year-old grapevines of the same cultivar (Tempranillo), maintained in large pots and grown under field conditions (Flexas et al., 1999a, see Fig. 1A).

Figure 1B shows that the response of stomatal conductance to Ψ was also different in field-grown and potted grapevines, possibly due to differences in the root system, osmotic adjustment and/or stomatal sensitivity to drought. Interestingly, when ETR was plotted against gs, a single hyperbolic function satisfactorily fitted data from both field-grown and potted plants (Flexas et al., 2002a) (Fig. 1C). From gs values of 400 down to about 150 mmol H2O m⁻² s⁻¹, ETR is little affected. Lower gs values lead to steep reductions of...
ETR. A study with 22 different grapevine cultivars, rooted in pots and grown under field conditions (Bota et al., 2001), also revealed that ETR correlated better with gs than with either leaf relative water content (RWC) or \( \Psi \) (Flexas et al., 2002a). On the basis of these observations, as well as on theoretical considerations given in the Introduction, we hypothesized that the use of gs as an integrative parameter reflecting the water stress condition of the plant would help to generalize a pattern of response of different photosynthetic processes to drought. To test this hypothesis, we related different photosynthetic parameters, studied in both field- and pot-grown grapevines between 1994 and 2000, to the corresponding light-saturated gs (Flexas et al., 2002a). These parameters included \( A_n \), the estimated gross photosynthesis (\( A_g \)), ETR, the ratios ETR/\( A_n \) and ETR/\( A_g \), leaf dark respiration (\( R_D \)), pre-dawn \( F_v/F_m \), non-photochemical quenching of chlorophyll fluorescence at midday (NPQ) and parameters derived from analyses of \( A_n-C_i \) curves, such as the apparent carboxylation efficiency (\( e' \)), leaf light respiration (\( R_L \)), CO2 compensation point (\( G_c \)) and the CO2-saturated rate of photosynthesis (\( A_{sat} \)). All parameters were found to be highly significantly correlated to gs, and accurately fitted data from both field-grown and potted plants, as well as data from 23 different cultivars (Flexas et al., 2002a).

Drought usually leads to erroneous calculation of \( C_i \) due to patchy stomatal closure (Downton et al., 1988; Terashima et al., 1988) and different cuticular conductance to water vapour and CO2 (Boyer et al., 1997). These limitations were taken into account and estimated, and the true \( C_i \) was recalculated accordingly (Osmond et al., 1997a; Escalona et al., 1999; Flexas et al., 2002a). Therefore, the \( C_i \) data used in the present paper should be free of errors, except for the low accuracy of gas-exchange determinations at very low gs.

Irrespective of the origin of the data (year, season, irrigation treatment, field- or pot-grown plants), significant regression patterns were observed between each parameter and gs. Three regions could be differentiated on these regressions along a gradient of gs during the development of drought. Decreases in gs from 0.4 to 0.15 mol H2O m\(^{-2}\) s\(^{-1}\) (corresponding to a mild water stress) were paralleled by a decline in \( A_n \) and a progressive decline in the sub-stomatal CO2 concentration. This suggested that stomatal limitations to photosynthesis were dominant. The ratio ETR/\( A_n \) increased, mirroring the decline in \( C_i \), which suggested an increased rate of photorespiration. At lower values of gs (0.15-0.05 mol H2O m\(^{-2}\) s\(^{-1}\)), \( C_i \) still decreased, but the electron transport rate and the carboxylation efficiency started to decline. At this stage, both stomatal and non-stomatal limitations were therefore important. Further reductions of gs (< 0.05 mol H2O m\(^{-2}\) s\(^{-1}\)) led to steeper reductions of \( A_n \), ETR and \( e' \), and to steep increases in \( C_i \), indicating that non-stomatal limitations to photosynthesis became dominant. Under these conditions pre-dawn \( F_v/F_m \),...
occasionally decreased. Although the ratio ETR/A\textsubscript{n} and $\Gamma$ increased exponentially with decreases in $g\textsubscript{s}$, the ratio ETR/A\textsubscript{g} remained almost constant through the entire range of $g\textsubscript{s}$, suggesting that the Mehler reaction did not increase substantially as stress progressed.

In summary, these results show that in addition to $A\textsubscript{n}$ and ETR, other important photosynthetic parameters were correlated to $g\textsubscript{s}$ in a simple manner, whereas their correlation to $\Psi$ and RWC was dependent on experimental conditions.

**DO THESE RELATIONSHIPS GIVE INSIGHTS INTO THE PROCESSES LIMITING PHOTOSYNTHESIS UNDER DROUGHT IN GRAPEVINES?**

The curves of best fit between four parameters ($A\textsubscript{n}$, ETR, $A\textsubscript{sat}$ and $\varepsilon$) and $g\textsubscript{s}$ are shown in Fig. 2. These parameters were selected because they represent very important components of photosynthesis: $A\textsubscript{n}$ is the actual rate of photosynthesis, ETR reflects the capacity for energy and reductant synthesis, $A\textsubscript{sat}$ may be related to the potential photosynthetic capacity and $\varepsilon$ reflects, to some extent, the activity and activation state of Rubisco.

Once these general relationships are established, one can evaluate the relative importance of each process in photosynthetic limitation at any given degree of water stress, represented by a value of $g\textsubscript{s}$ (Fig. 3). As drought progresses, the proportional decrease in the parameters studied was much less than the decline in stomatal conductance for any given interval of the latter. For instance, when $g\textsubscript{s}$ was halved, $A\textsubscript{n}$ decreased by only 30%. Therefore, during that interval, $C\textsubscript{i}$ decreased, whereas the intrinsic water use efficiency ($A\textsubscript{n}/g\textsubscript{s}$) and the rate of photorespiration increased (not shown). At the same time, $A\textsubscript{sat}$ decreased by 20% and ETR and $\varepsilon$ decreased by less than 10%. Therefore, over that range of $g\textsubscript{s}$ (i.e. mild drought), stomatal closure seems to be the main cause of decreased photosynthesis. This does not mean that non-stomatal limitations are absent, but simply that they are not the dominant factor limiting photosynthesis. For instance, decreasing $A\textsubscript{sat}$ suggests that the capacity for RuBP regeneration is adjusted progressively since early stomata closure.

Further reduction of $g\textsubscript{s}$ leads to more important reductions of all the parameters studied. When $g\textsubscript{s}$ is 100 mmol H\textsubscript{2}O m\textsuperscript{-2} s\textsuperscript{-1}, $A\textsubscript{n}$ decreases by 50%, $A\textsubscript{sat}$ by 35%, and ETR and $\varepsilon$ by 25–30%. When $g\textsubscript{s}$ equals 50 mmol H\textsubscript{2}O m\textsuperscript{-2} s\textsuperscript{-1}, $A\textsubscript{n}$ decreases by 70%, $A\textsubscript{sat}$ and $\varepsilon$ by 50%, and ETR by 40%. Below this threshold of $g\textsubscript{s}$, $C\textsubscript{i}$ increases (not shown), suggesting the predominance of non-stomatal limitations to photosynthesis.

These results in field-grown grapevines reveal a pattern of gradual response of photosynthesis to water stress, similar to that proposed by Lawlor (1995). After an early effect of drought resulting in partial stomatal closure, a metabolic adjustment takes place through limited RuBP-regeneration (possibly due to impaired ATP synthesis, see below). Further reductions of $g\textsubscript{s}$ as drought progresses lead to reduced photochemistry and carboxylation efficiency.

**WHAT ABOUT OTHER SPECIES?**

To further test the generality of the relationships between different photosynthetic parameters and light-saturated $g\textsubscript{s}$, six Mediterranean sclerophyllous trees and shrubs were subjected to progressive soil drying (Gulías et al., 2002).
We had previously shown that in one of these species, *Pistacia lentiscus* L., drought induced a cascade of photosynthetic regulations qualitatively similar to that of grapevines, first involving stomatal closure and, later, non-stomatal regulation (Gulló et al., 2002). Three of these species (*Quercus ilex*, *Rhamnus alaternus* and *R. ludoviciana*) showed proportional decreases of gs and RWC in response to soil drying. In contrast, the other three (*Quercus pubescens*, *Pistacia lentiscus* and *P. terebinthus*) showed similar decreases of gs but their RWC remained almost constant (Flexas, Gulló, Abadía and Medrano, unpubl. res.). In spite of this distinct behaviour, all six species showed a similar pattern of dependency of different photosynthetic parameters on gs. We have superimposed results obtained for these six species over the relationships obtained for grapevines (Fig. 4), and also added to the figure results from other authors to increase the genetic and environmental variability. All the data points added are similar to the relationship for grapevine in respect to $A_n$, ETR and $\varepsilon$. This was surprising given that the species studied represent a substantial variety of life forms and photosynthetic characteristics. The data that fitted least well were those for $A_{sat}$ for the six sclerophyllous species (Fig. 4C). The fact that these species share with grapevines a common relationship between $A_n$ and $g_s$, while displaying such a divergence in their relationship between $A_{sat}$ and $g_s$, could reflect a higher mesophyll resistance in the sclerophyllous species. It has been shown that sclerophyllous and woody species generally have a substantially higher mesophyll resistance than more mesophytic species (Lloyd et al., 1992; Epron et al., 1995; Evans and von Caemmerer, 1996). Additionally, the data from other authors were not dissimilar to the relationships found in grapevines. The non-origin intercept of the data from Martin and Ruiz-Torres (1992) was probably due to the fact that the relationships were not obtained from the original data, but rather from a combination of the best-fit relationships given by the authors for the plots of $g_s$, $A_{sat}$ and $\varepsilon$ vs. $\Psi$.

From the present data it is concluded that, although there is wide variability among species and genotypes in the maximum values of photosynthesis and stomatal conductance, as well as in the variations of leaf $\Psi$ and RWC...
(Schulze and Hall, 1982; Vadell and Medrano, 1992; Bota et al., 2001), the photosynthesis to conductance ratio is largely maintained (see also Farquhar et al., 1987; Lloyd et al., 1992; Bota et al., 2001). Even when relationships between different photosynthetic parameters and \( g_s \) are influenced by the species (Fig. 4; see Schulze and Hall, 1982; Farquhar et al., 1987), the species-effect seems to be much less than that on photosynthesis and RWC or \( \Psi \).

**DO GAS-EXCHANGE DATA MATCH THE BIOCHEMICAL EVIDENCE?**

The present results support a quite generalized pattern of down-regulation of different photosynthetic parameters in response to drought when using light-saturated \( g_s \) as a reference parameter. Such a pattern can be used to analyse the relative importance of every process at any given degree of stress. Nevertheless, all the evidence presented to date derives from *in vivo* measurements of gas exchange and chlorophyll fluorescence, and the interpretation of the results ultimately lies in the model of Farquhar and co-workers (Farquhar, 1980; von Caemmerer and Farquhar, 1981) and its derivatives. The validation of this model still needs to be extended, especially in respect to long-term responses (Farquhar et al., 2001).

To test the validity of this gas-exchange model for the estimation of drought-depressed rates of certain biochemical reactions, the results presented here are compared with those of other authors in which destructive, biochemical determinations were made in control and water-stressed plants at the same time as gas-exchange measurements. In particular, two important assumptions of the gas-exchange model require validation. First, in the model, control of RuBP regeneration is ascribed to ETR but, as recognized by Farquhar et al. (2001), it could also be limited by other components of the photosynthetic carbon reduction cycle. Secondly, the apparent carboxylation efficiency (\( \epsilon \)) was thought to be controlled by Rubisco activity, but other mesophyll limitations to photosynthesis may also exert control over \( \epsilon \). It is important to address both aspects for the study of photosynthetic responses to drought.

*Decreased capacity for RuBP regeneration should come from decreased ATP synthesis under moderate water stress*

Decreased capacity for RuBP regeneration, as determined by the \( \text{CO}_2 \)-saturated rate of photosynthesis, has been shown many times to be an early response to drought, decreasing much earlier than \( \epsilon \) (von Caemmerer and Farquhar, 1984; Martin and Ruiz-Torres, 1992; Escalona et al., 1999; see Figs 3 and 4). Determination of RuBP content of leaves from water-stressed plants seems to confirm that decreased capacity for RuBP regeneration is an early response to drought (Giménez et al., 1992; Gunasekera and Berkowitz, 1993). Farquhar’s model of photosynthesis assumes that this may be due to decreased ETR. However, the introduction of chlorophyll fluorescence techniques has shown that under mild drought \( A_{\text{sat}} \) is usually reduced to a much greater extent than ETR (Figs 3 and 4). Tezara et al. (1999) have suggested that decreased ATP synthesis through ATPase impairment would lead to reduced RuBP regeneration. Whether impaired ATPase would also affect ETR or not depends on the precise mechanism of impairment, which is still not well understood, and other possible unknown metabolic adjustments. In spite of these uncertainties, there seems to be an agreement between gas exchange and biochemical literature. Clearly, both limited RuBP regeneration and impaired ATP synthesis still occur at high light-saturated \( g_s \) (over 150 mmol H\(_2\)O m\(^{-2}\) s\(^{-1}\)), i.e. in early phases of drought development (Younis et al., 1979; Turner et al., 1985; Havaux et al., 1987; Meyer and de Kouchkovsky, 1992; Tezara et al., 1999). To our knowledge, there is only one report (Ortiz-López et al., 1991) of no inhibition of ATPase even at lower \( g_s \). The causes for reduced ATP synthesis under mild drought remain to be determined.

*Decreased carboxylation capacity does not reflect only decreased Rubisco activity*

The wide use of \( A_{\text{sat}}-C_i \) curves has led to several reports showing a decrease in the apparent carboxylation efficiency (and thus, presumably, Rubisco activity) even at mild to moderate water stress in a number of species (Figs 3 and 4; see Martin and Ruiz-Torres, 1992; Antolín and Sánchez-Díaz, 1993; Faver et al., 1996; Escalona et al., 1999). However, assays of Rubisco activity from water-stressed leaves have generally led to the conclusion that both its activity and activation state remain unaffected until the stress is severe (Jones, 1973; Beadle and Jarvis, 1977; Sharkey and Seemann, 1989; Pliant and Federman, 1991; Parry et al., 1993; Lal et al., 1996; Tezara et al., 1999; Wingler et al., 1999; Parry et al., 2002). Inhibition of Rubisco activity at mild to moderate water deficits has been reported only occasionally (Castrillo and Calcagno, 1989; Holaday et al., 1992; Medrano et al., 1997).

Therefore, for the particular case of Rubisco activity, it seems that the photosynthetic model of Farquhar et al. (1980) does not match the biochemical determinations. This is illustrated in Fig. 5, which shows the relationship between \( g_s \) and both \( \epsilon \) measured in grapevines and Rubisco activity determined *in vitro* for different species, including grapevines, by different authors. The results are expressed as a percentage of the control (unstressed) values to facilitate comparison of different units used by different authors, as well as to compare \( \epsilon \) with Rubisco activity. Again, \( g_s \) proves to be a solid reference parameter, since it generalizes the response of Rubisco activity to drought among a wide range of species and conditions. It is clear that two different relationships are obtained, the differences initially increasing with decreasing \( g_s \). When \( g_s \) is between 50 and 150 mmol H\(_2\)O m\(^{-2}\) s\(^{-1}\), \( \epsilon \) is about 20–30 % lower than the measured Rubisco activity.

A possible explanation arises given that \( \epsilon \) is underestimated whenever \( C_i \) is proportionally overestimated. Although we took into account patchy stomatal closure and cuticular conductance when calculating \( C_i \) (see previous sections), a different problem, namely varying mesophyll resistance, would lead to large and variable differences between \( C_i \) and the actual \( \text{CO}_2 \) concentration at the carboxylation site (\( C_e \)), so \( \epsilon \) would no longer be represen-
We have shown that in general the drought-regulation of a wide range of parameters related to photosynthesis seems more dependent on stomatal conductance than on typical parameters reflecting leaf water status. As these relationships are similar for different plant species and different circumstances, one inherent implication could be that under drought, down-regulation of different photosynthetic processes depends more on CO₂ availability in the mesophyll (i.e. on stomatal closure) than on leaf water potential or leaf water content, as suggested previously (Sharkey, 1990). This could be understood as a direct adjustment of photosynthetic metabolism to CO₂ availability, which is well known to act as a regulator of Rubisco (Perchorowicz and Jensen, 1983; Meyer and Genty, 1999), nitrate reductase (Kaiser and Förster, 1989) and sucrose phosphate synthase (Vassey et al., 1991). Low CO₂ also promotes increased trans-thylakoid ΔpH, which induces increased NPQ. Nevertheless, these suggestions are merely based on statistical correlative evidence, and further studies are required to prove them. In particular, it remains to be determined if low CO₂ availability, or the pH changes resulting from it, are capable of promoting down-regulation of other important photosynthetic steps such as ATP synthesis.

Irrespective of the uncertainties raised about the mechanistic reasons for the strong dependence of any photosynthetic parameter on gs, it reveals an integrated down-regulation of the whole photosynthetic process as drought progresses, in accordance with theories of integrated ‘photosynthetic control’ (Foyer et al., 1990). This integrated regulation of photosynthesis is reinforced by this analysis made, so they are not conclusive. A more extensive analysis of the effects of drought on mesophyll resistance is therefore needed.

**IMPLICATIONS OF THE PRESENT RESULTS AND PRACTICAL APPLICATIONS**

Gas-exchange measurements, according to a current model (Epron et al., 1995; Valentini et al., 1996; Roupsard et al., 1996). To test this possibility, we estimated C₅ from combined gas-exchange and chlorophyll fluorescence measurements, according to a current model (Epron et al., 1995; Valentini et al., 1996), and assuming that all the reducing power generated by the electron transport chain is used for photosynthesis and photorespiration, with only a negligible proportion being consumed by the Mehler reaction and other processes. The data obtained suggested that mesophyll conductance was decreasing as gs declined (Flexas et al., 2002a). Thereafter, we converted Aᵦ₋Cᵦ curves to Aᵦ₋Cₑ curves, and recalculated ε on this new basis. Figure 6 shows the relationship obtained between ε and light-saturated gs, using this new approach and should be compared with Fig. 2D showing the relationship based on the typical Aᵦ₋Cᵦ approach. Clearly, the new relationship is much more similar to that between Rubisco activity and light-saturated gs obtained from the literature (Fig. 5).

These findings seem to confirm an early study by Beadle and Jarvis (1977), who showed a decreased mesophyll conductance in *Picea sitchensis* as drought progressed without any inactivation of Rubisco as determined *in vitro*. It is suggested that drought-induced down-regulation of mesophyll conductance to CO₂ is much more important than previously thought. Nevertheless, these results are simply based on a model that requires many assumptions to be made, so they are not conclusive. A more extensive analysis of the effects of drought on mesophyll resistance is therefore needed.
since a direct correlation was described between \( g_s \), determined at a given light intensity, and a fluorescence parameter which, in principle, may have little dependence on stomatal conductance, the steady-state chlorophyll fluorescence \( (F_s) \) (Fig. 7; see Ounis et al., 2001; Flexas et al., 2002b). Figure 8 shows a tentative scheme of such a photosynthetic control under drought, which can be summarized as follows. Under drought, 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, thereby increasing photorespiration and/or the Mehler reaction. Since these processes consume relatively less ATP than does photosynthesis, they should lead to a certain increase of trans-thylakoid \( \Delta pH \) (Schreiber and Neubauer, 1990; Osmond et al., 1997b). Impaired ATPase and/or reduced ETR may also interfere with the build-up of trans-thylakoid \( \Delta pH \). The xanthophyll de-epoxidation that follows increased \( \Delta pH \) should lead to increased NPQ. Thermal dissipation in the antenna becomes progressively more important and \( F_s \) is consequently lowered.

The relationship between \( F_s \) and \( g_s \) provides a method for remote sensing of water stress. In grapevines, \( F_s/F_o \) declines steeply when non-stomatal limitations become important (when \( g_s \) drops below 100–150 mmol H2O m\(^{-2}\) s\(^{-1}\), see Figs 3, 4 and 7). Under moderate water deficit, i.e. when photosynthesis is mainly limited by stomatal conductance, a complete recovery of the maximum \( A_o \) occurred just one night after irrigation (Flexas et al., 1999a). However, if \( g_s \) reaches values as low as 50 mmol H2O m\(^{-2}\) s\(^{-1}\), photosynthesis does 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 to maintain plants at a limit between severe water stress and luxurious water consumption, thus rationalizing use of irrigation water. This method is especially useful because it does not depend on measuring fluorescence during saturating flashes, even during remote sensing (Moya et al., 1998; Flexas et al., 2000, 2002b; Ounis et al., 2001).

**FIG. 7.** Relationship between steady-state chlorophyll fluorescence \( (F_s) \) and stomatal conductance in leaves of grapevine, measured at a PPFD of 500 \( \mu\text{mol photons m}^{-2}\ \text{s}^{-1} \) (from Ounis et al., 2001).

\[ F_s = 101.8 + 45.9 \ln(g_s) \]
\[ r^2 = 0.96 \ (n = 27) \]

**FIG. 8.** Proposed mechanism of leaf response to water stress, explaining the observed interrelationships among different photosynthetic processes. Numbers indicate a proposed order for reading the scheme. See text for more details.

**ACKNOWLEDGEMENT**

We thank David Lawlor for introducing us to research on drought, for helpful comments and advice, and for his friendship.

**LITERATURE CITED**


Medrano et al.—Regulation of Photosynthesis of C₃ Plants Under Drought


Terashima I, Wong SC, Osmond CB, Farquhar GD, 1988. Characterization of non-uniform photosynthesis induced by...


