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Regulation of Photosynthesis of C_3 Plants in Response to Progressive Drought: Stomatal Conductance as a Reference Parameter

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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 CO_2 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 C_3 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, C_3 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, 2002*a*, *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, 2002*a*, *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*., 1999*a*; 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 predawn leaf water potential differs among grapevines, and

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depends on conditions during plant growth and measurements, as well as on the cultivar examined (Flexas *et al*., 1998, 1999*a*, *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 (g_s) and photosynthesis is usually found (Wong *et al*., 1979; Farquhar *et al*., 2001). Since g_s 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 g_s 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 g_s. This was preferred to midday g_s 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*., 1999*a*).

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 (F_v/F_m), 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 CO_2 assimilation (A_n). 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, 1977*a*, *b*; Lawlor, 1976*a*, *b*; Lawlor and Pearlman, 1981) and is now well accepted (Wingler *et al*., 1999, 2000). We have recently demonstrated that O_2 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*., 1999*b*, 2002*a*). 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*., 1999*a*, 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 g_s, a single hyperbolic function satisfactorily fitted data from both field-grown and potted plants (Flexas *et al*., 2002*a*) (Fig. 1C). From g_s values of 400 down to about 150 mmol H_2O m^{-2} s^{-1}, ETR is little affected. Lower g_s values lead to steep reductions of

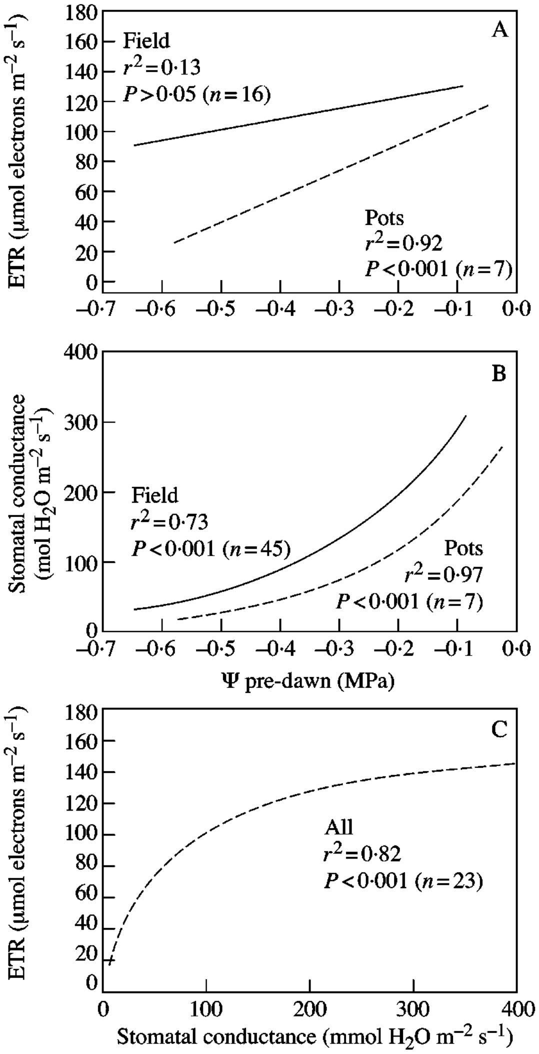

FIG. 1. Relationships between photosynthetic electron transport rate (ETR) and pre-dawn leaf water potential (Ψ)(A), and stomatal conductance and pre-dawn leaf water potential (B) in field-grown (solid lines) and potted (broken lines) grapevines. Only the regression fits are shown, indicating their correlation coefficient and significance (data from Flexas *et al.*, 1999*a*). When ETR was plotted against stomatal conductance (C), a single hyperbolic correlation was observed including field and potted plants (data from Flexas *et al.*, 2002*a*).

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 g_s than with either leaf relative water content (RWC) or Ψ (Flexas *et al.*, 2002*a*).

The primary correlation between A_n and g_s was already known to exist for both field- and pot-grown plants (Escalona *et al.*, 1999; Flexas *et al.*, 2002*a*). The most surprising of these new results was that the use of g_s also generalized the response of a parameter that, in principle, was not directly related to stomatal closure. That is, a secondary strong relationship was also found between ETR and g_s.

LIGHT-SATURATED STOMATAL CONDUCTANCE GENERALIZES THE RESPONSES OF MANY PHOTOSYNTHETIC PARAMETERS TO DROUGHT IN GRAPEVINES

Apart from ETR, pre-dawn F_v/F_m and the sub-stomatal CO_2 concentration (C_i) have also been shown in previous studies to be more dependent on g_s than on Ψ (Flexas *et al.*, 1998; Escalona *et al.*, 1999). On the basis of these observations, as well as on theoretical considerations given in the Introduction, we hypothesized that the use of g_s 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 g_s (Flexas *et al.*, 2002*a*). These parameters included A_n, C_i, 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 (ε), leaf light respiration (R_L), CO_2 compensation point (Γ) and the CO_2-saturated rate of photosynthesis (A_{sat}). All parameters were found to be highly significantly correlated to g_s, and accurately fitted data from both field-grown and potted plants, as well as data from 23 different cultivars (Flexas *et al.*, 2002*a*).

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 CO_2 (Boyer *et al.*, 1997). These limitations were taken into account and estimated, and the true C_i was re-calculated accordingly (Osmond *et al.*, 1997*a*; Escalona *et al.*, 1999; Flexas *et al.*, 2002*a*). 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 g_s.

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 g_s. Three regions could be differentiated on these regressions along a gradient of g_s during the development of drought. Decreases in g_s from 0·4 to 0·15 mol H_2O 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 CO_2 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 g_s (0·15–0·05 mol H_2O 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 g_s (< 0·05 mol H_2O m^{-2} s^{-1}) led to steeper reductions of A_n, ETR and ε, 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_n and Γ increased exponentially with decreases in g_s, the ratio ETR/A_g remained almost constant through the entire range of g_s, suggesting that the Mehler reaction did not increase substantially as stress progressed.

In summary, these results show that in addition to A_n and ETR, other important photosynthetic parameters were correlated to g_s in a simple manner, whereas their correlation to Ψ 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_n, ETR, A_{sat} and ε) and g_s are shown in Fig. 2. These parameters were selected because they represent very important components of photosynthesis: A_n is the actual rate of photosynthesis, ETR reflects the capacity for energy and reductant synthesis, A_{sat} may be related to the potential photosynthetic capacity and ε 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_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_s was halved, A_n decreased by only 30 %. Therefore, during that interval, C_i decreased, whereas the intrinsic water use efficiency (A_n/g_s) and the rate of photorespiration increased (not shown). At the same time, A_{sat} decreased by 20 % and ETR and ε decreased by less than 10 %. Therefore, over that range of g_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_{sat} suggests that the capacity for RuBP regeneration is adjusted progressively since early stomata closure.

Further reduction of g_s leads to more important reductions of all the parameters studied. When g_s is 100 mmol H_2O m^{-2} s^{-1}, A_n decreases by 50 %, A_{sat} by 35 %, and ETR and ε by 25–30 %. When g_s equals 50 mmol H_2O m^{-2} s^{-1}, A_n decreases by 70 %, A_{sat} and ε by 50 %, and ETR by 40 %. Below this threshold of g_s, C_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_s as drought progresses lead to reduced photochemistry and carboxylation efficiency. Photoinhibition eventually occurs under conditions of very severe drought and almost complete stomatal closure.

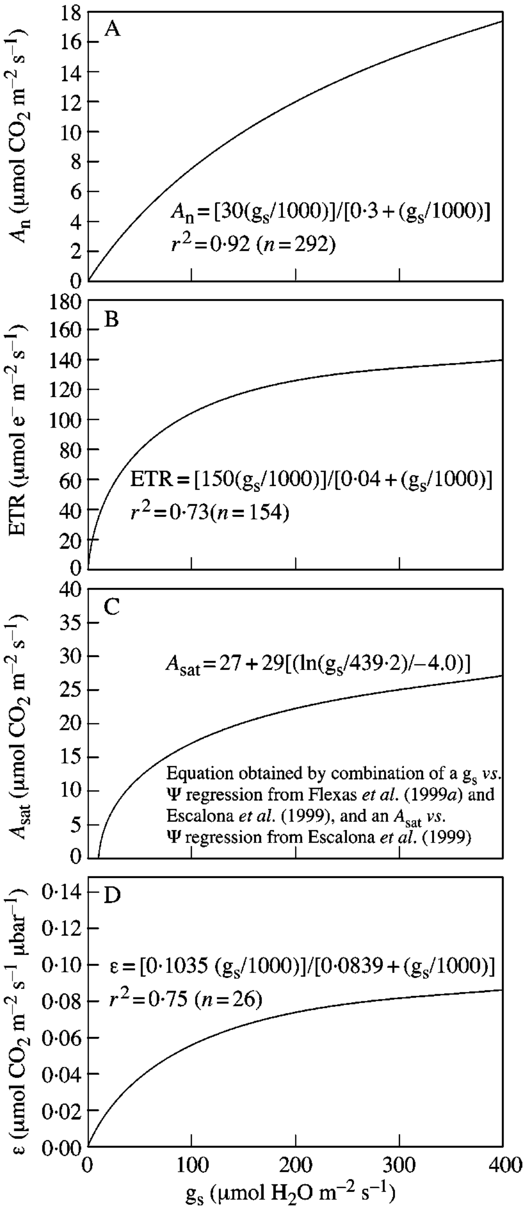

FIG. 2. Relationships between stomatal conductance (g_s) and: net CO_2 assimilation (A_n) (A), photosynthetic electron transport rate (ETR) (B), light- and CO_2-saturated net CO_2 assimilation (A_{sat}) (C), and apparent carboxylation efficiency, estimated as the initial slope of A_n–C_i curves (ε) (D). Data correspond to field-grown grapevines, and only the best-fitting correlation curves are shown, all of them being hyperbolic and highly significant. Leaf temperatures ranged from 28·5 to 39·3 °C (data from Flexas, 2000).

WHAT ABOUT OTHER SPECIES?

To further test the generality of the relationships between different photosynthetic parameters and light-saturated g_s, six Mediterranean sclerophyllous trees and shrubs were subjected to progressive soil drying (Gulías et al., 2002).

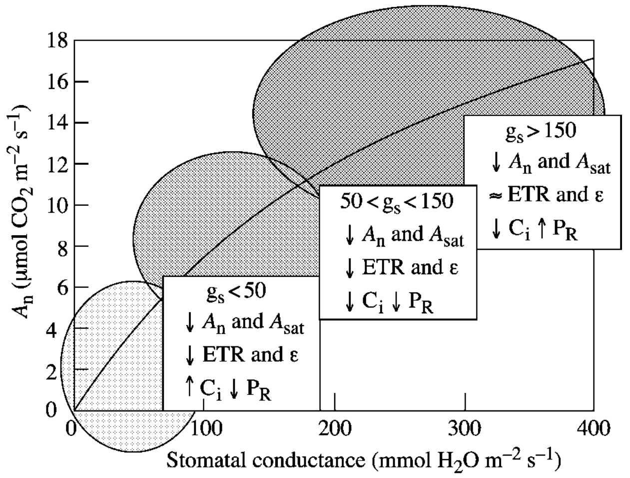

FIG. 3. Schematic pattern of response of photosynthesis in grapevines to drought, using g_s as a reference parameter. Three main regions are distinguished, and the down-regulation of different photosynthetic parameters is indicated for every region.

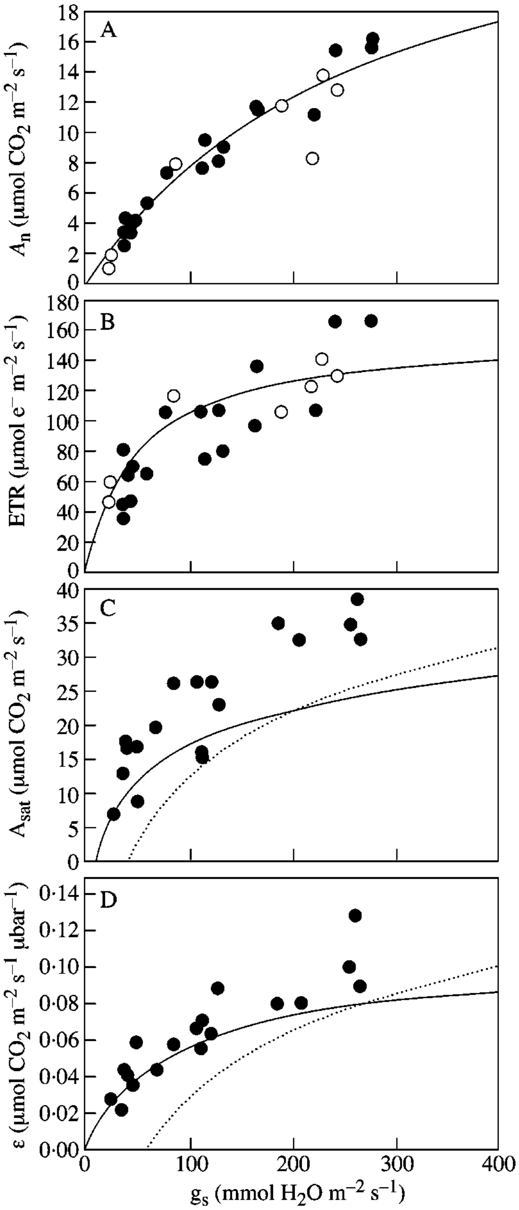

FIG. 4. This is the same as Fig. 2, after adding some data from the literature on other species to achieve a broad range of species and plant types. In all plots (A–D), data from Gulías *et al.* (2002) on six different Mediterranean sclerophyllous shrubs (filled circles) have been added. In A and B, data from other authors and species have been added (open circles). These include data on three different tropical understorey plants (from Ishida *et al.*, 1999), on the evergreen sclerophyllous shrub, *Pistacia lentiscus* (from Flexas *et al.*, 2001), and on alfalfa (*Medicago sativa*) plants (Antolín and Sánchez-Díaz, 1993). In C and D, curves of best fit (dotted lines) between the plotted parameters obtained by Martin and Ruiz-Torres (1992) in wheat (*Triticum aestivum*) are shown.

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 (Gulías *et al.*, 2002). Three of these species (*Quercus ilex*, *Rhamnus alaternus* and *R. ludovici-salvatoris*) showed proportional decreases of g_s and RWC in response to soil drying. In contrast, the other three (*Quercus pubescens*, *Pistacia lentiscus* and *P. terebinthus*) showed similar decreases of g_s but their RWC remained almost constant (Flexas, Gulías, 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 g_s. 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 ε. 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 ε *vs.* Ψ.

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 Ψ 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 Ψ.

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 (ε) was thought to be controlled by Rubisco activity, but other mesophyll limitations to photosynthesis may also exert control over ε. 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 CO_2-saturated rate of photosynthesis, has been shown many times to be an early response to drought, decreasing much earlier than ε (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_{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_2O 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_n–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; Plaut 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 ε 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 ε 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_2O m^{-2} s^{-1}, ε is about 20–30 % lower than the measured Rubisco activity.

A possible explanation arises given that ε 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 CO_2 concentration at the carboxylation site (C_c), so ε would no longer be represen-

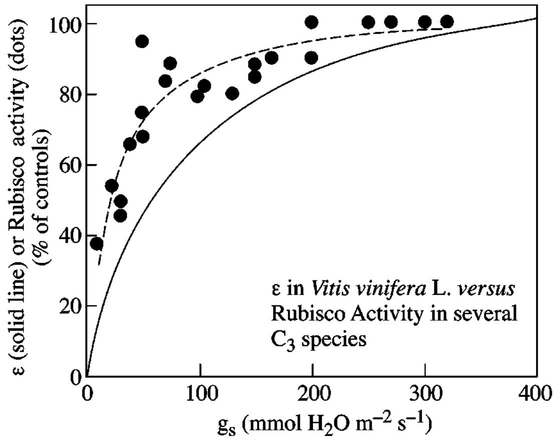

FIG. 5. Relationship between apparent carboxylation efficiency (ε) and stomatal conductance (g_s) in field-grown grapevines (solid line). Only the curve of best fit is plotted. ε is expressed as a % of the maximum observed values. Data of initial and/or total Rubisco activity have been added to the figure (filled circles), expressed as a % of maximum values for comparison. We selected data on Rubisco activity from the available literature in which g_s was given. These data are from the following species and references: *Helianthus annus* (Pancovic *et al.*, 1999; Tezara *et al.*, 1999), *Hordeum vulgare* (Lal *et al.*, 1996; Wingler *et al.*, 1999, 2000), *Medicago sativa* (Antolín and Sánchez-Díaz, 1993), *Trifolium subterraneum* (Medrano *et al.*, 1997), *Triticum aestivum* (Holaday *et al.*, 1992), *Vicia faba* (Lal *et al.*, 1996) and *Vitis vinifera* (Bota, unpubl. res.). Broken line shows the curve of best fit for Rubisco activity data.

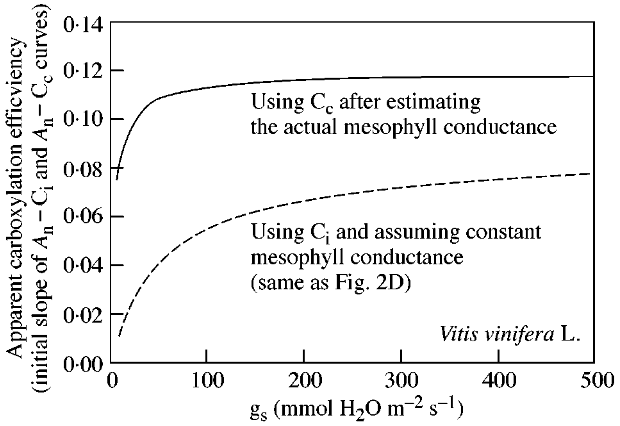

FIG. 6. Effect of changing mesophyll conductance in the calculation of the apparent carboxylation (ε). The graph shows ε as a function of stomatal conductance in field-grown, drought-stressed grapevines, calculated directly from A_n–C_i curves (broken line) or after calculating C_c, from A_n–C_c curves (solid line). Data are from Flexas *et al.*, 2002*a*).

tative of the actual carboxylation efficiency. Increased mesophyll resistance, due either to changes in leaf internal anatomy or impaired carbonic anhydrase, has been suggested to occur under water stress (Beadle and Jarvis, 1977; Cornic *et al.*, 1989; Renou *et al.*, 1990; Lal *et al.*, 1996; Roupsard *et al.*, 1996). To test this possibility, we estimated C_c from combined gas-exchange and chlorophyll fluorescence measurements, according to a current model (Epron *et al.*, 1995; Valentini *et al.*, 1995), 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 g_s declined (Flexas *et al.*, 2002*a*). Thereafter, we converted A_n–C_i curves to A_n–C_c curves, and recalculated ε on this new basis. Figure 6 shows the relationship obtained between ε and light-saturated g_s using this new approach and should be compared with Fig. 2D showing the relationship based on the typical A_n–C_i approach. Clearly, the new relationship is much more similar to that between Rubisco activity and light-saturated g_s 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 CO_2 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.

IMPLICATIONS OF THE PRESENT RESULTS AND PRACTICAL APPLICATIONS

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 CO_2 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 CO_2 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 CO_2 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 CO_2 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 g_s, 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

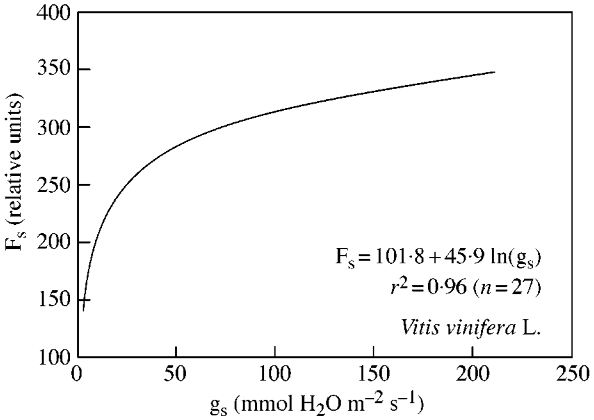

FIG. 7. Relationship between steady-state chlorophyll fluorescence (F_s) and stomatal conductance in leaves of grapevine, measured at a PPFD of 500 μmol photons m^{-2} s^{-1} (from Ounis *et al.*, 2001).

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.*, 2002*b*). 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 CO_2 availability in the chloroplast. CO_2 assimilation is reduced and the CO_2 : O_2 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 ΔpH (Schreiber and Neubauer, 1990; Osmond *et al.*, 1997*b*). Impaired ATPase and/or reduced ETR may also interfere with the build-up of trans-thylakoid ΔpH. The xanthophyll de-epoxidation that follows increased ΔpH 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 H_2O 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_n occurred just one night after irrigation (Flexas *et al.*, 1999*a*). However, if g_s reaches values as low as 50 mmol H_2O 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, 2002*b*; Ounis *et al.*, 2001).

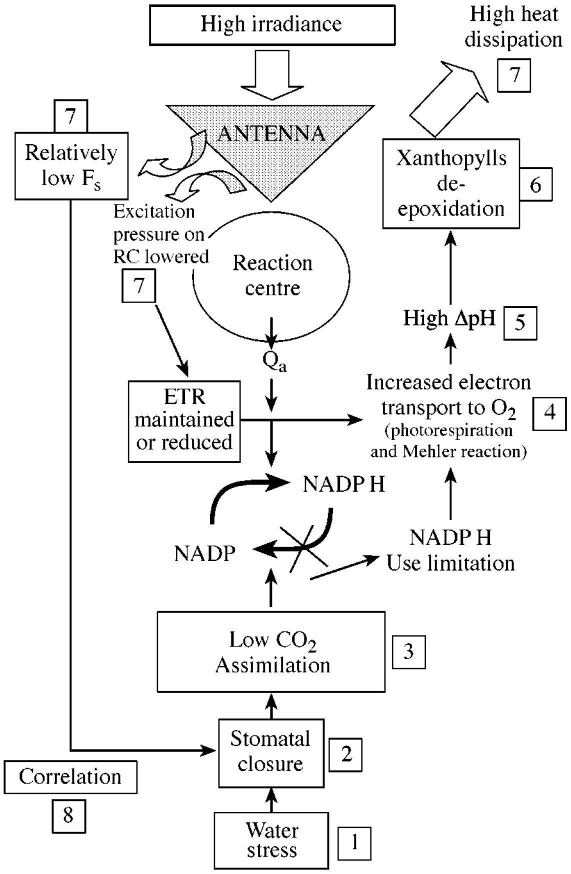

FIG. 8. Proposed mechanism of leaf response to water stress, explaining the observed interrelationships among different photosynthetic processes. Numbers indicate a proposed order for reading the scheme. See text for more details.

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LITERATURE CITED

Antolín MC, Sánchez-Díaz M. 1993. Effects of temporary droughts on photosynthesis of alfalfa plants. *Journal of Experimental Botany* **44**: 1341–1349.

Beadle CL, Jarvis PG. 1977. Effects of shoot water status on some photosynthetic partial processes in Sitka spruce. *Physiologia Plantarum* **41**: 7–13.

Bota J, Flexas J, Medrano H. 2001. Genetic variability of photosynthesis and water use in Balearic grapevine cultivars. *Annals of Applied Biology* **138**: 353–361.

Boyer JS. 1976. Photosynthesis at low water potentials. *Philosophical Transactions of the Royal Society B* **273**: 501–512.

Boyer JS, Wong SC, Farquhar GD. 1997. CO_2 and water vapour exchange across leaf cuticle (epidermis) at various water potentials. *Plant Physiology* **114**: 185–191.

Castrillo M, Calcagno AM. 1989. Effects of water stress and rewatering on ribulose 1,5-bisphosphate carboxylase activity, chlorophyll and protein contents in two cultivars of tomato. *Journal of Horticultural Science* **64**: 717–724.

Chaves MM. 1991. Effects of water deficits on carbon assimilation. *Journal of Experimental Botany* **42**: 1–16.

Choné X, van Leeuwen C, Dubourdieu D, Gaudillère JP. 2001. Stem water potential is a sensitive indicator of grapevine water status. *Annals of Botany* **87**: 477–484.

Cornic G. 1994. Drought stress and high light effects on leaf photosynthesis. In: Baker NR, Bowyer JR, eds. *Photoinhibition of photosynthesis: from molecular mechanisms to the field*. Oxford: BIOS Scientific Publishers.

Cornic G. 2000. Drought stress inhibits photosynthesis by decreasing stomatal aperture – not by affecting ATP synthesis. *Trends in Plant Science* **5**: 187–188.

Cornic G, Massacci A. 1996. Leaf photosynthesis under drought stress. In: Baker NR, ed. *Photosynthesis and the environment*. The Netherlands: Kluwer Academic Publishers.

Cornic G, Le Gouallec J-L, Briantais JM, Hodges M. 1989. Effect of dehydration and high light on photosynthesis of two C_3 plants (*Phaseolus vulgaris* L. and *Elatostema repens* (Lour.) Hall f.). *Planta* **177**: 84–90.

Cornic G, Fresneau C. 2002. Photosynthetic carbon reduction and carbon oxidation cycles are the main electron sinks for photosystem II activity during a mild drought. *Annals of Botany* **89**: 887–894.

Correia MJ, Pereira JS, Chaves MM, Rodrigues ML, Pacheco C.A. 1995. ABA xylem concentrations determine maximum daily leaf conductance of field-grown *Vitis vinifera* L. plants. *Plant Cell and Environment* **18**: 511–521.

Dai Z, Edwards GE, Ku MSB. 1992. Control of photosynthesis and stomatal conductance in *Ricinus communis* L. (castor bean) by leaf to air vapor pressure deficit. *Plant Physiology* **99**: 1426–1434.

Davies WJ, Zhang J. 1991. Root signals and the regulation of growth and development of plants in drying soil. *Annual Review of Plant Physiology and Plant Molecular Biology* **42**: 55–76.

Davies WJ, Gowing DJG. 1999. Plant responses to small perturbations in soil water status. In: Press MC, Scholes JD, Barker MG. *Physiological Plant Ecology*. Oxford: Blackwell Sciences, 67–89.

Downton WJS, Loveys BR, Grant WJR. 1988. Non-uniform stomatal closure induced by water stress causes putative non-stomatal inhibition of photosynthesis. *New Phytologist* **110**: 503–509.

Epron D, Godard D, Cornic G, Genty B. 1995. Limitation of net CO_2 assimilation rate by internal resistances to CO_2 transfer in the leaves of two tree species (*Fagus sylvatica* L. and *Castanea sativa* Mill.). *Plant Cell and Environment* **18**: 43–51.

Escalona JM, Flexas J, Medrano H. 1999. Stomatal and non-stomatal limitations of photosynthesis under water stress in field-grown grapevines. *Australian Journal of Plant Physiology* **26**: 421–433.

Evans JR, von Caemmerer S. 1996. Carbon dioxide diffusion inside leaves. *Plant Physiology* **110**: 339–346.

Farquhar GD, von Caemmerer S, Berry JA. 1980. A biochemical model of photosynthetic CO_2 assimilation in leaves of C_3 species. *Planta* **149**: 78–90.

Farquhar GD, von Caemmerer S, Berry JA. 2001. Models of photosynthesis. *Plant Physiology* **125**: 42–45.

Farquhar GD, Hubick KT, Terashima I, Condon AG, Richards RA. 1987. Genetic variation in the relationship between photosynthetic CO_2 assimilation rate and stomatal conductance to water loss. In: Biggens J, ed. *Progress in photosynthesis research Vol IV*. Dordrecht: Martinus Nijhoff Publishers.

Faver KL, Gerik TJ, Thaxton PM, El-Zik KM. 1996. Late season water stress in cotton: II. Leaf gas exchange and assimilation capacity. *Crop Science* **36**: 922–928.

Flexas J, Medrano H. 2002*a*. Drought-inhibition of photosynthesis in C_3 plants: stomatal and non-stomatal limitations revisited. *Annals of Botany* **89**: 183–189.

Flexas J, Medrano H. 2002*b*. Photosynthetic responses of C_3 plants to drought. In: Hemantaranjan A, ed. *Advances in Plant Physiology IV*. Jodhpur: Scientific Publishers.

Flexas J, Escalona JM, Medrano H. 1998. Down-regulation of photosynthesis by drought under field conditions in grapevine leaves. *Australian Journal of Plant Physiology* **25**: 893–900.

Flexas J, Escalona JM, Medrano H. 1999*a*. Water stress induces different levels of photosynthesis and electron transport rate regulations in grapevines. *Plant, Cell and Environment* **22**: 39–48.

Flexas J, Badger M, Chow WS, Medrano H, Osmond CB. 1999*b*. Analysis of the relative increase in photosynthetic O_2 uptake when photosynthesis in grapevine leaves is inhibited following low night temperatures and/or water stress. *Plant Physiology* **121**: 675–684.

Flexas J, Briantais J-M, Cerovic Z, Medrano H, Moya I. 2000. Steady-state and maximum chlorophyll fluorescence responses to water stress in grapevine leaves: a new remote sensing system. *Remote Sensing of the Environment* **73**: 283–297.

Flexas J, Gulías J, Jonasson S, Medrano H, Mus M. 2001. Seasonal patterns and control of gas exchange in local populations of the Mediterranean evergreen shrub *Pistacia lentiscus* L. *Acta Oecologica* **22**: 33–43.

Flexas J, Bota J, Escalona JM, Sampol B, Medrano H. 2002*a*. Effects of drought on photosynthesis in grapevines under field conditions: an evaluation of stomatal and mesophyll limitations. *Functional Plant Biology* (in press).

Flexas J, Escalona JM, Evain S, Gulías J, Moya I, Osmond CB, Medrano H. 2002*b*. Steady state chlorophyll fluorescence (Fs) measurements as a tool to follow variations of net CO_2 assimilation and stomatal conductance during water-stress in C_3 plants. *Physiologia Plantarum* **114**: 231–240.

Foyer CH, Furbank R, Harbinson J, Horton P. 1990. The mechanisms contributing to photosynthetic control of electron transport by carbon assimilation in leaves. *Photosynthesis Research* **25**: 83–100.

Giménez C, Mitchell VJ, Lawlor DW. 1992. Regulation of photosynthesis rate of two sunflower hybrids under water stress. *Plant Physiology* **98**: 516–524.

Gollan T, Turner NC, Schulze E-D. 1985. The responses of stomata and leaf gas exchange to vapour pressure deficits and soil water content. III. In the sclerophyllous woody species *Nerium oleander*. *Oecologia* **65**: 356–362.

Gulías J, Flexas J, Abadía A, Medrano H. 2002. Photosynthetic responses to water deficit in six Mediterranean sclerophyll species: possible factors explaining the declining distribution of an endemic Balearic species (*Rhamus ludovici-salvatoris*). *Tree Physiology* (in press).

Gunasekera D, Berkowitz GA. 1993. Use of transgenic plants with Rubisco antisense DNA to evaluate the rate limitation of photosynthesis under water stress. *Plant Physiology* **103**: 629–35.

Haraux F, de Kouchkovsky Y. 1998. Energy coupling and ATP synthase. *Photosynthesis Research* **57**: 231–251.

Havaux M, Canaani O, Malkin S. 1987. Inhibition of photosynthetic activities under slow water stress measured *in vivo* by the photoacoustic method. *Physiologia Plantarum* **70**: 503–510.

Heber U, Bligny R, Streb P, Douce R. 1996. Photorespiration is essential for the protection of the photosynthetic apparatus of C_3 plants against photoinactivation under sunlight. *Botanica Acta* **109**: 307–315.

Holaday AS, Ritchie SW, Nguyen HT. 1992. Effects of water deficit on gas-exchange parameters and ribulose 1,5-bisphosphate carboxylase activation in wheat. *Environmental and Experimental Botany* **32**: 403–410.

Hubbard RM, Ryan MG, Stiller V, Sperry JS. 2001. Stomatal conductance and photosynthesis vary linearly with plant hydraulic conductance in ponderosa pine. *Plant, Cell and Environment* **24**: 113–121.

Ishida A, Nakano T, Matsumoto Y, Sakoda M, Hoe Ang L. 1999. Diurnal changes in leaf gas exchange and chlorophyll fluorescence in tropical tree species with contrasting light requirements. *Ecological Research* **14**: 77–88.

Jones HG. 1973. Moderate-term water stresses and associated changes in some photosynthetic parameters in cotton. *New Phytologist* **72**: 1095–1105.

Kaiser WM, Förster J. 1989. Low CO_2 prevents nitrate reduction in leaves. *Plant Physiology* **91**: 970–974.

Kozaki A, Takeba G. 1996. Photorespiration protects C_3 plants from photooxidation. *Nature* **384**: 557–560.

Lal A, Ku MSB, Edwards GE. 1996. Analysis of inhibition of photosynthesis due to water stress in the C_3 species *Hordeum vulgare* and *Vicia faba*: electron transport, CO_2 fixation and carboxylation capacity. *Photosynthesis Research* **49**: 57–69.

Lawlor DW. 1976*a*. Water stress induced changes in photosynthesis, photorespiration, respiration and CO_2 compensation concentration of wheat. *Photosynthetica* **10**: 378–387.

Lawlor DW. 1976*b*. Assimilation of carbon into photosynthetic intermediates of water-stressed wheat. *Photosynthetica* **10**: 431–439.

Lawlor DW. 1983. Integration of biochemical processes in the physiology of water stressed plants. In: Marcelle R, Clijters H, von Puche M, eds. *Effects of stress on photosynthesis*. The Hague-Boston-London: Martinus Nijhoff/Dr W. Junk Publishers.

Lawlor DW. 1995. The effects of water deficit on photosynthesis. In: Smirnoff N, ed. *Environment and plant metabolism. Flexibility and acclimation*. Oxford: BIOS Scientific Publisher.

Lawlor DW. 2002. Limitation to photosynthesis in water-stressed leaves: stomal *vs.* metabolism and the role of ATP. *Annals of Botany* **89**: 871–885.

Lawlor DW, Fock H. 1975. Photosynthesis and photorespiratory CO_2 evolution of water-stressed sunflower leaves. *Planta* **126**: 247–258.

Lawlor DW, Fock H. 1977*a*. Photosynthetic assimilation of $^{14}CO_2$ by water-stressed sunflower leaves in two oxygen concentrations and the specific activity of products. *Journal of Experimental Botany* **28**: 320–328.

Lawlor DW, Fock H. 1977*b*. Water stress induced changes in the amounts of some photosynthetic assimilation products and respiratory metabolites of sunflower leaves. *Journal of Experimental Botany* **28**: 329–337.

Lawlor DW, Khanna-Chopra R. 1984. Regulation of photosynthesis during water stress. In: Sybesma C, ed. *Advances in photosynthesis research Vol IV*. The Hague-Boston-Lancaster: Martinus Nijhoff/Dr W. Junk Publishers.

Lawlor DW, Pearlman JG. 1981. Compartmental modelling of photorespiration and carbon metabolism of water stressed leaves. *Plant, Cell and Environment* **4**: 37–52.

Lawlor DW, Uprety DC. 1993. Effects of water stress on photosynthesis of crops and the biochemical mechanism. In: Abrol YP, Mohanty P, Govinjee, eds. *Photosynthesis: photoreactions to plant productivity*. New Dehli: Oxford and IBH Publishing Co. PVT. Ltd.

Lloyd J, Syversten JP, Kriedemann PE, Farquhar GD. 1992. Low conductances for CO_2 diffusion from stomata to the sites of carboxylation in leaves of woody species. *Plant, Cell and Environment* **15**: 873–899.

Martin B, Ruiz-Torres NA. 1992. Effects of water-deficit stress on photosynthesis, its components and component limitations, and on water use efficiency in wheat (*Triticum aestivum* L.). *Plant Physiology* **100**: 733–739.

Medrano H, Parry MA, Socias X, Lawlor DW. 1997. Long term water stress inactivates Rubisco in subterranean clover. *Annals of Applied Biology* **131**: 491–501.

Meyer S, de Kouchkovsky Y. 1992. ATPase state and activity in thylakoids from normal and water-stressed lupin. *FEBS Letters* **303**: 233–236.

Meyer S, Genty B. 1999. Heterogeneous inhibition of photosynthesis over the leaf surface of *Rosa rubiginosa* L. during water stress and abscisic acid treatment: induction of a metabolic component by limitation of CO_2 diffusion. *Planta* **210**: 126–131.

Moya I, Camenen L, Latouche G, Mauxion C, Evain S, Cerovic Z. 1998. An instrument for the measurement of sunlight excited plant fluorescence. In: Garab G, ed. *Photosynthesis: mechanisms and effects, Vol. V*. Dordrecht: Kluwer Academic Publishers.

Oren R, Sperry JS, Katul GG, Pataki DE, Ewers BE, Phillips N, Schäfer KVR. 1999. Survey and synthesis of intra- and interspecific variation in stomatal sensitivity to vapour pressure deficit. *Plant, Cell and Environment* **22**: 1515–1526.

Ort DR, Oxborough K, Wise RR. 1994. Depressions of photosynthesis in crops with water deficits. In: Baker NR, Bowyer JR, eds. *Photoinhibition of photosynthesis: from molecular mechanisms to the field*. Oxford: BIOS Scientific Publishers.

Ortiz-López A, Ort DR, Boyer JS. 1991. Photophosphorylation in attached leaves of *Helianthus annuus* at low water potentials. *Plant Physiology* **96**: 1018–1025.

Osmond CB, Flexas J, Neff R. 1997*a*. Imaging chlorophyll fluorescence *in vivo* with an almost portable system. In: Ball M, Clark-Walker GD, Farquhar GD, Gunning BES, Morgan IG, Osmond CB, eds. *Research School of Biological Sciences. Annual Report 1997*. Canberra: RSBS.

Osmond CB, Maxwell K, Björkman O, Badger M, Leegood R. 1997*b*. Too many photons: photorespiration, photoinhibition and photooxidation. *Trends in Plant Science* **4**: 119–121.

Ounis A, Evain S, Flexas J, Tosti S, Moya I. 2001. Adaptation of the PAM-fluorometer for remote sensing of chlorophyll fluorescence. *Photosynthesis Research* **68**: 113–120.

Pankovic D, Sakac, Z, Kevresan, S, Plesnicar, M. 1999. Acclimation to long-term water deficit in the leaves of two sunflower hybrids: photosynthesis, electron transport and carbon metabolism. *Journal of Experimental Botany* **50**: 127–138.

Parry MAJ, Delgado E, Vadell J, Keys AJ, Lawlor DW, Medrano H. 1993. Water stress and the diurnal activity of ribulose-1,5-bisphosphate carboxylase in field grown *Nicotiana tabacum* genotypes selected for survival at low CO_2. *Plant Physiology and Biochemistry* **31**: 113–120.

Parry MAJ, Andralojc PJ, Khan S, Lea PJ, Keys AJ. 2002. Rubisco activity: effects of drought stress. *Annals of Botany* **89**: 833–839.

Pei Z-M, Ghassemian M, Kwak CM, McCourt P, Schroeder JI. 1998. Role of farnesyltransferase in ABA regulation of guard cell anion channels and plant water loss. *Science* **282**: 287–290.

Perchorowicz JT, Jensen RG. 1983. Photosynthesis and activation of ribulose bisphosphate carboxylase in wheat seedlings. Regulation by CO_2 and O_2. *Plant Physiology* **71**: 955–960.

Plaut Z, Federman E. 1991. Acclimation of CO_2 assimilation in cotton leaves to water stress and salinity. *Plant Physiology* **97**: 515–522.

Quick WP, Chaves MM, Wendler R, David M, Rodrigues ML, Passaharinho JA, Pereira JS, Adcock MD, Leegood RC, Stitt M. 1992. The effect of water stress on photosynthetic carbon metabolism in four species grown under field conditions. *Plant, Cell and Environment* **15**: 25–35.

Raschke K. 1979. Movements of stomata. In: Haupt W, Feinleib ME, eds. *Encyclopedia of plant physiology New Series vol. 7. Physiology of movements*. Berlin: Springer-Verlag.

Renou J-L, Gerbaud A, Just D, André M. 1990. Differing substomatal and chloroplastic CO_2 concentrations in water-stressed wheat. *Planta* **182**: 415–419.

Roupsard O, Gross P, Dreyer E. 1996. Limitation of photosynthetic activity by CO_2 availability in the chloroplasts of oak leaves from different species and during drought. *Annales des Sciences Forestieres* **53**: 243–54.

Salleo S, Nardini A, Pitt F, Lo Gullo MA. 2000. Xylem cavitation and hydraulic control of stomatal conductance in Laurels (*Laurus nobilis* L.). *Plant, Cell and Environment* **23**: 71–79.

Schreiber U, Neubauer C. 1990. O_2-dependent electron flow, membrane energisation and the mechanism of non-photochemical quenching of chlorophyll fluorescence. *Photosynthesis Research* **25**: 279–293.

Schulze E-D, Hall AE. 1982. Stomatal responses, water loss and CO_2 assimilation rates of plants in contrasting environments. In: Lange OL, Nobel PS, Osmond CB, eds. *Encyclopedia of plant physiology Vol. 12 B. Physiological plant ecology II*. Berlin-Heidelberg-New York: Springer-Verlag.

Schurr U, Gollan T, Schulze E-D. 1992. Stomatal responses to drying soil in relation to changes in the xylem sap composition of *Helianthus annuus*. II. Stomatal sensitivity to abscisic acid imported from the xylem sap. *Plant, Cell and Environment* **15**: 561–567.

Sharkey TD. 1990. Water stress effects on photosynthesis. *Photosynthetica* **24**: 651.

Sharkey TD, Seeman JR. 1989. Mild water stress effects on carbon-reduction cycle intermediates, ribulose bisphosphate carboxylase activity, and spatial homogeneity of photosynthesis in intact leaves. *Plant Physiology* **89**: 1060–1065.

Socías FX, Correia MJ, Chaves MM, Medrano H. 1997. The role of abscisic acid and water relations in drought responses of subterranean clover. *Journal of Experimental Botany* **48**: 1281–1288.

Tang A-C, Kawamitsu Y, Kanechi M, Boyer JS. 2002. Photosynthetic oxygen evolution at low water potential in leaf discs lacking an epidermis. *Annals of Botany* **89**: 861–870.

Tardieu F, Davies WJ. 1992. Stomatal response to ABA is a function of current plant water status. *Plant Physiology* **98**: 540–545.

Tardieu F, Simmonneau T. 1998. Variability among species of stomatal control under fluctuating soil water status and evaporative demand: modelling isohydric and anisohydric behaviours. *Journal of Experimental Botany* **49**: 419–432.

Terashima I, Wong SC, Osmond CB, Farquhar GD. 1988. Characterization of non-uniform photosynthesis induced by abscisic acid in leaves having different mesophyll anatomies. *Plant and Cell Physiology* **29**: 385–394.

Tezara W, Mitchell VJ, Driscoll SD, Lawlor DW. 1999. Water stress inhibits plant photosynthesis by decreasing coupling factor and ATP. *Nature* **401**: 914–917.

Turner LB, Wellburn AR. 1985. Changes in adenylate nucleotide levels in the leaves of *Capsicum annuum* during water stress. *Journal of Plant Physiology* **120**: 111–122.

Tyree MT. 1999. Water relations and hydraulic architecture. In: Pugnaire FI, Valladares F, eds. *Handbook of functional plant ecology*. New York: Marcel Dekker Inc.

Vadell J, Medrano H. 1992. Effect of drought on subterranean clover. 2. Genetic variability of photosynthesis, transpiration and stomatal conductance. *Photosynthetica* **27**: 433–440.

Vadell J, Cabot C, Medrano H. 1995. Diurnal time course of leaf gas exchange rates and related characters in drought-acclimated and irrigated *Trifolium subterraneum. Australian Journal of Plant Physiology* **22**: 461–469.

Valentini R, Epron D, De Angelis P, Matteucci G, Dreyer E. 1995. *In situ* estimation of net CO_2 assimilation, photosynthetic electron flow and photorespiration in Turkey oak (*Quercus cerris* L.) Leaves: diurnal cycles under different levels of water supply. *Plant, Cell and Environment* **18**: 631–640.

Vassey TL, Quick WP, Sharkey TD, Stitt M. 1991. Water stress, carbon dioxide and light effects on sucrose phosphate synthase activity in *Phaseolus vulgaris*. *Physiologia Plantarum* **81**: 37–44.

von Caemmerer S, Farquhar GD. 1981. Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. *Planta* **153**: 376–387.

von Caemmerer S, Farquhar GD. 1984. Effects of partial defoliation, changes of irradiance during growth, short-term water stress and growth at enhanced $p(CO_2)$ on photosynthetic capacity of leaves of *Phaseolus vulgaris* L. *Planta* **160**: 320–329.

Wingler A, Lea PJ, Quick WP, Leegood RC. 2000. Photorespiration: metabolic pathways and their role in stress protection. *Philosophical Transactions of the Royal Society B* **355**: 1517–1529.

Wingler A, Quick WP, Bungard RA, Bailey KJ, Lea PJ, Leegood RC. 1999. The role of photorespiration during drought stress: an analysis utilizing barley mutants with reduced activities of photorespiratory enzymes. *Plant, Cell and Environment* **22**: 361–373.

Wong SC, Cowan IR, Farquhar GD. 1979. Stomatal conductance correlates with photosynthetic capacity. *Nature* **282**: 424–426.

Younis HM, Boyer JS, Govindjee. 1979. Conformation and activity of chloroplast coupling factor exposed to low chemical potential of water in cells. *Biochimica et Biophysica Acta* **548**: 328–340.