

Drought-inhibition of Photosynthesis in C₃ Plants: Stomatal and Non-stomatal Limitations Revisited

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Received: 27 April 2001 Returned for revision: 13 August 2001 Accepted: 22 October 2001

There is a long-standing controversy as to whether drought limits photosynthetic CO₂ assimilation through stomatal closure or by metabolic impairment in C₃ plants. Comparing results from different studies is difficult due to interspecific differences in the response of photosynthesis to leaf water potential and/or relative water content (RWC), the most commonly used parameters to assess the severity of drought. Therefore, we have used stomatal conductance (*g*) as a basis for comparison of metabolic processes in different studies. The logic is that, as there is a strong link between *g* and photosynthesis (perhaps co-regulation between them), so different relationships between RWC or water potential and photosynthetic rate and changes in metabolism in different species and studies may be 'normalized' by relating them to *g*. Re-analysing data from the literature using light-saturated *g* as a parameter indicative of water deficits in plants shows that there is good correspondence between the onset of drought-induced inhibition of different photosynthetic sub-processes and *g*. Contents of ribulose biphosphate (RuBP) and adenosine triphosphate (ATP) decrease early in drought development, at still relatively high *g* (higher than 150 mmol H₂O m⁻² s⁻¹). This suggests that RuBP regeneration and ATP synthesis are impaired. Decreased photochemistry and Rubisco activity typically occur at lower *g* (<100 mmol H₂O m⁻² s⁻¹), whereas permanent photoinhibition is only occasional, occurring at very low *g* (<50 mmol H₂O m⁻² s⁻¹). Sub-stomatal CO₂ concentration decreases as *g* becomes smaller, but increases again at small *g*. The analysis suggests that stomatal closure is the earliest response to drought and the dominant limitation to photosynthesis at mild to moderate drought. However, in parallel, progressive down-regulation or inhibition of metabolic processes leads to decreased RuBP content, which becomes the dominant limitation at severe drought, and thereby inhibits photosynthetic CO₂ assimilation.

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Key words: C₃ plants, drought, water stress, photosynthesis, stomatal conductance, photochemistry, carboxylation, photophosphorylation, RuBP regeneration, Rubisco.

INTRODUCTION

There is a long-standing controversy as to whether drought mainly limits photosynthesis through stomatal closure (Sharkey, 1990; Chaves, 1991; Ort *et al.*, 1994; Cornic and Massacci, 1996) or by metabolic impairment (Boyer, 1976; Lawlor, 1995). Evidence that impaired ATP synthesis is the main factor limiting photosynthesis even under mild drought (Boyer, 1976; Tezara *et al.*, 1999) has further stimulated debate (Cornic, 2000; Lawlor and Cornic, 2002).

Comparing results from different authors is difficult due to interspecific differences in the response of photosynthesis to leaf water potential and/or relative water content (RWC), the parameters most commonly used to assess the degree of drought (Tardieu and Simmoneau, 1998). To overcome this, we have exploited the relationship between stomatal conductance (*g*) and photosynthetic CO₂ assimilation (Wong *et al.*, 1979), since an early and progressive effect of drought is stomatal closure (Boyer, 1976; Sharkey, 1990; Chaves, 1991; Ort *et al.*, 1994; Lawlor, 1995; Cornic and Massacci, 1996). We have recently demonstrated (Flexas *et al.*, 2002; Medrano *et al.*, 2002) that, by relating photosynthetic parameters to their corresponding light-saturated *g*, a pattern of responses is revealed which is

independent of acclimation processes and only slightly dependent on the cultivars and species. For instance, the relationships between different photosynthetic parameters and the absolute values of *g* in grapevines (*Vitis vinifera*) and several Mediterranean sclerophyll shrubs were very similar. This applied even when maximum *g* reached approx. 500 mmol H₂O m⁻² s⁻¹ in grapevines, and only 200 mmol H₂O m⁻² s⁻¹ in sclerophyll shrubs (Medrano *et al.*, 2002). The relationship between different photosynthetic parameters and *g* was not observed with relative water content or leaf water potential, i.e. decreased photosynthesis caused by drought occurred at different leaf water status in different species, albeit at similar stomatal conductance. Based on these previous findings and using data from the literature, we have analysed at what values of *g*—and thus at different severity of drought—some photosynthetic metabolic processes are impaired.

MATERIALS AND METHODS

In order to see if *g*, relative water content or water potential provide a clearer basis or reference for the effects of drought on photosynthetic response to drought, we analysed the literature cited in Table 1.

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TABLE 1. References used for the analysis of each photosynthetic sub-process and in the construction of Fig. 2)

Photosynthetic sub-process	References	Species	
RuBP availability	Flexas, 2000	<i>Vitis vinifera</i>	
	Giménez <i>et al.</i> , 1992	<i>Helianthus annuus</i>	
	Gunasekera and Berkowitz, 1993	<i>Nicotiana tabacum</i>	
	Santakumary and Berkowitz, 1991	<i>Spinacia oleracea</i>	
	Sharkey and Badger, 1982	<i>Xanthium strumarium</i>	
	Sharkey and Seeman, 1989	<i>Phaseolus vulgaris</i>	
	Stuhlfauth <i>et al.</i> , 1990	<i>Digitalis lanata</i>	
	Tezara <i>et al.</i> , 1999	<i>Helianthus annuus</i>	
	Vu <i>et al.</i> , 1987	<i>Glycine max</i>	
	Wingler <i>et al.</i> , 1999	<i>Hordeum vulgare</i>	
	ATP synthesis	Havaux <i>et al.</i> , 1987	<i>Nicotiana tabacum</i>
Lawlor, 1983		<i>Triticum aestivum</i>	
Meyer and de Kouchkovsky, 1992		<i>Lupinus albus</i>	
Sharkey and Badger, 1982		<i>Xanthium strumarium</i>	
Tezara <i>et al.</i> , 1999		<i>Helianthus annuus</i>	
Younis <i>et al.</i> , 1979		<i>Spinacia oleracea</i>	
Photochemistry		Björkman and Powles, 1984	<i>Nerium oleander</i>
	Brestic <i>et al.</i> , 1995	<i>Phaseolus vulgaris</i>	
	Damesin and Rambal, 1995	<i>Quercus pubescens</i>	
	Demmig <i>et al.</i> , 1988	<i>Nerium oleander</i>	
	Faria <i>et al.</i> , 1998	<i>Quercus ilex</i> , <i>Q. suber</i> , <i>Olea europaea</i> , <i>Eucalyptus globulus</i>	
	Flexas, 2000; Flexas <i>et al.</i> , 1998, 1999a, 1999b	<i>Vitis vinifera</i>	
	Lal <i>et al.</i> , 1996	<i>Vicia faba</i> , <i>Hordeum vulgare</i>	
	Meyer and Genty, 1999	<i>Rosa rubiginosa</i>	
	Munné-Bosch and Alegre, 2000	<i>Melissa officinalis</i>	
	Munné-Bosch <i>et al.</i> , 1999	<i>Lavandula stoechas</i> , <i>Rosmarinus officinalis</i>	
	Pankovic <i>et al.</i> , 1999	<i>Helianthus annuus</i>	
	Wingler <i>et al.</i> , 1999	<i>Hordeum vulgare</i>	
	Rubisco activity	Antolín and Sánchez-Díaz, 1993	<i>Medicago sativa</i>
		Castrillo and Calcagno, 1989	<i>Lycopersicon esculentum</i>
		Holaday <i>et al.</i> , 1992	<i>Triticum aestivum</i>
Lal <i>et al.</i> , 1996		<i>Vicia faba</i> , <i>Hordeum vulgare</i>	
Medrano <i>et al.</i> , 1997		<i>Trifolium subterraneum</i>	
Pankovic <i>et al.</i> , 1999		<i>Helianthus annuus</i>	
Plaut and Federman, 1991		<i>Gossypium hirsutum</i>	
Tezara <i>et al.</i> , 1999		<i>Helianthus annuus</i>	
Vu and Yelenosky, 1988		<i>Citrus sinensis</i>	
Vu <i>et al.</i> , 1987		<i>Glycine max</i>	
Wingler <i>et al.</i> , 1999		<i>Hordeum vulgare</i>	
Permanent photoinhibition		Angelopoulos <i>et al.</i> , 1996	<i>Olea europaea</i>
		Brodribb, 1996	<i>Acacia melanoxylon</i> , <i>Eucalyptus tenuiramis</i> , <i>Podocarpus lawrencii</i>
	Faria <i>et al.</i> , 1998	<i>Quercus ilex</i> , <i>Q. suber</i> , <i>Olea europaea</i> , <i>Eucalyptus globulus</i>	
	Flexas <i>et al.</i> , 1998; Flexas, 2000	<i>Vitis vinifera</i>	
	Méthy <i>et al.</i> , 1996	<i>Quercus pubescens</i>	
	Ramanjulu <i>et al.</i> , 1998	<i>Morus alba</i>	
	Valladares and Percy, 1997	<i>Heteromeles arbutifolia</i>	
Ci inflexion point	Brodribb, 1996	<i>Acacia melanoxylon</i> , <i>Eucalyptus tenuiramis</i> , <i>Podocarpus lawrencii</i>	
	Epron and Dreyer, 1993	<i>Quercus robur</i> , <i>Q. petraea</i>	
	Faver <i>et al.</i> , 1996	<i>Gossypium hirsutum</i>	
	Flexas, 2000	<i>Vitis vinifera</i>	
	Giménez <i>et al.</i> , 1992	<i>Helianthus annuus</i>	
	Jensen <i>et al.</i> , 1996	<i>Brassica napus</i>	
	Johnson <i>et al.</i> , 1987	<i>Triticum ssp.</i>	
	Lal <i>et al.</i> , 1996	<i>Vicia faba</i> , <i>Hordeum vulgare</i>	
	Luo, 1991	<i>Abutilon theophrasti</i>	
	Martin and Ruiz-Torres, 1992	<i>Triticum aestivum</i>	
	Nicolodi <i>et al.</i> , 1988	<i>Medicago sativa</i>	
	Ramanjulu <i>et al.</i> , 1998	<i>Morus alba</i>	
	Shangguan <i>et al.</i> , 1999	<i>Triticum aestivum</i>	

The species analysed in every reference are indicated in the right-hand column.

Photosynthetic metabolism was divided into five sub-processes implicated as important sites of inhibition of photosynthetic metabolism under drought. The sub-processes were: (1) ribulose 1,5-bisphosphate (RuBP) regener-

ation capacity (Giménez *et al.*, 1992; Gunasekera and Berkowitz, 1993) as indicated by the RuBP content in leaves; (2) ATP synthesis (Younis *et al.*, 1979; Meyer and de Kouchkovsky, 1992; Tezara *et al.*, 1999) as indicated by

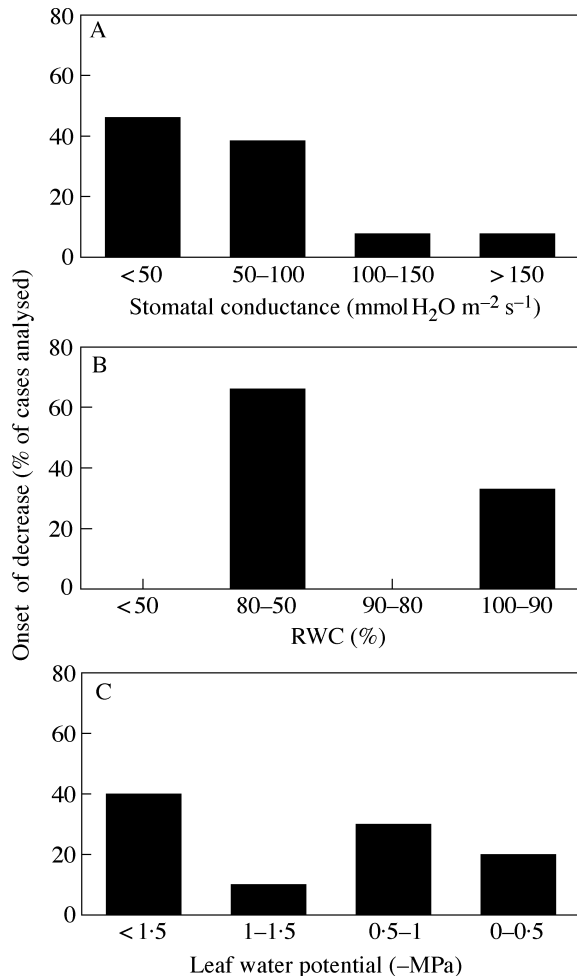


FIG. 1. Analysis of Rubisco activity under drought. The y-axis shows the percentage (%) of the studies from the literature in which the activity of Rubisco first decreased in relation to intervals of (A) light-saturated stomatal conductance, (B) of relative water content (RWC) and (C) of leaf water potential.

the ATP content of leaves or ATP synthase activity (photophosphorylation) or the amount of ATP synthase; (3) leaf photochemistry (Cornic and Massacci, 1996; Flexas *et al.*, 1999a, b) as indicated by chlorophyll *a* fluorescence; (4) ribulose 1,5-bisphosphate carboxylase/oxidase (Rubisco) activity (Castrillo and Calcagno, 1989; Medrano *et al.*, 1997; Tezara *et al.*, 1999); and (5) permanent photoinhibition (Björkman and Powles, 1984; Valladares and Percy, 1997). In addition, the change in sub-stomatal CO_2 concentration (C_i) with progressive drought was also analysed as an indicator of the predominance of stomatal or non-stomatal limitations to photosynthesis (Ort *et al.*, 1994; Cornic and Massacci, 1996). We related the C_i inflexion point between decreasing and increasing C_i to the value of g .

The data were grouped according to the change in each of these five sub-processes (Table 1), irrespective of the methods used to assess the effects of drought in each experiment (usually gas exchange or photoacoustic meas-

urements, coupled with determinations of chlorophyll *a* fluorescence, on leaves, followed by destructive sampling and biochemical analyses). Changes in Rubisco activity and RuBP regeneration derived from CO_2 -response curves of photosynthesis (A/C_i curves) were not considered, since they assume that regulation under non-stressed conditions is applicable to stressed. In addition, they are difficult to compare with biochemical assessments (Medrano *et al.*, 2002).

For each study and sub-process, the threshold of g below which the sub-processes was impaired by the drought treatment (i.e. the value of g at which each process started to become inhibited) was estimated. When g was not given, it was derived from the relationship between g and leaf water potential obtained for the same species under similar conditions either by the same or other authors. When there were uncertainties about the values of g , these studies were not included in the analysis.

Finally, for simplicity and because only approximate g values were usually available (or impossible, for example, to determine accurately from the figures given), the occurrence of inhibition of each sub-process (expressed as a percentage of the total number of cases analysed) was related to four discrete intervals of g . These were: $g > 150 \text{ mmol H}_2\text{O m}^{-2} \text{s}^{-1}$ (i.e. control plants to mild drought); $150 \text{ mmol H}_2\text{O m}^{-2} \text{s}^{-1} > g > 100 \text{ mmol H}_2\text{O m}^{-2} \text{s}^{-1}$ (i.e. moderate drought); $100 \text{ mmol H}_2\text{O m}^{-2} \text{s}^{-1} > g > 50 \text{ mmol H}_2\text{O m}^{-2} \text{s}^{-1}$ (i.e. severe drought); $g < 50 \text{ mmol H}_2\text{O m}^{-2} \text{s}^{-1}$ (i.e. very severe drought). When the data were available, results were also related to discrete intervals of relative water content and leaf water potential.

This method determines the onset of changes in metabolism with progressive drought, by comparison with unstressed plants (the control). If the changes in a particular process occur with only small increase in stress, they appear in the range of $g > 150 \text{ mmol H}_2\text{O m}^{-2} \text{s}^{-1}$ (i.e. control plants to mild drought). This is because the g values of the control plants are not distinguished from mildly stressed plants. It means that the onset of metabolic changes occurs with very limited drought as g starts to decrease.

RESULTS AND DISCUSSION

Using different values of stomatal conductance, g , as a reference to analyse the effects of drought on photosynthetic metabolism provides a clearer pattern of the changes in different parts of metabolism in response to drought than using relative water content or leaf water potential. This is illustrated in Fig. 1 for Rubisco activity. When plotted as a function of g intervals, Rubisco activity starts to decrease when g drops below $100 \text{ mmol H}_2\text{O m}^{-2} \text{s}^{-1}$ (Fig. 1A). However, when plotted as a function of the RWC intervals proposed by Lawlor (1995) to reflect different stages of drought effects on photosynthesis, no clear pattern was observed (Fig. 1B). Rubisco activity decreased in 65 % of studies at RWC between 80 and 50 %, but in a substantial proportion (35 %) of cases, Rubisco activity was lost at very high RWC (90–100 %). With leaf water potential as a reference (Fig. 1C), the pattern of response was even less clear, with Rubisco activity inhibited over a range of water

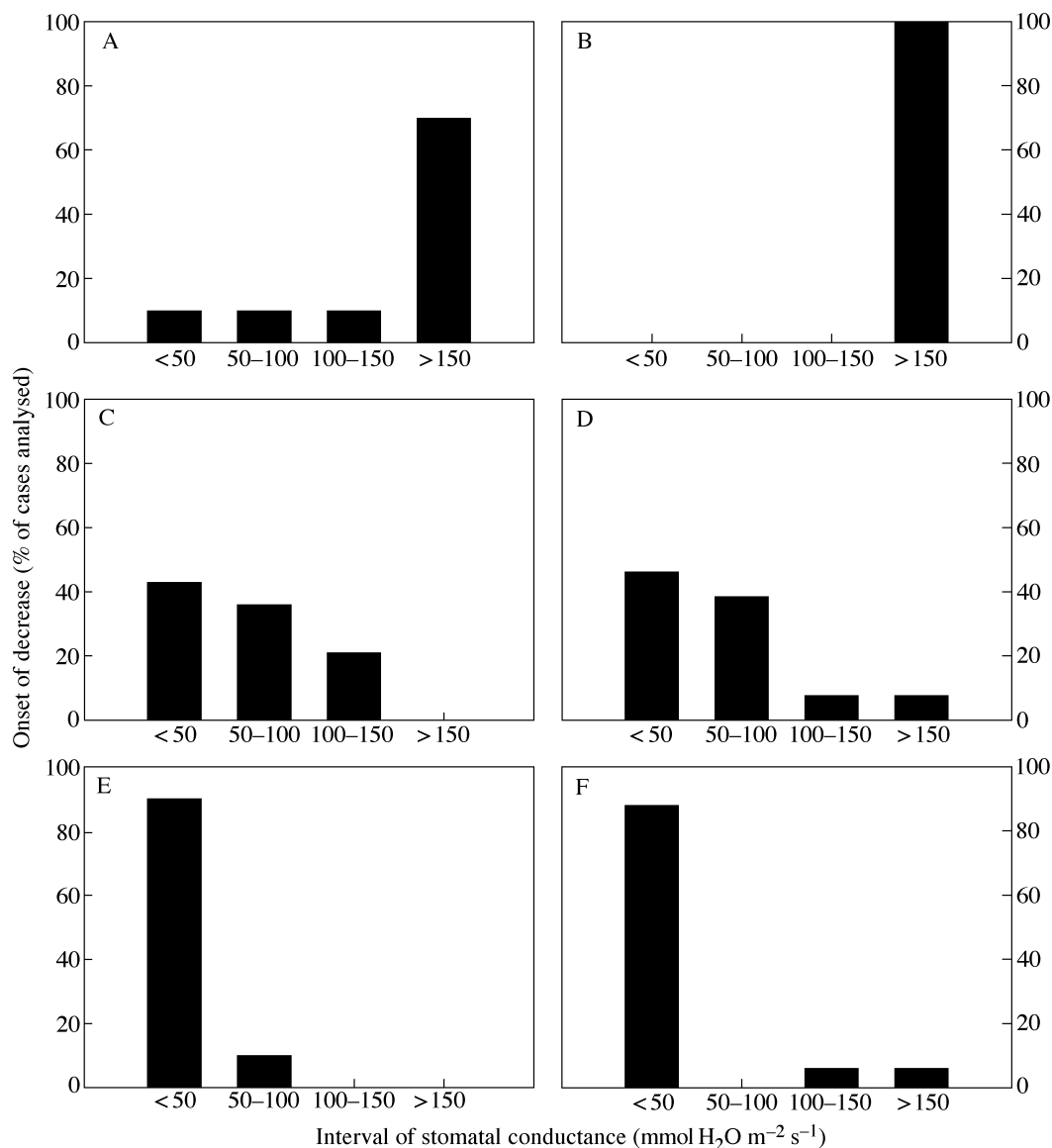


FIG. 2. Occurrence of the onset of drought-induced decrease of metabolic processes as a function of the corresponding light saturated stomatal conductance (g), from the literature (Table 1). The y-axis shows the percentage (%) of the studies (the number is shown as n) in which the decrease occurred at different intervals of g . For simplicity, those studies in which no effect of drought on metabolism occurred are not included but are mentioned in the text. The effects on metabolism are represented by: A, RuBP content (RuBP regeneration, $n = 10$); B, ATP content (ATP synthesis, $n = 6$); C, Photochemistry ($n = 14$); D, Rubisco activity ($n = 13$); E, Permanent photoinhibition ($n = 10$); F, Appearance of the C_i inflexion point ($n = 17$).

potentials. Other photosynthetic processes showed similar responses to g , RWC and leaf water potential (not shown). Using RWC or water potential as references, only photochemistry and permanent photoinhibition showed a degree of correspondence similar to that observed when using g . Photochemistry decreased mainly between 80 and 50 % RWC with leaf water potentials below -1 MPa. However, permanent photoinhibition occurred at RWC between 80 and 50 % as well, but at leaf water potentials only below -1.5 MPa. Following this primary evaluation, we used g as a reference parameter to analyse the literature.

The results of this analysis are given in Fig. 2. Clearly, decreased RuBP (Fig. 2A) and impaired ATP synthesis

(Fig. 2B) have been most frequently reported to occur in early phases of drought, when g is still relatively large (>150 $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$). Important exceptions are the studies of Sharkey and Seeman (1989), in which RuBP content of *Phaseolus vulgaris* was unaffected at g around 100 $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$, and of Ortiz-López *et al.* (1991), in which inhibition of ATPase in *Helianthus annuus* did not occur even at very low g (approx. 50 $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$).

Decreased photochemistry (Fig. 2C) and Rubisco activity (Fig. 2D) are commonly reported to occur at severe stress, and in our analysis this corresponded to $g < 100$ $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$. Only in the study of Munné-Bosch *et al.* (1999) in *Rosmarinus officinalis*, were electron transport rates un-

affected even when g dropped to 75 mmol H₂O m⁻² s⁻¹. We found only two reports, both using *Phaseolus vulgaris*, showing unaltered Rubisco activity at $g < 100$ mmol H₂O m⁻² s⁻¹ (Sharkey and Seeman, 1989; Brestic *et al.*, 1995).

Permanent photoinhibition (Fig. 2E) was only occasional. Indeed, in about half the references analysed permanent photoinhibition did not occur; when it did, it was at very low g (<50 mmol H₂O m⁻² s⁻¹) (see Epron and Dreyer, 1993; Faria *et al.*, 1998; Flexas and Medrano, 1998).

As stomata close, the CO₂ inside the leaf, C_i, initially declines with increasing stress and then increases as drought becomes more severe (Lawlor, 1995). According to Cornic and Massacci (1996), C_i estimated from fluorescence decreases to compensation point under drought and can be estimated accurately. If C_i is high, this reflects the inaccuracies in the C_i calculation under drought (i.e. heterogeneous stomatal closure, cuticular conductance, etc.), which tend to overestimate C_i. The decrease in C_i indicates that stomatal limitations dominate, with moderate drought, irrespective of any metabolic impairment. However, at a certain stage of water stress, shown by a threshold value of g , C_i frequently increases, indicating the predominance of non-stomatal limitations to photosynthesis. In most cases the point at which C_i starts to increase, which we call the C_i inflexion point, occurs at g around 50 mmol H₂O m⁻² s⁻¹. Only Nicolodi *et al.* (1988) in *Medicago sativa* and Luo (1991) in *Abutilon theophrasti* observed the C_i inflexion point at higher g .

The results of this literature survey analysing the effects of drought on photosynthesis are consistent with a gradual pattern of response of photosynthesis to water stress similar to that proposed by Lawlor (1995). After an early partial closure of stomata, metabolic limitation, caused by either damage (i.e. permanent) or adjustment (i.e. reversible 'down-regulation') occurs. The limitation at large g , when drought is mild, is often impaired ATP synthesis and thus ATP-limited regeneration of RuBP. Further reduction of g as drought increases leads to reduced photochemical activity. The analysis shows that, as it is the Rubisco activity, this loss is more progressive with increasing drought than sometimes suggested (Lawlor, 1995; Lawlor and Cornic, 2002). Photoinhibition eventually occurs under conditions of very severe drought and almost complete stomata closure. The C_i inflexion point is also observed predominantly at low g .

This pattern of metabolic changes supports the assertion by Cornic (2000) that stomatal closure is the primary cause of the reduction in photosynthetic rate under mild drought, but shows that metabolic damage or down-regulation—this analysis cannot distinguish between them—is progressive and commences with small changes in g under mild drought. In particular, decreased ATP content, implying impaired synthesis [and thus supporting the observations of Younis *et al.* (1979) and Tezara *et al.* (1999) of impaired photophosphorylation and loss of ATP synthase, respectively] is important. To our knowledge, only one reference (Ortiz-López *et al.*, 1991) reported no inhibition of ATPase under mild to moderate drought. A major consequence of loss of ATP would be limited RuBP regeneration under mild drought, shown clearly as an early effect of drought by our

analysis. Nevertheless, despite the decreased capacity of these metabolic processes, decreased C_i confirms the predominance of stomatal limitation in restricting photosynthetic rate in the early phase of water loss. However, the metabolic changes are responsible for loss of photosynthetic potential during this phase (Lawlor and Cornic, 2002).

Our analysis does not include the effects of drought on nitrate reductase and sucrose phosphate synthase, enzymes shown in a number of studies to be inhibited under water stress. This is because too few analyses with information on g are available. The activities of these enzymes can be restored by placing the water-stressed plant in high CO₂ for a number of hours (Sharkey, 1990; Cornic and Massacci, 1996). This strongly suggests that CO₂ availability in the chloroplast, mainly regulated by g , may serve as a signal to trigger metabolic adjustments in the leaf in response to water deficit. This would be consistent with the observed response of the different photosynthetic processes to g . ATP synthesis is probably not restored by elevated CO₂ (Tang *et al.*, 2002), suggesting that the enzyme is not impaired, directly or indirectly, by low CO₂ concentration. Instead, increased magnesium concentration has been shown to inhibit ATP synthase (Tang *et al.*, 2002). Alternatively, inhibition of ATP synthesis, and not lowered C_i, may be responsible for impairments to metabolism, which cannot be regulated by adjustments in metabolism. One of the major goals for future research on drought effects on photosynthesis should be to confirm how general are the responses that have been identified (Lawlor, 1995; Lawlor and Cornic, 2002). From an analysis of the literature over the widest range of drought and for a number of species with different responses to drought, we have shown that changes in metabolism occur despite stomatal closure. It is still uncertain if these are the consequences of damage to or adjustment (down-regulation) in metabolism, and better understanding of the mechanisms is required.

ACKNOWLEDGEMENTS

We thank Drs D. W. Lawlor, M. A. J. Parry and W. Tezara for critical reading and useful comments on the manuscript.

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