Limitations to leaf photosynthesis in field-grown grapevine under drought — metabolic and modelling approaches

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Abstract. The effects of a slowly-imposed drought stress on gas-exchange, chlorophyll a fluorescence, biochemical and physiological parameters of Vitis vinifera L. leaves (cv. Aragonez, syn. Tempranillo) growing in a commercial vineyard (South Portugal) were evaluated. Relative to well-watered plants (predawn water potential, $\Psi_{PD} = –0.13 \pm 0.01$ MPa), drought-stressed plants ($\Psi_{PD} = –0.97 \pm 0.01$ MPa) had lower photosynthetic rates (ca 70%), stomatal conductance, and PSII activity (associated with a higher reduction of the quinone A pool and lower efficiency of PSII open centres). Stomatal limitation to photosynthesis was increased in drought-stressed plants relative to well-watered plants by ca 44%. Modelled responses of net photosynthesis to internal CO$_2$ indicated that drought-stressed plants had significant reductions in maximum Rubisco carboxylation activity (ca 32%), ribulose-1,5-bisphosphate regeneration (ca 27%), and triose phosphate (triose-P) utilization rates (ca 37%) relative to well-watered plants. There was good agreement between the effects of drought on modelled biochemical parameters, and in vitro activities of key enzymes of carbon metabolism, namely Rubisco, glyceraldehyde-3-phosphate dehydrogenase, ribulose-5-phosphate kinase and fructose-1,6-bisphosphate phosphatase. Quantum yields measured under both ambient (35 Pa) and saturating CO$_2$ (100 Pa) for drought-stressed plants were decreased relative to well-watered plants, as well as maximum photosynthetic rates measured at light and CO$_2$ saturating conditions (three times ambient CO$_2$ levels). Although stomatal closure was a strong limitation to CO$_2$ assimilation under drought, comparable reductions in electron transport, CO$_2$ carboxylation, and utilization of triose-P capacities were also adaptations of the photosynthetic machinery to dehydration that slowly developed under field conditions. Results presented in this study confirm that modelling photosynthetic responses based on gas-exchange data can be successfully used to predict metabolic limitations to photosynthesis.

Keywords: drought, enzymes of carbon metabolism, gas-exchange, modelling, photosynthesis, Vitis vinifera.

Introduction

There is ongoing discussion on how drought affects photosynthesis, namely on the relative roles of restricted diffusion of CO$_2$ into the leaf due to stomata closure and inhibition of CO$_2$ metabolism (Tezara et al. 1999; Cornic 2000). Recent studies have shown that both the photochemical apparatus and CO$_2$ assimilation capacity are quite resistant to drought stress, and that stomatal closure, with a reduction in mesophyll CO$_2$ availability, is the main factor responsible for reductions in CO$_2$ assimilation (stomatal effects) under mild drought (Cornic and Massacci 1996; Chaumont et al. 1997; Correia et al. 1999). However, other studies suggest a non-stomatal limitation of CO$_2$ assimilation via a direct effect of drought on ATP synthase, with a reduction of ATP production (Lawlor 1995; Tezara et al. 1999) and ribulose-1,5-bisphosphate (RuBP) regeneration (Gunasekera and Berkowitz 1993). The reduction in the maximum photosynthetic capacity under long-term drought allows photosynthesis to operate near break point of the RuBP- and CO$_2$-limited regions of the $A/C_i$ (where $A$ is net CO$_2$ assimilation; $A_{max}$ maximum net CO$_2$ assimilation; $C_{ext}$ external CO$_2$ partial pressure; $C_{i}$ intercellular CO$_2$ partial pressure; ETP potential evapotranspiration; FruBPase, fructose-1,6-bisphosphate phosphatase; $Fv'/Fm'$ efficiency of PSII open centres; G3PDH, glyceraldehyde-3-phosphate dehydrogenase; $g_s$ stomatal conductance; $J_{max}$ maximum electron transport rate; PPFD, photosynthetic photon flux density; QA primary quinone receptor of PSII; $1-q_p$ reduction state of the QA pool; RuBP, ribulose-1,5-bisphosphate; Ru5PK, ribulose-5-phosphate kinase; TPU, triose-P utilization; V$C_{max}$ maximum Rubisco activity; $\Phi_{PSII}$ quantum yield of PSII; $\Psi_{PD}$ predawn water potential.

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assimilation and \( C_i \) is the intercellular partial pressure of  
\( \text{CO}_2 \) curve (Lambers et al. 1998). This adaptation  
mechanism maximizes the effectiveness of both light and dark  
reactions under stress. However, this downregulation of  
photosynthesis in response to water stress is not fully  
understood (Lambers et al. 1998). Differences among  
species and in the rates of imposition of water deficits, as  
well as the superimposition of other environmental stresses,  
may also play a role in the relative importance of stomatal vs  
non-stomatal limitations under drought (Chaves 1991). In  
grapevine (\( Vitis \ vinifera \ L. \)), water stress aggravates photo-  
inhibition of the photochemical apparatus under elevated  
irradiances, causing stomatal closure and downregulation of  
carbon assimilation (Correia et al. 1990; Quick et al. 1992;  
Rodrigues et al. 1993; Chaumont et al. 1997; Flexas et al.  
1998, 1999b). Non-stomatal effects have been suggested for  
grapevine responses to drought under field conditions, as  
estimated from coupled gas-exchange and fluorescence  
measurements (Flexas et al. 1998, 1999b; Escalona et al.  
1999). However, unresponsiveness of net \( \text{CO}_2 \) assimilation  
relative to \( C_i \) suggests that the occurrence of non-stomatal  
effects under drought stress may be, to some degree,  
attributable to patchy stomatal aperture in heterobaric  
leaves, although these effects may be minimized when water  
deficits are imposed slowly (Mott and Buckley 2000). The  
increasing importance of cuticular transpiration under water  
deficit may also add to the erroneous overestimation of \( C_i \)  
(Dowtown et al. 1988; Raschke et al. 1990; Meyer and  

Our hypothesis in the present study is that by measuring  
the activities of key enzymes of the \( \text{CO}_2 \) assimilation  
pathway (involved in carboxylation, regeneration of RuBP  
and utilization of triose-P) we may override the ambiguity in  
estimating non-stomatal effects of water stress, by using net  
\( \text{CO}_2 \) assimilation responses to internal \( \text{CO}_2 \). We therefore  
used both biochemical and modelling approaches to  
differentiate between stomatal vs metabolic responses of  
grapevine to water stress driven by a slow and seasonal drought  
under field conditions.

**Materials and methods**

**Plant material and growth conditions**

Fourteen-year-old grapevine plants (\( Vitis \ vinifera \ L. \) cv. Aragonez syn.  
Tempranillo) were selected from an irrigation experiment in a  
commercial vineyard in the South of Portugal (Alentejo). Vine spacing  
was 1.2 m within rows and 2.5 m between rows. Plants were trained on  
a vertical trellis with three fixed wires (50, 90 and 130 cm above the  
ground) and a pair of movable foliage wires for upwards shoot  
positioning. The vines were spur pruned on a bilateral Royat Cordon  
(16 buds per vine). Well-watered plants were drip irrigated from the  
end of May onwards every 3–4 d with 80% of potential  
evapotranspiration (ETp, as estimated by the Penman-Monteith  
method), while drought-stressed plants were rain fed, suffering  
progressive soil water depletion during the growing season as  
described by Lopes et al. (2001). All measurements were performed in  
July (mid-Mediterranean summer).

**Plant water status**

\( \Psi_{PD} \) was measured in 4–6 individual mature leaves using a pressure  
chamber (Model 1000; PMS Instrument Co., Corvallis, OR, USA).

**Gas exchange and fluorescence**

\( A/C_i \) response curves were generated using a portable LI 6400 infra-red  
gas analyser [IRGA (Li-Cor, Lincoln, NE, USA)] with a constant leaf  
temperature of 25°C and 1200 \( \mu \text{mol} \) photons \( \text{m}^{-2} \text{s}^{-1} \) supplied by the  
LI 6400-02B light system (Li-Cor). Relative humidity inside the  
cuvette was kept at 70 ± 2%. Daily courses of gas exchange between  
the leaf and the atmosphere were performed with the LI 6400 under  
naturally occurring photosynthetic photon flux density (PPFD) and air  
temperature. Maximum net \( \text{CO}_2 \) assimilation and incident light  
quantum yield rates were calculated from light response curves  
measured at 35 and 100 Pa ambient \( \text{CO}_2 \) using the portable LI 6400  
IRGA. Chlorophyll \( a \) fluorescence was measured with a PAM 2000  
fluorimeter (Walz, Effeltrich, Germany), and the quantum yield of  
PSII (\( \Phi_{PSII} \)), reduction state of the primary acceptors (1-\( q_P \)), and  
efficiency of PSII open centres (\( F'_v/F_{m'} \)) were calculated as described  
in Maroco et al. (1998).

**Biochemical modelling and relative stomatal limitation**

The net \( \text{CO}_2 \) assimilation biochemical model for \( C_i \) plants of Farquhar  
et al. (1980) can be written as:

\[
A = V_C - 0.5V_O - R_d = V_C(1 - \frac{0.5O}{\tau C_i}) - R_d
\]

(1)

where \( V_C \) and \( V_O \) are the rates of carboxylation and oxygenation of  
Rubisco, respectively, \( R_d \) is the mitochondrial respiration, \( O \) and \( C_i \) are  
the oxygen and \( \text{CO}_2 \) partial pressures in intercellular spaces,  
respectively, and \( \tau \) is the specificity of Rubisco for \( \text{CO}_2/\text{O}_2 \). According  
to Farquhar et al. (1980), with modifications by Sharkey (1985), \( V_C \) is a  
minimum function of the carboxylation rates, limited by either  
(i) kinetic properties and amount of Rubisco (\( W_C \)), (ii) the rate of RuBP  
regeneration (\( W_j \)), or (iii) the availability of inorganic phosphate (\( W_p \)).  
That is \( V_C = \text{minimum}(W_C, W_j, W_p) \).

The rate of carboxylation limited by the amount and kinetic  
properties of Rubisco is given by:

\[
W_C = \frac{V_{Cmax} \cdot C_i}{C_i + K_C(1 + O/K_O)}
\]

(2)

where \( V_{Cmax} \) is the maximum rate of carboxylation, and \( K_C \) and \( K_O \) are  
the Michaelis-Menten constants for the carboxylation and oxygenation  
processes, respectively.

The rate of carboxylation limited by the regeneration of RuBP is a  
function of the rate of electron transport, and is given by:

\[
W_j = \frac{J \cdot C_i}{4(C_i + O/\tau)}
\]

(3)

where it is assumed that four electrons are enough to generate the  
three ATP and two NADPH required in the Calvin cycle to regenerate RuBP,  
and \( J \) is the rate of electron transport through PSII given by:

\[
J = \frac{\alpha \cdot I}{\sqrt{\left[ 1 + \frac{\alpha^2 I^2}{J_{max}^2} \right]}}
\]

(4)

where \( \alpha \) is the fraction of incident-light photons that are converted into  
electrons, and \( J_{max} \) is the maximum, light saturated, rate of electron  
transport.
Finally, the rate of carboxylation limited by inorganic phosphate availability inside the chloroplast is given by:

\[ W_P = 3TPU + \frac{V_C \cdot O}{2 \cdot C_i \cdot \tau} \]  

where TPU is the rate of triose-P utilization (for sucrose and starch synthesis) (Sharkey, 1985).

Michaelis-Menten constants \( (K_C \) and \( K_o \)), \( \alpha \), \( \tau \), and temperature-dependence of the model parameters were corrected as described by Harley et al. (1992). According to these authors, the temperature dependence of \( K_C \) and \( K_o \), \( \alpha \), and \( \tau \) is described by an exponential function of the type \( \text{Parameter} = \exp(-\Delta H/RT) \) where \( c \) is a scaling constant characteristic for each parameter, \( \Delta H \) is the activation energy for the parameter, \( R \) is the ideal gas constant, and \( T \) is the leaf temperature in degrees Kelvin [see Harley et al. (1992) for the values of the constants used]. \( V_{\text{Cmax}} \) and \( R_P \) were estimated by fitting the model to measured \( A/C_i \) data for \( C_i < 20 \text{ Pa} \) because, in this \( C_i \) range, \( V_C \) is limited only by \( W_C \) if light is saturating, as it was in this case. Finally, \( J_{\text{max}} \) and \( T PU \) were estimated by fitting the model, with previously estimated \( V_{\text{Cmax}} \) and \( R_P \) to the complete \( A/C_i \) data using a non-linear curve fitting routine from SAS (version 6.12; SAS Institute, Cary, NC, USA).

The limitation to photosynthesis imposed by closed stomatal conductance, both under well-watered and drought conditions, was estimated through the relative stomatal limitation (RSL) calculated from \( A/C_i \) response curves using the equation:

\[ \text{RSL} = \frac{A_{C_i=35\text{Pa}} - A_{C_i=35\text{Pa}}}{A_{C_i=35\text{Pa}}} \times 100\% \]  

Estimates of net CO\(_2\) assimilation at \( C_i = 35 \text{ Pa} \) and at \( C_i = 35 \text{ Pa} \) (\( A_{C_i} \) and \( A_{C_i} \), respectively) were obtained from previously fitted Eqn 1 to \( A/C_i \) (or \( A_g \)) data.

**Protein, sugar and enzyme assays**

Six leaf discs (5.22 cm\(^2\)) from two different leaves per plant were harvested in the light, frozen in liquid N\(_2\) and stored at –80°C until assay. For protein determination and enzyme activities, the frozen leaf discs were ground to a fine slurry in 1.5 mL of an extraction solution containing 200 mM Tris-HCl (pH 8.0), 10 mM MgCl\(_2\), 6H\(_2\)O, 10 mM NaHCO\(_3\), 10 mM β-mercaptoethanol, 2 mM dithiothreitol, 2% Triton X-100, 4% (v/v) ‘Complete-protease inhibitor cocktail with EDTA’, 10% polyvinylpyrrolidone, and 10% glycerol. The extract was centrifuged at 16000 g for 4 min at 4°C, and the supernatant collected. The pellet was resuspended in 0.2 mL of the extraction solution, and after a 16000 g centrifugation at 4°C for 2 min, both supernatants were combined and used for total soluble protein determination and enzyme activity measurements. Solubilized protein was measured using Bio-Rad’s protein assay kit according to the manufacturers instructions (Bio-Rad, Hercules, CA, USA), and protein integrity was followed by SDS-PAGE.

Enzyme activities were measured spectrophotometrically by following the oxidation of NADH at 340 nm and 21°C as described by Leegood (1993) for Rubisco (EC 4.1.1.39), and by Maroco et al. (1999) for glyceraldehyde-3-phosphate dehydrogenase (G3PDH; EC 1.2.1.13), ribulose-5-phosphate kinase (Ru5PK; EC 2.7.1.19) and fructose-1,6-bisphosphate phosphatase (FruBPass; EC 3.1.3.11). All chemicals and coupling enzymes were from Sigma (St Louis, MO, USA) except for the ‘Complete’ cocktail, which was from Roche (Mannheim, Germany). The presence of very acidic vacuoles as well as an abundance of phenolic compounds, which increase in concentration under drought stress conditions (data not shown), made the extraction of active enzymes from the leaves of grapevines very difficult. Rubisco solubilization required a high percentage (up to 2%) of a mild detergent (suggesting that Rubisco is somehow associated with the insoluble thylakoid membranes) and several protease inhibitors, especially for drought-stressed leaves (data not shown, but see, for example, Kanna-Chopra et al. 1999). Soluble sugars and starch were measured enzymatically as described in Stitt et al. (1983, 1989).

**Sampling and statistical analysis**

All measurements and sample collections were carried out on the sun-exposed side of the vines, in two recently fully-expanded leaves per plant and four plants per treatment. Data are shown as means ± s.e. in both tables and figures. Statistically significant differences between treatments were analysed by Student \( t \)-tests with \( \alpha = 0.05 \).

**Results**

**Plant water status**

Well-watered plants maintained \( \Psi_{PD} \) at about –0.2 MPa throughout the growing season, while in drought-stressed plants, \( \Psi_{PD} \) decreased from mid-May to the end of July at a rate of 0.01 MPa d\(^{-1}\). At the time the experiments were performed (mid-summer; at veraison), \( \Psi_{PD} \) was –0.13 ± 0.01 MPa for well-watered plants, and –0.97 ± 0.01 MPa for drought-stressed plants. At this date, the accumulated \( \Delta \text{ETp} \) was around 600 mm, and accumulated irrigation was around 200 mm (Fig. 1).

**Net CO\(_2\) assimilation, stomatal conductance and PSII yield**

Relative to well-watered controls, drought-stressed plants had lower CO\(_2\) exchange rates (\( A \) (Fig. 2a; around 70% less throughout the day)) which were associated with lower

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**Fig. 1.** Seasonal evolution of accumulated potential evapotranspiration (\( \Delta \text{ETp} \); from bud-break), accumulated irrigation for the well-watered controls and rain (\( \alpha \)), and seasonal evolution of the predawn leaf water potential (\( \Psi_{PD} \)) in drought-stressed (DS) vs well-watered (WW) plants (\( \delta \)).
stomatal conductance \([g_s; \text{Fig. 2c; around four times lower}]\) and quantum yield of PSII reaction centres \([\Phi_{PSII} \text{ (Fig. 2b; 35\% less, on average, throughout the day)}]\). The lower \(\Phi_{PSII}\) of drought-stressed plants was associated with a higher reduction state of the primary acceptors (the QA pool) \([1-q_p; \text{Fig. 2f; 50\% more throughout the day)}]\) and lower efficiency of open PSII centres \([F_v'/F_m'; \text{Fig. 2d; about 20\% less, on average, throughout the day)}]\). A midday depression of photosynthesis was observed in the drought-stressed plants. In well-watered plants, stomatal closure occurred late in the day in response to reduced light levels. This was not the case for drought-stressed plants, where stomatal conductance remained very low and constant throughout the day (Fig. 2c). As a result, drought-stressed plants had higher intrinsic water use efficiency \([A/g_s \text{ (Fig. 2e)}]\).

**Soluble and insoluble sugars**

Sucrose, fructose and glucose, as well as starch, concentrations per unit leaf area were decreased under drought stress (Fig. 3). There was a statistically significant reduction

![Graph showing daily courses of various parameters](image-url)
Limitations to leaf photosynthesis in grapevine under drought

in starch during the course of the day in drought-stressed, relative to well-watered, plants (Fig. 3a; 53% reduction, on average, throughout the day). Statistically significant differences were also observed at midday for glucose and fructose, and by the end of the day for sucrose (Fig. 3b). Relative to insoluble sugars (starch), the reductions observed in soluble sugars in drought-stressed plants throughout the day were much smaller (only 15%).

Modelled biochemical and stomatal responses

Biochemical modelling of A/Ci response curves suggests that Rubisco maximal activity, RuBP regeneration capacity, and triose-P utilization capacity were decreased in drought-stressed plants relative to well-watered plants (Fig. 4). Model estimates of maximum Rubisco carboxylation capacity (V_{\text{Cmax}}) were decreased by 32%, RuBP regeneration capacity (which can be estimated by J_{\text{max}}; see, for example, Wullschleger 1993 and Wohlfahrt 1999) was decreased by up to 27%, and triose-P utilization capacity (TPU) was reduced by ca 37% in drought-stressed plants relative to well-watered plants (Table 1). There were no significant differences between treatments for estimated dark-respiration rates. Consistent with model estimates, quantum yields of incident PPFD under ambient CO₂ (35 Pa) were reduced by 57% in drought-stressed plants relative to well-watered plants, while maximum rates of photosynthesis under saturating light (2000 μmol photons m⁻² s⁻¹) and CO₂ (100 Pa) were decreased by 64%. Both maximum CO₂ assimilation and quantum yield increased under very high CO₂ (100 Pa), but the percent reductions observed in drought-stressed plants as compared with well-watered plants were similar to those observed at 35 Pa of CO₂ for A_{\text{max}} only (Table 1). Elevated CO₂ did not relieve the inhibition of quantum yield by drought, suggesting that photochemical inhibition did occur, as also indicated by Φ_{\text{PSII}} values estimated through chlorophyll fluorescence. Finally, the relative stomatal limitation of photosynthesis increased from 22% in well-watered plants to 31% in drought-stressed plants (Table 1).
In vitro activities of key enzymes of the Calvin cycle

Total soluble protein and total chlorophyll were reduced by 25 and 20%, respectively, in drought-stressed plants relative to well-watered controls (Table 2). The decrease in total chlorophyll was proportional to the reduction observed in Chlorophyll $a$ and $b$, which resulted in non-significant differences in the Chlorophyll $a/b$ ratio. The in vitro activity of Rubisco (a key enzyme in the carboxylation phase of the Calvin cycle), which relates to $V_{C_{\text{max}}}$ estimated by the biochemical model, was reduced by 37% in drought-stressed plants relative to well-watered controls (Fig. 5). Drought induced a similar reduction (36%) in the activity of G3PDH (a key enzyme in the regenerative phase of the Calvin cycle), while the activity of Ru5PK (a key enzyme in the reductive phase of the Calvin cycle), which is related to $J_{\text{max}}$, was reduced by 40% relative to well-watered plants. Finally, the activity of FruBPase (a key enzyme in the utilization of triose-P, leading to a diminished supply of inorganic phosphate to the Calvin cycle, is also consistent with the slow-down in growth generally observed in drought-stressed leaves of grapevine (this study) and in sucrose phosphate synthase activity observed by Vassey et al. (1991) in Phaseolus vulgaris. Our model estimates of $C_3$ photosynthesis (Fig. 4; Table 1) show that, in addition to increased relative stomatal limitation, drought stress is responsible for the reduction in maximum Rubisco carboxylation activity and electron transport, and therefore RuBP regeneration. The reduction observed in the utilization of triose-P, leading to a diminished supply of inorganic phosphate to the Calvin cycle, is also consistent with the slow-down in growth generally observed in drought-stressed plants (Chaves 1991).

Although genotypic variations may explain differences in the degree of stomatal vs non-stomatal limitations on photosynthesis during drought (Quick et al. 1992; Wohlfahrt et al. 1999), most conclusions have been based on coupled gas-exchange and Chlorophyll $a$ fluorescence data. It must

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Protein (g m$^{-2}$)</th>
<th>$a$ (g m$^{-2}$)</th>
<th>$b$ (g m$^{-2}$)</th>
<th>Total (g m$^{-2}$)</th>
<th>Ratio $a/b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>WW</td>
<td>9.16 ± 1.14$^a$</td>
<td>0.16 ± 0.01$^a$</td>
<td>0.089 ± 0.005$^a$</td>
<td>0.25 ± 0.01$^a$</td>
<td>1.87 ± 0.05$^a$</td>
</tr>
<tr>
<td>DS</td>
<td>6.86 ± 0.80$^a$</td>
<td>0.13 ± 0.01$^b$</td>
<td>0.06 ± 0.003$^b$</td>
<td>0.20 ± 0.01$^b$</td>
<td>1.83 ± 0.06$^a$</td>
</tr>
</tbody>
</table>
be emphasized that effects deduced from gas-exchange data (including our model estimates) may be overestimated, due to erroneous calculations of Ci resulting from stomatal patchiness and cuticular transpiration, especially under drought-stress conditions (Beyschlag et al. 1992; Meyer and Genty 1998; Mott and Buckley 2000).

To eliminate ambiguity in model estimates caused by possible errors in Ci calculation, we measured the in vitro activities of key enzymes of the Calvin cycle and triose-P utilization, namely Rubisco, G3PDH, Ru5PK and FruBPase, which can be related directly to the model estimates (Farquhar et al. 1980; Wullschleger 1993; Wohlfahrt et al. 1999). There were striking reductions in maximum Rubisco, G3PDH, Ru5PK and FruBPase activities per unit of leaf area in drought-stressed plants (Fig. 5), which were correlated with a significant reduction in total protein in the same plants. Reductions in soluble protein may result from increased protease activity, which is normally the case under drought (Kanna-Chopra et al. 1999). In addition, disequilibrium between source and sink carbohydrates, deficient nitrogen assimilation, and increased carbon:nitrogen ratio, which occur under stress conditions, are known to repress the expression of genes that encode photosynthetic enzymes (Paul and Driscoll 1997; Nielsen et al. 1999). This type of response is likely to occur under a slowly-induced water stress. With rapid imposition of water stress, effects on CO2 assimilation may be caused both by responses involving stomatal closure (Tezara et al. 1999; Cornic 2000) and imbalances of key metabolites of the pathway due to the lack of CO2 in the chloroplast (Gunasekera and Berkowitz 1993; Lawlor 1995; Tezara et al. 1999). In this study, we did not address processes associated with the rapid imposition of stress, rather the ones observed during acclimation to water stress developing slowly under field conditions.

Differences in estimated and measured activities of the enzymes analysed in this study (compare Table 1 and Fig. 5) may be due to the use of model constants and kinetic properties of Rubisco which were determined for spinach (Harley et al. 1992). Possible effects of drought over kinetic properties of Rubisco that, again, were estimated for non-stressed spinach plants, may also play a role in the differences observed between modelled and measured activities of the enzymes studied.

The reductions observed in both modelled and measured activities of key enzymes of the carbon assimilation pathway associated with drought stress were of comparable magnitude, suggesting that model estimates are appropriate to evaluate relative (control vs stressed) non-stomatal effects under drought. The influence of stomatal patchiness is probably not important under the conditions of the slowly-developed water stress that the plants in our study endured (Wullschleger 1993; Theobald et al. 1998).

In conclusion, data gathered in this study suggest that while the limitation of CO2 assimilation due to stomatal closure in response to slowly-imposed drought is significant; there is also a proportional reduction in Rubisco maximum carboxylation capacity, RuBP regeneration, and triose-P utilization. Reductions in the biochemical capacity for carbon assimilation and utilization under long-term drought is proportional, and may be caused by the shorter-term decrease in CO2 availability following increased stomatal limitation. However, maximum quantum yield of PSII photochemistry is not greatly affected under these conditions.

Fig. 5. In vitro activities of key enzymes of carbon metabolism (Rubisco, G3PDH, Ru5PK and FruBPase) in well-watered (open bars) and drought-stressed (closed bars) plants. Values are means ± s.e. Statistically significant differences occur for P<0.05.
while downregulation of PSII activity during the day plays an important role in the tight regulation between photochemical and carbon assimilation reactions under the long-term photosynthetic adaptation to water stress.

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