Drought-induced hydraulic limitations constrain leaf gas exchange recovery after precipitation pulses in the C\textsubscript{3} woody legume, \textit{Prosopis velutina}

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Summary

- The hypothesis that drought intensity constrains the recovery of photosynthesis from drought was tested in the C\textsubscript{3} woody legume \textit{Prosopis velutina}, and the mechanisms underlying this constraint examined.
- Hydraulic status and gas exchange were measured the day before a 39 mm precipitation pulse, and up to 7 d afterwards. The experiment was conducted under rainout shelters, established on contrasting soil textures and with different vegetation cover at the Santa Rita Experimental Range in southeastern Arizona, USA.
- Rates of photosynthesis and stomatal conductance after re-watering, as well as the number of days necessary for photosynthesis to recover after re-watering, were negatively correlated with predawn water potential (\(R^2 = 0.83, 0.64\) and \(0.92\), respectively).
- Photosynthetic recovery was incomplete when the vascular capacity for water transport had been severely impaired (percentage loss of hydraulic conductance > 80\%) during the drought, which largely increased stomatal limitations. However, changes in biochemical capacity or in mesophyll conductance did not explain the observed pattern of photosynthesis recovery. Although the control that hydraulic limitations impose on photosynthesis recovery had been previously inferred, the first empirical test of this concept is reported here.

Key words: cavitation, hydraulic limitations to gas exchange, photosynthesis recovery from drought, precipitation pulses, soil–plant–atmosphere continuum, water stress.

Introduction

Precipitation is one of the most important factors controlling primary productivity in terrestrial ecosystems, and increases in importance as mean annual precipitation decreases (Huxman \textit{et al.}, 2004a). Accordingly, ecosystems in areas with low mean annual precipitation are predicted to be most susceptible to anticipated changes in rainfall associated with climate warming (de Dios \textit{et al.}, 2007). The rate of photosynthetic carbon assimilation (\(A\)), a key process related to primary productivity, varies widely over the growing season in arid and semiarid ecosystems, and often responds significantly to changes in resource supply associated with pulsed inputs of growing season precipitation (Sala & Lauenroth, 1982; Williams & Ehleringer, 2000; Huxman \textit{et al.}, 2004b).

Differences in growing conditions, such as soil texture, vegetation cover and atmospheric humidity, along with the high spatial and temporal variance of precipitation in arid and semiarid regions, produce a highly heterogeneous mosaic of water availability which may change dramatically even over very short timescales of hours and days (Reynolds \textit{et al.}, 2004). This variation in water availability results in substantial variation of the photosynthetic gas exchange response following precipitation inputs during the growing season in these environments (Huxman \textit{et al.}, 2004b; Reynolds \textit{et al.}, 2004; Ignace \textit{et al.}, 2007; Patrick \textit{et al.}, 2007; Resco \textit{et al.}, 2008).

Temporal up-scaling of leaf photosynthetic fluxes in these ecosystems is problematic because of the different time lags observed for different components of leaf gas exchange regulation (Tuzet \textit{et al.}, 2003). In response to a precipitation pulse,
stomatal conductance \((g_s)\), \(A\) and plant water potential may be temporarily decoupled from each other, although the underlying mechanism has not yet been elucidated (Yan et al., 2000; Tuzet et al., 2003; Resco et al., 2008).

These uncertainties arise partly from our incomplete understanding of how gas exchange recovers from drought after new pulses of precipitation. In recent years, a general model of drought effects on photosynthesis limitations has been proposed, based on the interplay between biochemical, stomatal and mesophyll limitations (Flexas et al., 2006). Stomatal limitations to photosynthesis are regarded as the prevailing limiting factor except when: plants are operating in the asymptotic part of the \(A=g\) relationship; and \(g_s\) drops below 0.05–0.1 mol m\(^{-2}\) s\(^{-1}\). Biochemical limitations are generally thought to take over after these thresholds. However, decreases in mesophyll conductance \((g_m)\), the conductance of \(CO_2\) from the substomatal cavity to the site of carboxylation, has been increasingly reported as another dynamic factor limiting photosynthesis (Flexas et al., 2008).

Although photosynthesis responses to drought are relatively well understood, there is a surprising paucity of studies on photosynthesis recovery from drought (Flexas et al., 2006). \(A\) and \(g_s\) typically increase in response to a biologically significant precipitation pulse, until they reach a ‘peak’ value, and then return to values comparable to those before the irrigation. Peak response rates of \(A\) and \(g_s\) \((A_p\) and \(g_{Sp}\) respectively) following a precipitation pulse, as well as the time lag \((\tau)\) necessary to reach that peak value, are often reported to depend upon the intensity of water stress antecedent to the pulse (Huxman et al., 2004b). However, the mechanisms underlying this relationship are currently being debated. The Flexas et al. (2006) model of photosynthesis responses to drought predicts that incomplete recovery of photosynthesis occurs when, as a result of previous drought, biochemical capacity needs to be restored. When \(g_s\) drops below 0.05 mol m\(^{-2}\) s\(^{-1}\), the concentration of antioxidants compounds in photosynthetic tissues increases while the carboxylation capacity is impaired (Flexas et al., 2006). Hence, incomplete photosynthesis recovery may occur when the plant needs to repair its carboxylation capacity after drought-mediated oxidative stress. Alternatively, stomatal limitations may prevail if the plant has suffered a large percentage loss of hydraulic conductance (PLC) during the drought (Wheeler & Holbrook, 2007). Stomata close when vascular supply diminishes, and incomplete photosynthesis recovery is to be expected when the plant has experienced a large PLC, because xylem refilling under tension is problematic (Clearwater & Goldstein, 2005; Lovisolo et al., 2008). Finally, Galmes et al. (2007) observed that incomplete recovery of \(g_{Sm}\) after re-watering was also important in limiting photosynthesis recovery, because a large resistance to \(CO_2\) diffusion from the substomatal cavity to the chloroplast diminishes the substrate for carboxylation, and \(g_{Sm}\) is not driven solely by physical diffusion but seems to be controlled by plasma membrane intrinsic proteins (aquaporins) and/or by the activity of carbonic anhydrase (Bernacchi et al., 2002), which, in turn, are highly sensitive to drought (Kaldenhoff et al., 2008).

In this study, we monitored the dynamics of \(A\) and \(g_s\) in the C\(_3\) woody legume Prosopis velutina Woot (mesquite) over a period spanning the day before and up to 7 d after a large precipitation pulse. Mesquite was chosen for this study because of its historic encroachment into grasslands in southwestern North America and its documented impacts on community structure and ecosystem processes (Scholes & Archer, 1997; McClaran et al., 2003; Williams et al., 2006; Yepez et al., 2007; Knapp et al., 2008). Photosynthesis recovery was assessed after two months of imposed drought and at the peak of the summer rainy season on clay loam and sandy loam soils, on different aged seedlings of \(P.\) velutina, as well as on plants growing on bare ground or with interspecific competition from perennial C\(_4\) grasses. This variety of growing environments allowed us to evaluate drought recovery under contrasting amounts of antecedent water stress. For expediency, ‘antecedent’ will be used throughout the text to indicate values measured the day before the irrigation input \((\text{abbreviated as } p^-)\), whereas ‘peak’ denotes the day on which the highest value of \(A\) in response to the irrigation pulse was recorded \((\text{abbreviated as } p^+)\), unless otherwise noted.

The first goal of this study was to quantify the importance of antecedent conditions on constraining the recovery of \(A_p\), \(g_p\) and \(\tau_A\) \((\text{the number of days between the precipitation pulse and } A_p\text{ after re-watering (Ogle & Reynolds, 2004)})\). The second goal was to test whether the constraint exerted by antecedent conditions on drought recovery is imposed by biochemical, stomatal or mesophyll limitations in \(P.\) velutina. The third goal was to test the generality of this relationship in other desert species for which data were available from the literature.

**Materials and Methods**

**Study sites**

Field measurements were conducted under experimental rainout shelters installed on sandy loam and clay loam soils at the Santa Rita Experimental Range in southeastern Arizona, USA (English et al., 2005). The rainout shelters and the experimental plots they covered were established in 2002 as part of a global change experiment to investigate mesquite seedling establishment under different seasonal precipitation regimes (50% increase or decrease of the long-term average in summer or winter precipitation), soil textures (sandy loam and clay loam) and grass cover (bare ground, native or invasive C\(_4\) grass). A cohort of 30 seeds was planted yearly on each plot and, because of highly variable treatment-induced patterns of seedling establishment, we were unable to assess seedling physiology across all treatment combinations. Two separate seedling cohorts (1 and 4 yr of age for the present study) were available for intensive physiological measurements. Adequate numbers of
1- and 4-yr-old seedlings were available in grass-free, bare plots on each of the two soil surfaces, but in plots dominated by the native C₄ grass *Heteropogon contortus* (L.) Beauv only 1-yr-old seedlings were available for measurements and only on the sandy loam surface (Table 1). A detailed description of the demographic patterns is provided by Resco (2008).

The rainout shelters excluded natural precipitation (mean annual precipitation of 394 mm at the sandy loam site and 430 mm at the clay loam site (Frovolini et al., 2005)) from each plot. On June 10, 2006, after 2 months of imposed drought, we applied a 39 mm irrigation pulse to all of the experimental plots on the sandy loam and clay loam surfaces. Another 39 mm pulse was applied to the plots at the sandy loam site on August 1, 2006, at the peak of the summer rainy season, during a period of frequent experimental irrigation. The experiment was conducted on these highly contrasting soil textures, vegetation covers and different parts of the year to ensure large differences in the antecedent stress (Fig. 2, Table 1).

### Photosynthetic gas exchange measurements

Spot gas exchange measurements (n = 3–5) were performed at 07:30 h the day before the irrigation and 1, 3, 5 and 7 d afterwards with a portable photosynthesis system (LI-6400, Li-Cor Inc., Lincoln, NE, USA). Environmental conditions inside the leaf chamber were set to match early morning conditions. Light intensity, block temperature and CO₂ concentration were 700 µmol m⁻² s⁻¹, 30°C and 400 ppm, respectively. Leaf-to-air vapor pressure deficit (D) was maintained in the range 1.5–3.5 kPa, depending on environmental conditions. Leaves were marked and the same leaf was measured each day of the pulse period.

To quantify the importance of antecedent water stress for constraining the pulse response, we measured predawn water potential (Ψᵦ), an integrated indicator of water availability in the rhizosphere when nocturnal transpiration is negligible, the day before the irrigation pulse. To test whether this constraint originated from limitations in leaf biochemical capacity, CO₂ diffusion through the mesophyll, or through the stomata, we estimated maximal leaf carboxylation capacity (Vᵦₙ₅₉) and mesophyll conductance (gᵦₚₖ), and measured gᵦₚₖ the day before the watering. A possible role for hydraulic limitations as driver of the pulse response was evaluated by estimating PLC at midday, through a previously established relationship between xylem tension and PLC (see later). Ψᵦ, Vᵦₙ₅₉, gᵦₚₖ and gᵦₚₖ were also monitored 1, 3, 5 and 7 d after the precipitation pulse, to characterize differences in drought recovery across treatments.

Ψᵦ and midday water potential (Ψᵦₚₖ) measurements were taken on additional seedlings not used for gas exchange within each experimental plot. They were determined on leaves of 1-yr-old *P. velutina* seedlings using Peltier thermocouple psychrometers (PST-55 Wescor Inc., Logan, UT, USA), and with a Scholander-type pressure chamber (PMS Instruments, Corvallis, OR, USA) on the 4-yr-old seedlings (n = 3). No statistical difference in estimated Ψ from these two techniques was found in an independent test (Resco et al., 2008). A slight modification to the original design of the PST-55 was necessary to measure leaves in these soil psychrometers. We enclosed leaves inside an isolated custom-built chamber, and immersed the chamber in a water bath at 25°C until equilibration occurred. Further details are provided by Resco et al. (2008) and by the manufacturer's website (http://www.wescor.com).

Response curves relating net photosynthetic rate (A) to variation in the leaf intercellular (Cₐ) and chloroplast (Cₚ) concentration of CO₂ were developed the day before the watering between 06:00 and 10:00 h, following Long & Bernacchi (2003) at saturating light (1500 µmol m⁻² s⁻¹) using the same leaves on which spot gas exchange measurements had been made. Leaves were allowed to acclimate to chamber conditions at a CO₂ concentration (Cₚ) of 400 ppm, after which gas exchange parameters were recorded. Gas exchange rates were then determined sequentially as Cₚ was reduced to 300, 200, 100 and 50 ppm, and then as Cₚ was returned to 400 ppm and then sequentially at 600, 800, 1000, 1200, 1400, 1600, 1800

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**Table 1** Characteristics of the different species and treatments reported in this study, and codes used in figures

<table>
<thead>
<tr>
<th>Code</th>
<th>Species</th>
<th>Data source</th>
<th>Ecosystem</th>
<th>Soil texture</th>
<th>Ground cover</th>
<th>Age</th>
<th>Measurement</th>
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<td>This study</td>
<td>Sonoran</td>
<td>Sandy loam</td>
<td><em>Heteropogon contortus</em> stand</td>
<td>1 yr</td>
<td>After drought</td>
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<td>Sonoran</td>
<td>Sandy loam</td>
<td>Bare ground</td>
<td>1 yr</td>
<td>After drought</td>
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<td>Sonoran</td>
<td>Sandy loam</td>
<td>Bare ground</td>
<td>4 yr</td>
<td>After drought</td>
</tr>
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<td>This study</td>
<td>Sonoran</td>
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<td><em>H. contortus</em> stand</td>
<td>1 yr</td>
<td>Rainy season</td>
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<td>1 yr</td>
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<td>Rainy season</td>
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<td>1 yr</td>
<td>After drought</td>
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<td>Bare ground</td>
<td>4 yr</td>
<td>After drought</td>
</tr>
<tr>
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<td>NA</td>
<td>Adult</td>
<td>After drought</td>
</tr>
<tr>
<td>Ps</td>
<td><em>Pascopyrum smithii</em></td>
<td>Schomp (2007)</td>
<td>Mixed Prairie</td>
<td>NA</td>
<td>Native vegetation</td>
<td>Adult</td>
<td>Rainy season</td>
</tr>
</tbody>
</table>

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and 2000 ppm. Each curve was developed within 30–40 min. \( V_{\text{max}} \) was estimated from the Farquhar et al. (1980) photosynthesis model, following the assumptions and model-fitting approach of Sharkey et al. (2007).

Mesophyll conductance to \( \text{CO}_2 \) transfer (\( g_m \)) was estimated with the ‘variable’ \( f \) method on the same leaves used to develop \( A/C_i \) curves. This method compares electron transport rate (\( f \)) measured from chlorophyll fluorescence (Genty et al., 1989) with \( f \) estimated with the Farquhar et al. (1980) model. The difference between both estimates is assumed to be related to \( g_m \); see Harley et al. (1992) and Long & Bernacchi (2003) for a detailed description of the method. Four parameters are required to obtain \( f \) from chlorophyll fluorescence: (i) the photochemical efficiency of photosystem II (\( \Phi_{\text{PSII}} \)), which was determined using a portable pulse-modulated fluorometer (FMS2, Hansatech Instruments, King’s Lynn, UK) immediately after development of each \( A/C_i \) curve, and after acclimating each leaf for 10 min at the irradiance value at which the previous \( A/C_i \) curve had been measured (Maxwell & Johnson, 2000; Loik & Holl, 2001); (ii) leaf absorptance, which was assumed to be 0.86 for \( \text{Prosopis} \) (Asner et al., 1998); (iii) irradiance (same as in the \( A/C_i \) curve); and (iv) the fraction of absorbed irradiance that reaches PSII, which was assumed to be 0.5 for \( \text{C}_4 \) plants (Ögren & Evans, 1993). Warren (2006) provides a critical analysis on the limitations of using this approach to estimate \( g_m \).

Vulnerability to cavitation

Direct estimates of PLC in the limited population of \( \text{Prosopis} \) seedlings were not possible to attain, because of the destructive nature of the measurement. Hence, we had to estimate PLC from vulnerability to cavitation (VC) curves, which relate PLC to xylem tension (\( P_x \)) in 1-yr-old \( \text{Prosopis} \) seedlings growing on sandy loam (closed squares, continuous line) and clay loam (open squares, dashed line) sites. Different lines result from fitting the Weibull function \( y = 100(1 - \exp \left(-\left(\frac{x}{a}\right)^b\right)) \), where \( a \) and \( b \) are 2.98 and 1.19 for sandy loam seedlings and 5.40 and 1.54 for clay loam seedlings, respectively.

In Fig. 6, but observed no significant difference in the relationship reported between PLC with the drought recovery parameters (not shown).

Plants were harvested under water, at least 10 cm below the root collar, and transported to the laboratory in wet paper towels inside zip bags. In the laboratory, they were allowed to air dry to different stages, from 1 up to 10 d (−0.1 to −14 MPa). The whole plant was kept inside a zip bag the night before measurement, to allow for equilibration of spatial gradients in water potential. Before the conductivity measurement, a segment centered on the root collar was cut under water. Hydraulic conductivity was measured in this segment as the ratio between the flow of deionized water (measured by XYLEM, Bronkhorst, France (Cruiziat et al., 2002)) and the gravity-induced pressure gradient (10 kPa). Maximum conductivity was estimated after flushing the segment at high pressure (100 kPa, refer to the XYLEM manual for further details (http://www.bronkhorst.fr/fr/produits/xylem_ embolimetre)). Xylem tension was measured with a Scho
tlander type pressure chamber (PMS Instruments, Corvallis, OR, USA) in the above-ground part of the plant and VC curves were fitted through a Weibull function (Sperry et al., 1998, Fig. 1). PLC was then estimated by substituting \( \Psi_{\text{md}} \) for \( P_x \) in the equations given in Fig. 1. \( \Psi_{\text{md}} \) measures the minimum water potential throughout the day, and we thus estimated the maximum PLC that might occur.

Water potential in the leaves is necessarily more negative than in the stem for water to flow. Because \( \Psi_{\text{md}} \) was measured in leaves and not in the stem, we may have consistently overestimated stem PLC. However, this study was performed in rather short mesquite plants (< 40 cm), and spatial gradients...
in $\Psi$ from the stem to the leaf will likely be in the order of a few tenths of a kPa (Tyree & Ewers, 1991), a negligible error for the accuracy necessary in this study (Fig. 1).

Data analyses

To quantify the importance of antecedent water stress ($\Psi_{pd,D-1}$) in constraining the pulse response in mesquite and in other desert species, we conducted data searches on Web of Science (http://portal.isiknowledge.com/) for studies that were performed on C$_3$ plants; that reported the pulse response of spot gas exchange at leaf level for at least the day before the watering and up to the day when $A_p$ and $g_p$ occurred; and that maintained comparable light intensities inside the photosynthesis chamber. Unfortunately, only one study (Gillespie & Loik, 2004; Loik, 2007) matched our criteria. Loik (2007) reported results for two shrubs from the southwestern Great Basin desert – *Purshia tridentata* (Rosaceae) Pursh and *Artemisia tridentata* (Asteraceae) Nutt. We also incorporated the results from an unpublished thesis (Schomp, 2007), where a pulse response study was conducted in mixed-grass prairie in southeastern Wyoming as a component of the Prairie Heating and CO$_2$ Enrichment (PHACE) experiment (http://www.phace.us/). Schomp (2007) reported results for the C$_3$ perennial grass *Pascopyrum smithii* (Poaceae) (Rybd.) A. Love (Table 1).

We examine the relationship between $\Psi_{pd,D-1}$ and $A_p$, $g_p$, and $\tau_A$ through least-squares fitting. Because the different species included in this analysis had different photosynthetic capacities (Table 1), we normalized $A_p$ as percentage photosynthesis recovery:

$$\% \text{ recovery} = 100 \left( \frac{\bar{A}_p}{\bar{A}_{max,s}} \right) \quad \text{Eqn 1}$$

where $\bar{A}_p$ is the average ($n = 3$–$5$) of the value of $A_p$ for species $s$; and $\bar{A}_{max,s}$ is the maximum of the mean assimilation rates under optimum conditions (during the peak of the rainy season) for species $s$: 22 µmol m$^{-2}$ s$^{-1}$ for mesquite (this study), 18 µmol m$^{-2}$ s$^{-1}$ for *A. tridentata* (Gillespie & Loik, 2004), 15 µmol m$^{-2}$ s$^{-1}$ for *P. tridentata* (Gillespie & Loik, 2004) and 20 µmol m$^{-2}$ s$^{-1}$ for *P. smithii* (Schomp, 2007; V. Resco, unpublished).

Then, we evaluate in mesquite whether the relationship between drought recovery and antecedent stress is mediated by antecedent stomatal limitations ($g_{D-1}$), antecedent $V_{cmax}^m$ ($V_{cmax,D-1}$) or antecedent mesophyll conductance ($g_{m,D-1}$), by examining whether these parameters correlate with $A_p$. To understand why higher-stressed plants did not attain the same $A_p$ as lower-stressed plants, we tested for differences in $g_m$, $V_{cmax}^m$ and $g_m$ across treatments when $A_p$ is reached ($g_{p,s}$, $V_{cmax,p,s}$ and $g_{m,p,s}$, respectively), through analysis of variance. Finally, because $g_p$ could be influenced by both PLC and leaf-to-air vapor pressure deficit ($D$), we partitioned the effects of these two through stepwise regression following the model selection criteria proposed in Crawley (2007). Nonlinear curve fits were just chosen to establish empirical relationships, but without exploring the potentially relevant biological information stored in the parameters. We used R 2.5.0 (R Development Core Team, Vienna, Austria) in all of our statistical analyses.

Results

Constraints on drought recovery imposed by antecedent drought stress

Because this study was performed on plants growing on different sites and under different plant covers, the underlying assumption is that a comparable degree of hydration across treatments was attained after the application of the 39 mm irrigation pulses, such that variance in $A_p$ does not merely reflect differences in post-irrigation $\Psi_{pd}$. Indeed, Fig. 2c shows that there is no significant relationship between peak $\Psi_{pd}$ and antecedent $\Psi_{pd}$ ($P = 0.63$). Moreover, the relationship between peak $\Psi_{pd}$ and $A_p$ was not significant ($P = 0.56$, Fig. 2b), indicating that the results from this experiment are not an artifact originating from post-irrigation differences in plant water status.

Antecedent water stress, as indicated by $\Psi_{pd,D-1}$, explained 83, 92 and 64% of the variance in $A_p$, $\tau_A$ and $g_p$, respectively (Figs 2, 3), suggesting that antecedent conditions exert substantial control on drought recovery.

No differences in antecedent values of $V_{cmax}$ and $g_m$ across treatments were observed (Table 2, Fig. 4). However, we did observe differences in antecedent $g_p$ (Table 2, Fig. 4), such that $g_{p,D-1}$ was lower in the seedlings growing at the sandy loam site in June than in the other treatments (Fig. 4). The control of antecedent stress on drought recovery does not seem to be mediated by nonstomatal limitations, as no significant relationship ($P > 0.3$) was observed between $V_{cmax,D-1}$ or $g_{m,D-1}$ with $A_p$ (Fig. 5b,c) in *P. velutina* seedlings. However, the degree of antecedent $g_p$ was significantly correlated ($P < 0.01$, $R^2 = 0.53$) with photosynthesis recovery from drought (Fig. 5a).

Stomatal limitations in our system may result from either high $D$ and/or PLC. Stomatal conductance responds rather rapidly to variations in $D$, and no direct mechanistic link is to be expected between gas exchange after re-watering and the $D$ that occurred during drought. However, the effects of increasing PLC after a prolonged period of drought may last for some time, even after re-watering, if complete recovery of hydraulic capacity is not achieved. Indeed, PLC$_{p-1}$ was significantly correlated with $A_p$ ($R^2 = 0.83$, Fig. 6c), $\tau_A$ ($R^2 = 0.97$, Fig. 6b) and $g_p$ ($R^2 = 0.82$, Fig. 6a), suggesting that limited hydraulic conductivity caused the observed differences in the dynamics of photosynthesis recovery from drought.

Antecedent water stress also imposed an important constraint on $A_p$ and $g_p$ in *A. tridentata*, *P. tridentata* and *P. smithii*, and no interspecific differences were apparent, since these values
fell within the 95% confidence interval for the Prosopis data (Figs 2a and 3b). However, \( \tau_A \) was up to 4 d shorter in these species than in \textit{P. velutina} (Fig. 3a).

Effects of post-irrigation conditions on photosynthetic recovery

We only observed significant differences in \( g_s \) across treatments after the application of the 39 mm precipitation pulses, but not in peak values of \( V_{\text{cmax}} \) and of \( g_m \) (Table 2). The effect of peak stomatal limitations on \( A_p \) could be the result of the previously reported differences in antecedent PLC, but also of differences in peak \( D \) (\( D_p \)) across seasons, as \( D_p \) was significantly lower in June than in August (\( P < 0.01 \)). To partition the effect of PLC\(_{D-1}\) from that of \( D_p \) on stomatal conductance, we performed a stepwise regression where we compared a model with PLC\(_{D-1}\) as the only independent

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Table 2

Analysis of variance testing for significant differences in maximum carboxylation capacity (\( V_{\text{cmax}} \)), mesophyll conductance (\( g_m \)) and stomatal limitations to photosynthesis (\( l \)) across eight treatments (Table 1, Fig. 4), the day before the pulse (pre-pulse) and when \( A_p \) was reached (post-pulse)

<table>
<thead>
<tr>
<th>Period</th>
<th>Variables</th>
<th>( df )</th>
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<tr>
<td></td>
<td>( g_m )</td>
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<tr>
<td></td>
<td>( l )</td>
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<td>Post-pulse</td>
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</table>
variable (Fig. 6b) with another regression model where both $D_p$ and PLC$_{D-1}$ were independent variables. $D_p$ and PLC$_{D-1}$ were not significantly correlated with each other ($P > 0.05$). The inclusion of $D_p$ did not significantly improve the performance of the model ($P = 0.13$), and we thus concluded that the effect of different $D$ on $g_p$ across seasons was overridden by that of PLC$_{D-1}$.

**Discussion**

Photosynthesis limitations and recovery from drought

Our results partially support the generality of a recently proposed conceptual model on the changes in photosynthetic limitations with drought (Flexas et al., 2006), which predicts that stomatal limitations prevail except under severe stress (defined as $g_s < 0.05 \text{ mol m}^{-2} \text{s}^{-1}$), when biochemical limitation starts to operate. We failed to observe any statistical difference in $V_{c_{\text{max}}}^{\text{D-1}}$ or in $g_m$ across treatments before the irrigation pulse (Table 2), although antecedent stomatal conductance varied from 0.06 to 0.33 mol m$^{-2}$ s$^{-1}$.

A prediction from this photosynthesis model is that recovery from drought will be complete and immediate when no reductions in biochemical capacity are experienced, because a relaxation in $g_s$ is often assumed after the water input (Flexas et al., 2006; Galmes et al., 2007). Stomatal aperture is thought to be regulated by changes in epidermis and guard cell water potential, which, in turn, are affected by $D$ as well as by xylem hydraulic conductance (Brodribb et al., 2003;
Buckley, 2005), amongst others. Whereas an increase in relative humidity usually accompanies a large precipitation pulse, and this would alleviate atmospheric stress, our data indicate that photosynthetic gas exchange recovery may be neither complete nor fast when a large proportion of the hydraulic capacity has been lost as a result of drought. For *P. velutina*, photosynthesis limitation in response to a pulse remains at around or below 10–20%, and the time lag necessary to reach this peak assimilation value is between 1 and 3 d, when the percentage loss of hydraulic conductance antecedent to the pulse is < 80%. However, an incomplete recovery of photosynthesis from drought was observed when antecedent PLC was above 80% (Fig. 6).

Previous studies had suggested thresholds for incomplete drought recovery in terms of a minimum stomatal conductance (Flexas et al., 2006; Galmes et al., 2007). However, we have shown that proposing this threshold as a function of $g_s$, instead of PLC, may be misleading: complete and timely photosynthetic recovery is to be expected if low $g_{s,D-1}$ is the result of large antecedent $D$ (alone or in combination with a low $\Psi_{pd}$, which does not lead to substantial cavitation), but incomplete drought recovery will follow when large PLC_{D-1} is motivating the low antecedent stomatal conductance.

With the goal of predicting the time lag necessary to reach peak gas exchange, one must take into account the relationship between water infiltration and soil texture. A regression model was able to explain 95% of the variation in $\tau_A$ as a function of PLC_{D-1} for the 'sandy loam seedlings', whereas the 'clay loam seedlings' fell out of this regression line (Fig. 6a). This is likely because of the longer time required for water infiltration and for water potential to change in finer-textured soils (Hillel, 2004), the observed correlation between the time necessary to reach $A_p$ with the time necessary to reach peak $\Psi_{pd}$ ($P < 0.05, R^2 = 0.63$), and differences in rooting depth across soil textures (Resco, 2008).

The accumulation of abscisic acid (ABA) signaling stomatal closure has previously been reported as another mechanism affecting photosynthetic gas exchange recovery from drought (Davies & Zhang, 1991). However, high ABA concentrations are not likely to persist for > 3 d after water stress is relieved (Davies & Zhang, 1991). An ABA-mediated response would not explain either the long $\tau_A$ or the low $A_p$ observed 5–7 d after watering in the seedlings with the highest PLC_{D-1} (Fig. 6).

Our results support the findings of Fuchs & Livingston (1996), who suggested that woody plants rely more on hydraulic signals, whereas ABA regulation is probably more common in herbaceous plants.

Our results also support a rapidly increasing body of literature showing the dependence of photosynthetic gas exchange on plant hydraulic properties (Brodribb & Feild, 2000; Sperry, 2000; Maherali et al., 2006; Sack & Holbrook, 2006; Brodribb et al., 2007), although previous studies focused mainly on interspecific comparisons between different parameters related to maximum photosynthetic and hydraulic capacities. Moreover, considering the reported paucity of studies on photosynthetic drought recovery (Flexas et al., 2006; Galmes et al., 2007), this may be the first report linking biochemical, mesophyll and stomatal limitation with plant hydraulic architecture. Indeed we provide, to the best of our knowledge, the first study demonstrating that hydraulic limitations determine peak rates of $A_p, g_s$ and $\tau_A$ associated with the short-term responses to precipitation pulses.
Do antecedent drought-induced hydraulic limitations constrain photosynthetic recovery across species?

We observed that the degree of antecedent water stress explains up to 87% of the variance in the pulse response in *P. velutina*. We failed to find significant differences in the relationship between $A_{\text{max}}$ and $g_s$ with $\Psi_{\text{pd, D}_1}$ (Figs 2, 3) as a function of species identity, as values for *A. tridentata*, *P. tridentata* and *P. smithii* fell within the 95% confidence interval. This result may seem surprising at first, as these species are likely to have different VC curves. However, when antecedent drought stress is so severe that $\Psi_{\text{pd}}$ drops to the values reported here (~3.8 to ~4.6 MPa), PLC values of all four species are likely to converge at rather large values. Indeed, photosynthesis recovery of these three species was around or above 40%, which, according to our model for *Prosopis*, would imply a $\Delta LC_{\text{D}_1}$ of 80% or higher. Published VC curves for some of these species, although growing at different sites, support this possibility (Kolb & Sperry, 1999).

Our estimate of percentage photosynthesis recovery is very sensitive to the maximum photosynthetic rates for a given species ($A_{\text{max}}$, Eqn 1). We conducted a sensitivity analysis which indicated that values for the recovery of photosynthesis in *A. tridentata*, *P. tridentata* and *P. smithii* fell within the 95% confidence interval in Figs 2 and 3 when the error in $A_{\text{max}}$ was up to 15%.

Galmes et al. (2007) and Flexas et al. (2006) observed an incomplete photosynthetic recovery from drought when antecedent $g_s$ dropped below 0.15 mol m$^{-2}$ s$^{-1}$ across a range of phylogenetically and functionally diverse plant species. However, they did not observe any reductions in the leaf biochemical capacity until $g_s$ was smaller than 0.05 mol m$^{-2}$ s$^{-1}$, and stomatal limitations seemed to prevail after re-watering. Hence, it could be hypothesized that hydraulic limitations developed during drought, as $\Delta LC_{\text{D}_1}$ rises when $g_s_{\text{D}_1}$ drops below 0.15 mol m$^{-2}$ s$^{-1}$, are a widespread mechanism limiting photosynthetic recovery, at least, until biochemical limitations arise. Unfortunately, the paucity of studies in the literature on this important topic prevent us from developing any further synthetic advancements. It is an important research need to elucidate the link between stem hydraulics and photosynthesis recovery from drought.

Understanding photosynthesis responses to precipitation may prove extremely useful for the temporal up-scaling of leaf fluxes. Ignace et al. (2007) showed that in two C4 grasses, the cumulative carbon gain following precipitation pulses was, under some environmental conditions, highly predictable based upon $\Psi_{\text{pd, D}_1}$. Although predicting cumulative carbon gain was beyond the scope of this study, our results suggest that by understanding how hydraulics constrain photosynthesis, we may develop simpler quantitative models of leaf-level rates of gas exchange than current approaches (Tuzet et al., 2003; Schymanski et al., 2008), which would require less data input and parameterization, and without compromising accuracy.

Moreover, understanding plant responses to pulses may prove key to develop water-saving land management techniques, and to mitigate effects of anticipated global warming.

Conclusions

In this study, we observed that drought-induced hydraulic limitations strongly constrain photosynthetic recovery after re-watering in *P. velutina*. PLC$_{\text{D}_1}$ < 80% seems to be the threshold value after which photosynthesis recovery is incomplete, and this threshold roughly corresponds to $g_s_{\text{D}_1}$ < 0.15 mol m$^{-2}$ s$^{-1}$. Contrary to recent hypotheses, incomplete photosynthetic recovery may occur even without reductions in leaf biochemical capacity. There is a great need for further tests on the mechanisms constraining drought recovery.

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References


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