

# Stomatal responses to changes in vapor pressure deficit reflect tissue-specific differences in hydraulic conductance

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## ABSTRACT

The vapor pressure deficit ( $D$ ) of the atmosphere can negatively affect plant growth as plants reduce stomatal conductance to water vapor ( $g_{wv}$ ) in response to increasing  $D$ , limiting the ability of plants to assimilate carbon. The sensitivity of  $g_{wv}$  to changes in  $D$  varies among species and has been correlated with the hydraulic conductance of leaves ( $K_{leaf}$ ), but the hydraulic conductance of other tissues has also been implicated in plant responses to changing  $D$ . Among the 19 grass species, we found that  $K_{leaf}$  was correlated with the hydraulic conductance of large longitudinal veins ( $K_{lv}$ ,  $r^2 = 0.81$ ), but was not related to  $K_{root}$  ( $r^2 = 0.01$ ). Stomatal sensitivity to  $D$  was correlated with  $K_{leaf}$  relative to total leaf area ( $r^2 = 0.50$ ), and did not differ between  $C_3$  and  $C_4$  species. Transpiration ( $E$ ) increased in response to  $D$ , but 8 of the 19 plants showed a decline in  $E$  at high  $D$ , indicative of an 'apparent feedforward' response. For these individuals,  $E$  began to decline at lower values of  $D$  in plants with low  $K_{root}$  ( $r^2 = 0.72$ ). These results show the significance of both leaf and root hydraulic conductance as drivers of plant responses to evaporative demand.

**Key-words:** C3/C4 grasses; grass physiology; leaf hydraulic conductance; root hydraulic conductance; stomatal conductance; stomatal sensitivity.

## INTRODUCTION

Plants reduce stomatal conductance in response to large vapor pressure deficits ( $D$ ) between the leaf and atmosphere, leading to global reductions in ecosystem productivity (Zhao & Running 2010). By reducing stomatal conductance to water vapor ( $g_{wv}$ ), plants minimize water loss and maintain the hydration of plant cells as  $D$  increases. This response, however, also decreases the rate of  $CO_2$  diffusion into the leaf, lowering internal  $CO_2$  concentrations leading to decreased efficiency of carbon fixation. Although  $g_{wv}$  responds to multiple plant signals triggered by a variety of environmental drivers (Hartung, Sauter & Hose 2002; Bunce 2006; Domec *et al.* 2009), the hydraulic architecture of plants plays a vital role in the response of  $g_{wv}$  to changes in leaf hydration (Sperry *et al.* 2002). A better understanding of the coordination between hydraulic architecture and stomatal responses to changing  $D$

will provide insight into the diurnal and seasonal growth patterns of plants.

Identifying the mechanism in plants that detects changes in  $D$  is still an active area of research (Mott & Parkhurst 1991; Buckley 2005; Shope, Peak & Mott 2008), but the sensitivity of stomata to  $D$  has been accurately described using empirical relationships. The absolute reduction in  $g_{wv}$  to changing  $D$  (stomatal sensitivity) differs among plants, but is related along a common spectrum of maximum stomatal conductance. Stomatal sensitivity is quantified as the slope between  $g_{wv}$  and  $\ln(D)$  (Oren *et al.* 1999) and is closely related to the magnitude of  $g_{wv}$  at  $D = 1.0$  kPa (referred to as  $g_{ref}$ , Oren *et al.* 1999; Addington *et al.* 2004; Domec *et al.* 2009); the absolute change in  $g_{wv}$  is greater in plants with high  $g_{ref}$ . This relationship holds whether the variability in  $g_{ref}$  results from inherent species differences (Oren *et al.* 1999), photosynthetic pathway (Maherali, Johnson & Jackson 2003) or changes within individuals in response to soil moisture (Saliendra, Sperry & Comstock 1995; Addington *et al.* 2004) and atmospheric  $CO_2$  concentration (Wullschlegel *et al.* 2002; Maherali *et al.* 2003). Brodrribb & Jordan (2008) also showed that stomatal sensitivity was closely related to maximum  $g_{wv}$  relative to leaf hydraulic conductance, but the hydraulic conductivity of other plant tissues can mediate stomatal responses to  $D$  under different environmental conditions (Domec *et al.* 2009; Meinzer *et al.* 2010).

Differential stomatal responses of  $C_3$  and  $C_4$  species to increased air temperature can alter the relative abundance of these species in mixed  $C_3/C_4$  communities (Epstein *et al.* 2002; Polley, Johnson & Derner 2003; Morgan *et al.* 2011). If these functional groups differ in their stomatal sensitivity, then warmer temperatures in the future may differentially affect  $g_{wv}$  and, therefore, community dynamics between these two functional groups. The  $CO_2$  concentrating mechanism of  $C_4$  plants allows for lower  $g_{wv}$  and greater water use efficiency (Sage & Monson 1999), but this is coordinated with reduced hydraulic conductance per unit leaf area (LA) compared with  $C_3$  plants (Kocacinar & Sage 2003; Kocacinar *et al.* 2008). This suggests that the relationship between  $g_{wv}$  and hydraulic conductance may remain constant despite differences in photosynthetic pathway. We would expect stomatal conductance of  $C_4$  plants to be governed by the same principles of supply and demand of water as  $C_3$  plants (Brodrribb & Jordan 2008) such that both functional groups should align with the hydraulic model proposed by Oren *et al.* (1999), but results from the literature are not consistent. The stomatal

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sensitivity of a drought-tolerant C<sub>4</sub> grass was lower than expected compared with a C<sub>3</sub> grass at a given  $g_{\text{ref}}$  (Maherali *et al.* 2003), but the sensitivity among 2-C<sub>3</sub> and 2-C<sub>4</sub> plants (Morison & Gifford 1983) was related to maximum  $g_{\text{wv}}$  in a manner similar to that described by Oren *et al.* (1999). These relationships need to be tested among a larger set of C<sub>3</sub> and C<sub>4</sub> species to better establish whether there are differences in stomatal sensitivity between these functional groups.

Despite reduced  $g_{\text{wv}}$  in response to increasing  $D$ , transpiration ( $E$ ) typically increases, but the response varies among species and genotypes (Monteith 1995; Maherali *et al.* 2003; Fletcher, Sinclair & Allen 2007; Sinclair, Zwieniecki & Holbrook 2008). Most plants show a non-linear, asymptotic response of  $E$  to increasing  $D$ , but some plants show a reduction in  $E$  as  $D$  continues to increase, which has been referred to as an 'apparent feedforward' response (Farquhar 1978; Monteith 1995). The hydraulic conductance of leaves has been implicated in the response of  $E$  to high levels of  $D$ ; modeling efforts have shown that the apparent feedforward response could result from changes in leaf hydraulic conductance (Dewar 2002; Buckley 2005). Recently, Sadok & Sinclair (2010) suggested that root hydraulic conductance affects the point at which plants reach their maximum rate of  $E$  or begin to reduce  $E$  in response to  $D$ . High hydraulic conductance of the root system could minimize the water potential gradient from soil to leaf at high  $D$ , allowing plants to maintain higher rates of stomatal conductance and preventing a decline in  $E$

in response to high  $D$ . Root hydraulic conductance, however, has not been tested for its role in the response of  $E$  to high  $D$  across a range of species.

The objective of this study was to test whether relationships between plant responses to  $D$  and hydraulic conductance were consistent among a group of C<sub>3</sub> and C<sub>4</sub> species. We hypothesized that (1) stomatal sensitivity to  $D$  will be correlated to  $g_{\text{wv}}$  at low  $D$ ; (2) stomatal sensitivity to  $D$  will be correlated with the hydraulic conductance of leaves; and (3) C<sub>3</sub> and C<sub>4</sub> species will follow the same relationship of stomatal sensitivity despite their fundamentally different photosynthetic pathways. Lastly, we hypothesized that (4) the vapor pressure deficit at which  $E$  begins to decline in species with an apparent feedforward response will be correlated with root hydraulic conductance.

## MATERIALS AND METHODS

### Plant material

We grew 19 grass species, including both C<sub>3</sub> and C<sub>4</sub> functional types, to look for patterns in stomatal responses to changing  $D$  (species listed in Table 1). The species were selected to represent plants adapted to a variety of environments (e.g. canopy-dominant, shallow-upland soils; deep-lowland soils; wetlands; etc.) in order to maximize our chances of capturing a wide range of stomatal responses to  $D$ . Rhizomes were

**Table 1.** List of the 19 grass species selected for our study grouped by photosynthetic pathway. The hydraulic conductance of the leaf ( $K_{\text{leaf}}$ ), root ( $K_{\text{root}}$ ) and large longitudinal veins ( $K_{\text{lv}}$ ) was compared between the C<sub>3</sub> and C<sub>4</sub> species using a two-sample  $t$ -test, but there were no significant differences between the functional groups. Significant differences between the 'overall mean' values of hydraulic conductance for different tissues at the  $P < 0.05$  level are indicated with lowercase letters. The species that exhibited an 'apparent feedforward' response are also indicated

	Species	$K_{\text{leaf}}$ (mmol m <sup>-2</sup> s <sup>-1</sup> MPa <sup>-1</sup> )	$K_{\text{root}}$ (mmol m <sup>-2</sup> s <sup>-1</sup> MPa <sup>-1</sup> )	$K_{\text{lv}}$ (mmol m <sup>-2</sup> s <sup>-1</sup> MPa <sup>-1</sup> )	Apparent feedforward response
C <sub>3</sub> species	<i>Agrostis hyemalis</i> Walter	2.56	26.17	1.57	Yes
	<i>Bromus inermis</i> Leyss.	1.82	4.49	1.45	No
	<i>Dactylis glomerata</i> L.	1.06	3.75	0.44	No
	<i>Dichanthelium oligosanthes</i> Schult.	1.09	3.27	0.46	No
	<i>Festuca subverticillata</i> Pers.	1.91	38.39	1.22	Yes
	<i>Hordeum pusillum</i> Nutt.	0.44	20.89	0.46	Yes
	<i>Koeleria macrantha</i> Ledeb.	4.49	29.57	5.00	Yes
	<i>Pascopyrum smithii</i> Rydb.	1.46	28.16	7.22	Yes
	<i>Poa pratensis</i> L.	8.57	54.04	5.02	No
	C <sub>3</sub> mean	2.60	23.19	2.54	NA
C <sub>4</sub> species	<i>Andropogon gerardii</i> Vitman	14.27	nd	5.82	No
	<i>Bouteloua dactyloides</i> Nutt.	5.24	48.46	3.58	Yes
	<i>Chloris verticillata</i> Nutt.	7.24	27.06	1.08	No
	<i>Digitaria californica</i> Benth.	1.85	39.47	0.90	Yes
	<i>Panicum virgatum</i> L.	13.24	6.38	8.23	No
	<i>Setaria pumila</i> Poir.	2.47	14.55	1.14	No
	<i>Spartina pectinata</i> Bosc.	3.12	11.19	1.68	No
	<i>Sporobolus cryptandrus</i> Torr.	3.88	nd	2.30	No
	<i>Sporobolus heterolepis</i> A. Gray	1.33	2.39	1.55	Yes
	<i>Tripsacum dactyloides</i> L.	2.56	29.87	1.16	No
C <sub>4</sub> mean	5.52	22.42	2.74	NA	
Overall mean	4.14 <sup>a</sup>	21.79 <sup>b</sup>	2.65	NA	

NA, not applicable; nd, not determined.

collected from Konza Prairie Biological Station (KPBS; Manhattan, KS, USA) during May and June 2010, as grass tillers emerged from the soil and could be identified. Rhizomes were immediately transplanted into pots constructed of polyvinyl chloride (PVC) pipe (5 cm outside diameter, 40 cm long) filled with local soil. Plants were grown at full sunlight and watered daily until at least five mature leaves were fully expanded but before flowering was initiated. Since rhizomes were collected from May to June, plants did not synchronously reach this measurement stage, and so sampling was staggered throughout June to early August.

### D response curves

Plants were watered the evening before gas exchange measurements to ensure soil was at pot-holding capacity during measurements. Vapor pressure deficit response curves were created by measuring the transpiration rate ( $E$ ) of the entire above-ground portion of each plant at a range of  $D$ .  $E$  was measured using a custom chamber that enclosed the entire aboveground portion of each plant. The *Arabidopsis* whole plant chamber for the Li-Cor 6400 (Li-Cor Biosciences, Inc. Lincoln, NE, USA) was used to couple the PVC pot to an acrylic chamber via a custom-made compression fitting and the soil surface was sealed with plumber's putty to ensure soil evaporation was excluded from measurements of  $E$ . The chamber was constructed of cast acrylic with low water adsorption capacity (product 8486 K351; McMaster-Carr, Chicago, IL, USA). A fan (SanAce40 9GV0412K301; Sanyo Denki, Tokyo, Japan) was installed inside the chamber to mix the air and reduce boundary layer conductance of the leaves; wind speed within the chamber was  $\sim 4.8 \text{ m s}^{-1}$ . A fine wire thermocouple installed in the chamber was secured to the leaf to calculate  $D$  based on leaf temperature. Plants were illuminated at  $\sim 1000 \mu\text{mol m}^{-2} \text{ s}^{-1}$  photosynthetically active radiation (PAR) using a light-emitting diode (LED) light source (180 W LED light; Advanced LED Grow Lights, Bentonville, AR, USA).

$E$  was calculated based on the water vapour concentration differential of air entering and leaving the chamber. This differential was then multiplied by the flow rate through the chamber and normalized by leaf area (LA) for final determination of  $E$  ( $\text{mmol m}^{-2} \text{ s}^{-1}$ ). This calculation ignores a term that describes the effect of water vapor on the flow rate in the chamber resulting in a slight underestimate of  $E$  by 0.7–3.1% in our study (Von Caemmerer & Farquhar 1981). The flow rate through the chamber was measured by pressure differential (model 68075; Cole-Parmer Instrument Company, Vernon Hills, IL, USA) across a 1 m piece of stainless steel tubing (0.16 cm internal diameter). A calibration curve was generated to convert the pressure differential to flow rate using a mass flow meter reserved for calibration purposes (Type 1640; MKS Instruments, Inc., Andover, MA, USA). The  $\text{CO}_2$  and  $\text{H}_2\text{O}$  concentration differential across the chamber was measured with a Li-Cor 6262 (Li-Cor Biosciences, Inc.) in differential mode; air entering the chamber was plumbed through the reference cell and air leaving the chamber passed through the sample cell. Source air was supplied from a tank of dry air with a  $\text{CO}_2$  concentration of  $394 \mu\text{mol mol}^{-1}$ .  $D$  of chamber air was

adjusted by changing the flow rate of dry source air through the chamber (Gholipour *et al.* 2010). As the flow rate of source air was adjusted, the  $\text{CO}_2$  concentration ( $[\text{CO}_2]$ ) of chamber air also changed, increasing the  $[\text{CO}_2]$  by, on average, 74.8 ppm across the range of  $D$  values. Increasing the flow rate into the chamber increased  $[\text{CO}_2]$  and  $D$ , both of which cause reductions in  $g_{\text{wv}}$ .  $D$  increased by 386% compared with 27% for  $[\text{CO}_2]$  as flow rate was increased, and so changes in  $D$  likely had a larger impact than  $[\text{CO}_2]$  on changes in  $g_{\text{wv}}$ .

### Hydraulic conductivity

The hydraulic conductivity of grass leaves and roots was measured immediately following determination of the  $D$  response curves using the steady-state high pressure flow method (HPFM) described by Yang & Tyree (1994). Briefly, the flow rate through the leaves was determined by measuring the pressure differential across a high-resistance piece of tubing [polyether ether ketone (PEEK) tubing with internal diameters of 0.127, 0.178, 0.254 and 0.508 mm] placed upstream from the leaf being measured. A variety of hydraulic resistors were calibrated to accommodate a range of leaf conductance values so that the pressure differential across the resistor could be maintained between 5 and 30 kPa, and the pressure of water entering the leaf was kept between 15 and 30 kPa. Once flow through the leaves was measured, the pressure differential across the resistor and the pressure of water entering the leaf was recorded and used to calculate conductivity of the plant tissue. We considered the flow rate to be stable when the coefficient of variation  $< 5\%$  for 5 min, which typically took 20–30 min.

Hydraulic conductivity of whole leaves ( $k_{\text{leaf}}$ ,  $\text{mmol MPa}^{-1} \text{ s}^{-1}$ ) was measured by cutting leaves near the ligule under water using a razor blade and then placing the cut ends immediately inside a custom-made hydraulic chamber. Approximately half of the LA for each plant was used for this measurement. The path of water flow for this measurement was through the entire leaf, including both xylem and extra-xylary components. The leaf was kept submerged during the measurement and was illuminated at  $\sim 1000 \mu\text{mol m}^{-2} \text{ s}^{-1}$  PAR using a LED light source (180 W LED light). Following the measurement of  $k_{\text{leaf}}$  another transverse cut was made across the leaf to remove most of the distal portion of the leaf and measure axial hydraulic conductivity through the large longitudinal veins ( $k_{\text{lv}}$ ,  $\text{mmol MPa}^{-1} \text{ s}^{-1}$ ). This cut excluded the path of water through transverse veins, small longitudinal veins and the leaf mesophyll as water moves from the vascular bundle to epidermis (Altus & Canny 1985). The leaf segment used for this measurement was  $\sim 3$  cm long and after the flow through this leaf section reached steady state the pressures across the resistor and of water entering the leaves were again recorded. The flow rate was divided by the pressure of water entering the leaf and then normalized by LA to calculate leaf-specific hydraulic conductance of the leaves ( $K_{\text{leaf}}$ ,  $\text{mmol MPa}^{-1} \text{ s}^{-1} \text{ m}^{-2}$ ). To calculate leaf-specific  $K$  through the major longitudinal veins,  $k_{\text{lv}}$  was normalized by LA distal to the leaf segment ( $\text{mmol MPa}^{-1} \text{ s}^{-1} \text{ m}$ ). Following the measurement of  $K_{\text{lv}}$  but before removing the leaf sections from the chamber, we emptied the water and forced air into the chamber to

ensure no air bubbles were coming from the end of the leaf section or chamber gasket, which accomplished two tasks: (1) it ensured there were no leaks in the chamber seal that would have biased our results; and (2) it ensured there were no open vessel elements in the leaf section.

Once the measurement of hydraulic conductivity through the leaf tissue was initiated, root hydraulic conductivity ( $k_{\text{root}}$ ,  $\text{mmol MPa}^{-1} \text{s}^{-1}$ ) was measured on the entire root system in a manner similar to that just described. While holding the remaining plant and pot under water, the tiller was cut ~3 cm above the soil surface using a razor blade and immediately placed in the hydraulic chamber. Measurements then proceeded using the HPFM as described above for leaves. Following this measurement, the root system was rinsed thoroughly in the water bath and then gently washed under a faucet to remove all the soil. The root system was scanned for determination of root length using WinRHIZO software (Regent Instruments Inc., Quebec City, QC, Canada).

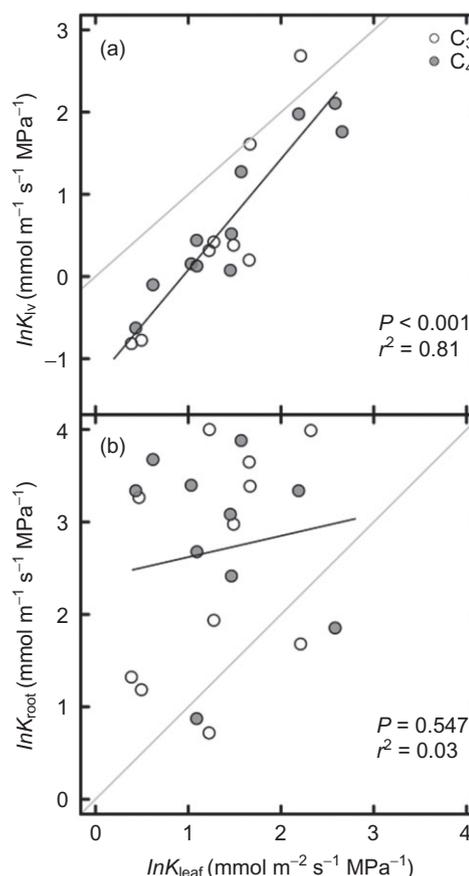
Following all measurements, the entire leaf canopy was scanned (at 600 dpi, Epson Perfection V500; Epson America Inc., Long Beach, CA, USA) and LA measured (ImageJ, Rasband 1997–2011) to normalize gas exchange and hydraulic measurements by LA. Finally, all leaf and root tissue was dried at 60 °C for ~48 h and then weighed ( $\pm 0.0001$  g, Pioneer PA214; Ohaus Corporation, Pine Brook, NJ, USA). All conductivity measurements were normalized to a water temperature of 20 °C.

## Statistics

All statistical analyses were carried out using the R open-source statistics package (R Development Core Team 2011). Two-sample *t*-tests were used for comparisons of hydraulic conductance between functional groups ( $C_3$  and  $C_4$ ) and linear regression analyses were performed using the ‘lm’ function. We calculated leaf conductance to water vapor ( $g_{\text{wv}}$ ) from  $E$  and  $D$  using ohm’s law, which includes both stomatal conductance and boundary layer conductance. Changes in  $g_{\text{wv}}$  in response to  $D$  primarily reflect changes in stomatal conductance as the air speed (and thus boundary layer conductance) in the chamber remained constant throughout the development of the  $D$  response curves. To identify species with an ‘apparent feedforward’ response to  $D$  we plotted  $g_{\text{wv}}$  by  $E$ . There is typically a negative linear relationship between these variables, but a change to a positive slope at high  $E$  indicates an apparent feedforward response (Monteith 1995, see Fig. 4b). For the species showing this response, we identified the vapor pressure deficit at which  $E$  reached its maximum value before beginning to decrease ( $D_{\text{Emax}}$ ). We calculated the stomatal sensitivity to  $D$  as  $dg_{\text{wv}}/d\ln D$  and this correlated with  $g_{\text{ref}}$ , which was calculated as  $g_{\text{wv}}$  at  $D = 1.0$  kPa.

## RESULTS

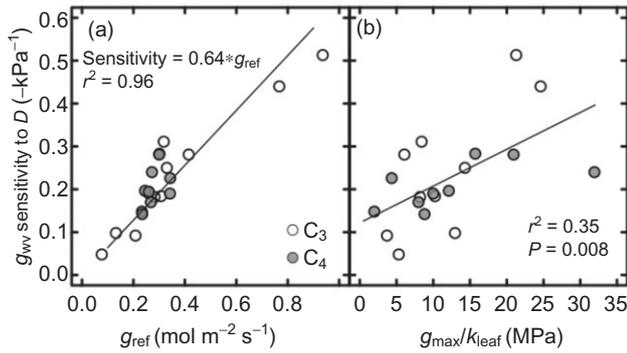
The  $C_3$  and  $C_4$  species measured in this study had very similar hydraulic properties.  $K_{\text{leaf}}$  varied from 1.06 to 14.27  $\text{mmol MPa}^{-1} \text{s}^{-1} \text{m}^{-2}$  among  $C_3$  and  $C_4$  grasses, but there was no significant difference between these two functional groups



**Figure 1.** Correlations between the leaf hydraulic conductance and (a) hydraulic conductance of the large longitudinal veins or (b) root hydraulic conductance. The natural logarithms of all values are shown, and the grey line represents a 1:1 relationship between the two variables.

for the species studied here ( $P = 0.2$ ). There was also no statistical difference between these functional groups for  $K_{\text{lv}}$  ( $P = 0.3$ ) or  $K_{\text{root}}$  ( $P = 0.9$ ).  $K_{\text{lv}}$  scaled closely with  $K_{\text{leaf}}$  (Fig. 1a) and no significant difference was present in the slopes of  $C_3$  (0.91–2.54, 95% confidence intervals) and  $C_4$  species (0.85–1.50, 95% confidence intervals).  $K_{\text{root}}$ , however, did not scale with  $K_{\text{leaf}}$  when species were pooled, nor when the functional groups were analysed independently (Fig. 1b).  $K_{\text{root}}$  was significantly larger than  $K_{\text{leaf}}$  ( $P < 0.001$ , Table 1) for all but four species (Fig. 1b).

The sensitivity of  $g_{\text{wv}}$  to  $D$  was tightly correlated with the magnitude of  $g_{\text{wv}}$  at  $D = 1.0$  kPa ( $g_{\text{ref}}$ , Fig. 2a) with a slope of 0.64 when the intercept was forced through zero. This slope was slightly larger than the predicted value of 0.6, but not significantly different (0.58–0.70, 95% confidence intervals). When the line was not forced through the origin (as in Oren *et al.* 1999), the slope decreased to 0.53, but was still not significantly different from the predicted value of 0.6 (0.42–0.63, 95% confidence intervals). Photosynthetic pathway was not a significant predictor of stomatal sensitivity to  $D$  ( $P = 0.56$ ), but stomatal sensitivity of  $C_4$  species was constrained to a smaller range of values (0.15–0.28, Fig. 2a) compared with the  $C_3$  species (0.05–0.51). Stomatal sensitivity was tightly

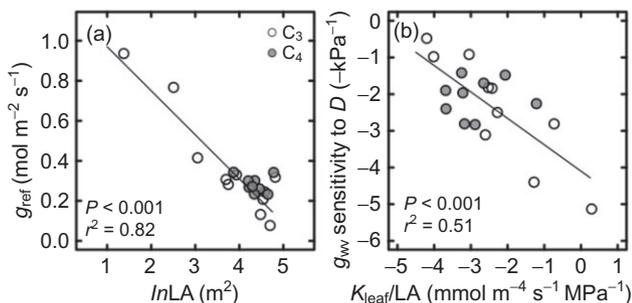


**Figure 2.** The sensitivity of stomatal conductance in response to changing  $D$  was related to the magnitude of  $g_{\text{wv}}$  at  $D = 1.0$  kPa ( $g_{\text{ref}}$ , a).  $g_{\text{ref}}$  was calculated from the regression of  $g_{\text{wv}}$  and  $\ln(D)$ . The sensitivity of  $g_{\text{wv}}$  to  $D$  was also related to the maximum stomatal conductance relative to  $K_{\text{leaf}}$  (b).

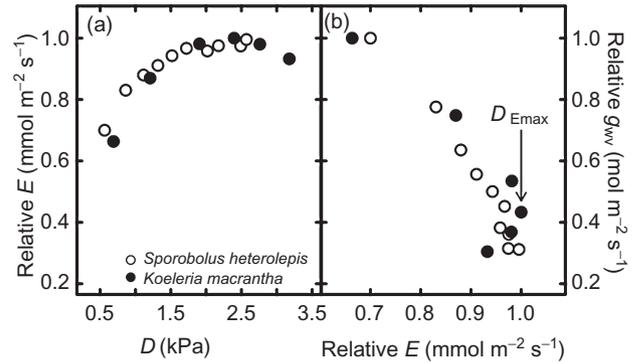
correlated with the magnitude of  $g_{\text{max}}$  relative to leaf hydraulic capacity ( $g_{\text{max}}/K_{\text{leaf}}$ , Fig. 2b). Plants with high  $g_{\text{wv}}$  relative to their ability to conduct water were more sensitive to increases in  $D$  ( $r^2 = 0.35$ ,  $P = 0.008$ ).

The variability in  $g_{\text{ref}}$  was negatively correlated with the total plant LA ( $\text{m}^2$ ) of the species measured (Fig. 3a). The relationship between LA and  $g_{\text{ref}}$  was identical between C<sub>3</sub> and C<sub>4</sub> species ( $P = 0.2$ ), although  $g_{\text{ref}}$  was again confined to a smaller range in the C<sub>4</sub> species measured (Fig. 3a). Although stomatal sensitivity was correlated with  $g_{\text{ref}}$ , the correlation between stomatal sensitivity and LA was only moderately significant ( $P = 0.06$ ). The ratio of hydraulic capacity of leaf tissue to total plant leaf area ( $K_{\text{leaf}}/\text{LA}$ ), however, was negatively correlated with stomatal sensitivity (Fig. 3b).

Two examples of  $D$  response curves are shown in Fig. 4a: an asymptotic response (open symbols) and an ‘apparent feedforward’ response (closed symbols). For both responses, there was an initial rapid increase in  $E$  as  $D$  was increased, and a corresponding decrease of  $g_{\text{wv}}$  (data not shown).  $E$  began to asymptote for many species in this study (at least for the range of  $D$  values measured), but  $E$  of some species declined in response to subsequent increases in  $D$  after reaching a maximum value, indicating an apparent feedforward



**Figure 3.** The correlation between: (a)  $g_{\text{ref}}$  and whole plant leaf area (LA), and (b) stomatal sensitivity and  $K_{\text{leaf}}/\text{LA}$ .  $K_{\text{leaf}}/\text{LA}$  is an estimate of tissue-specific hydraulic capacity relative to the total leaf area that must be supplied with water.



**Figure 4.** Vapor pressure response curves of two species to illustrate differential plant responses (a). The majority of species tested (11 of the 19) exhibited an asymptotic non-linear response of  $E$  to  $D$  (e.g. *Sporobolus heterolepis* represented with open symbols), but 8 species showed an ‘apparent feedforward’ response, where  $E$  began to decline at high  $D$  (e.g. *Koeleria macrantha* black symbols). The relationship between  $g_{\text{wv}}$  and  $E$  is shown to help identify an apparent feedforward relationship (b). Plants with this response initially had a negative linear slope between  $E$  and  $g_{\text{wv}}$ , which switched to a positive slope at high  $D$ . The vapor pressure deficit at maximum  $E$  ( $D_{\text{Emax}}$ ) was identified for the species showing an apparent feedforward response. In order for both species to be plotted on the same axis, the units of  $g_{\text{wv}}$  and  $E$  are presented as ‘relative’ to the maximum value for each variable so that all data points are in the range 0–1.

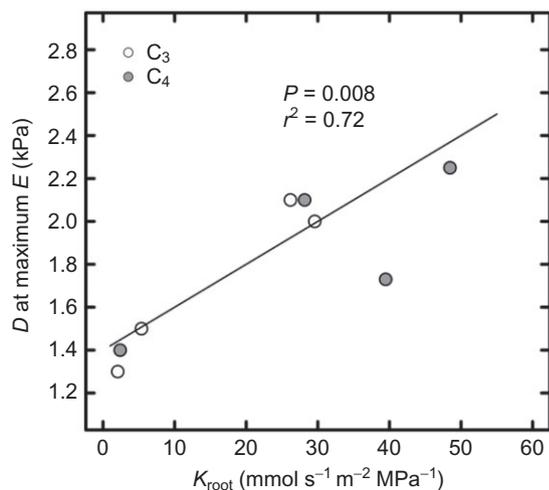
response. To identify the point where  $E$  began to decline,  $g_{\text{wv}}$  was plotted against  $E$  and the vapor pressure deficit when  $E$  reached its maximum value was selected as  $D_{\text{Emax}}$  (Fig. 4b). Species that did not show the apparent feedforward response showed a negative linear slope across the entire range of  $E$  values (Fig. 4b).

Eight of the 19 species tested exhibited an apparent feedforward response (Table 1), and the other 11 species showed an asymptotic response across the range of  $D$  values measured. Using only the species with an apparent feedforward response, we tested for correlations between  $D_{\text{Emax}}$ , stomatal sensitivity, and hydraulic conductance of leaves and roots.  $D_{\text{Emax}}$  was not correlated with stomatal sensitivity ( $P = 0.74$ ),  $g_{\text{ref}}$  ( $P = 0.54$ ) or  $K_{\text{leaf}}$  ( $P = 0.07$ ), but was significantly correlated with  $K_{\text{root}}$  ( $P = 0.008$ ; Fig. 5). Plants with low  $K_{\text{root}}$  reached the threshold where  $E$  declined at lower values of  $D$ . We did not have enough individuals to test for differences in the slopes between the two functional groups.  $K_{\text{root}}$  did not differ significantly ( $P = 0.98$ ) between species that did or did not exhibit an apparent feedforward response.

We found no correlations between  $K_{\text{root}}$  and any root morphological characteristics (root length, root mass) or tissue characteristics (root tissue density, specific root length) (Table 2). Additionally, there was no relationship between  $K_{\text{root}}$  and the root:shoot ratio of the plants studied in this experiment (Table 2).

## DISCUSSION

The sensitivity of stomatal conductance to vapor pressure deficit is a key regulator of the water use and carbon



**Figure 5.** Using only the species exhibiting an ‘apparent feedforward’ response, the vapor pressure deficit at maximum  $E$  ( $D_{E_{max}}$ ) was correlated with  $K_{root}$ . Plants with high hydraulic conductance of the root system reached  $D_{E_{max}}$  at larger vapor pressure deficits.

assimilation strategies of plants. The variability in stomatal sensitivity is related to the hydraulic conductance of leaves among eudicot leaves (Brodribb & Jordan 2008), but little data exist to relate stomatal sensitivity with leaf hydraulic conductance across a range of monocot leaves. Furthermore, many grasslands have a mix of both  $C_3$  and  $C_4$  species, which differ in their water use efficiencies. Here, we measured the hydraulic conductance of different tissues and stomatal sensitivity to  $D$  in order to compare responses among a wide range of grasses from mesic grasslands.  $C_3$  and  $C_4$  species shared common relationships between hydraulic conductance and responses to increasing  $D$ . Some of the relationships we identified are consistent with previous measurements on eudicot leaves, and new relationships were identified. Knowing these functional groups share common relationships of hydraulic capacity and stomatal sensitivity simplifies our ability to predict physiological responses and interactions of  $C_3$  and  $C_4$  species both now and in the future.

The hydraulic conductance of different tissues varied widely among the species measured in this study, but when grouped by functional type both  $C_3$  and  $C_4$  species had the same hydraulic capacity in leaves and roots. This differs from previous work that found the axial transport of water through the xylem was lower in  $C_4$  compared with  $C_3$  species (Kocacinar & Sage 2003; Kocacinar *et al.* 2008), but these studies specifically addressed the evolutionary significance of photosynthetic pathway on hydraulic conductance using contrasts between closely related  $C_3$  and  $C_4$  species. The focus of our study was to investigate differences in hydraulic conductance and stomatal sensitivity among  $C_3$  and  $C_4$  species common to mesic and semi-arid plant communities, and we did not control for phylogenetic relatedness. Within this context, we did not find a functional difference in hydraulic capacity among the selected  $C_3$  and  $C_4$  species.

We also found that the relationship between stomatal sensitivity and  $g_{ref}$  was identical for the  $C_3$  and  $C_4$  grasses

measured, and supported the empirical model described by Oren *et al.* (1999). The slope of the line between stomatal sensitivity and  $\ln(D)$  was not statistically different from the predicted slope of 0.6 when both  $C_3$  and  $C_4$  species were included in the regression. Because  $C_4$  species typically have lower  $g_{wv}$  and higher water use efficiency (Sage & Monson 1999), they would be expected to occupy a different region of the  $g_{ref}$  versus stomatal sensitivity spectrum compared with  $C_3$  species, but there is no reason to expect them to have a slope different from 0.6. The  $C_4$  species measured in this study had  $g_{ref}$  values that were confined to a smaller range than the  $C_3$  species (Fig. 3a), but mean  $g_{ref}$  was not significantly different. Previous work on stomatal responses of  $C_3$  and  $C_4$  species found that stomatal sensitivity was related to stomatal conductance (Morison & Gifford 1983), but a slope was not calculated. Within an individual  $C_4$  species (*Bromus japonicus*), the slope of  $g_{ref}$  versus stomatal sensitivity was 0.37 when  $g_{ref}$  was modified by changing atmospheric  $CO_2$  concentration (Maherali *et al.* 2003). This suggests that variability among species is likely, but across a wide range of  $C_3$  and  $C_4$  grasses, stomatal sensitivity scales closely with  $g_{ref}$ .

The response of plants to changing  $D$  was tightly linked with the hydraulic efficiency of leaves relative to water use and plant size. Stomatal sensitivity correlated with maximum  $g_{wv}$  relative to  $K_{leaf}$  (Fig. 3b), which has been shown for  $C_3$  eudicot leaves (Brodribb & Jordan 2008) but not for a range of  $C_4$  species.  $g_{max}/K_{leaf}$  is a tissue-specific measurement of the hydraulic capacity relative to the rate of water use, but stomatal conductance can be affected by plant size with no apparent change in  $K_{leaf}$ , as individuals with large leaf canopies tend to have lower stomatal conductance (Meinzer & Grantz 1990; Reich *et al.* 1993). We found a similar relationship between  $g_{ref}$  and total plant LA, which may explain why  $K_{leaf}/LA$  correlated better with stomatal sensitivity (Fig. 4b) than  $g_{max}/K_{leaf}$ . Because stomata respond to changes in water status of the leaf (Buckley 2005), it seems reasonable that plants with high tissue-specific hydraulic capacity relative to their total leaf canopy would be less sensitive to changes in  $D$ . The relationships observed here reinforce the tight link between hydraulic architecture and stomatal responses to abiotic factors, and reveal that  $C_3$  and  $C_4$  species share a common slope between hydraulic conductance and stomatal sensitivity to  $D$  despite fundamental differences in photosynthetic pathways.

The decline in  $E$  at high  $D$  was originally referred to as a ‘feedforward response’ (Farquhar 1978), but later modified

**Table 2.** Results from multiple linear regression analyses with  $K_{root}$  and root system characteristics. Significance was determined at the  $P < 0.05$  level.  $K_{root}$  was not correlated with any characteristics of the root system measured in this study

Dependent variable	Independent variable	P-value
$K_{root}$	Root length	0.56
	Specific root length	0.83
	Root density	0.78
	Root:shoot	0.91

to an 'apparent feedforward' response (Monteith 1995) because it did not always meet the requirements of a true feedforward mechanism (Franks, Cowan & Farquhar 1997). The decline in  $E$  at high  $D$  does not occur in all species (Monteith 1995; Franks *et al.* 1997), but could result from several mechanisms (reviewed in Buckley 2005) including changes in  $K_{\text{leaf}}$  (Dewar 2002; Buckley 2005). Reductions in  $K_{\text{leaf}}$  could result from xylem cavitation (Marenco *et al.* 2006; Blackman, Brodribb & Jordan 2009) or changes in conductance of tissue outside the xylem (Kim & Steudle 2007) as a result of declining water potential in the xylem or water status of leaf cells. In species that exhibit an apparent feedforward response, maintenance of leaf water status above the threshold when  $K_{\text{leaf}}$  declines would prevent the occurrence of the apparent feedforward response. Here, we show that plants with high  $K_{\text{root}}$  did not reach the point where  $E$  declined until higher levels of  $D$ .  $K_{\text{root}}$ , however, did not explain the presence or absence of an apparent feedforward response, as  $K_{\text{root}}$  did not differ between these groups of species. The effect of  $K_{\text{root}}$  on  $D_{\text{Emax}}$  is consistent with an apparent feedforward response, as high  $K_{\text{root}}$  would minimize the water potential gradient from the soil to the aboveground plant tissue allowing leaves to maintain  $E$  higher levels of  $D$ .  $K_{\text{root}}$  may be especially important for the grass growth form, as the hydraulic pathway is comprised mainly of root and leaf conductance. For woody species with a substantial portion of the hydraulic pathway in the stem, both root and stem hydraulic conductance may be important in determining the point at which  $E$  begins to decline in species exhibiting an apparent feedforward response.

The apparent feedforward response tends to be rare compared with the expected asymptotic response of  $E$  to  $D$ , but here we found that 8 of the 19 species tested displayed an 'apparent feed-forward response' (Table 1), which is a larger proportion than is typically reported for other multi-species comparisons (Monteith 1995; Franks *et al.* 1997). It is unclear why such a large portion of our species exhibited this response, but previous studies included both woody and herbaceous species, and few grass species. The data used in the analyses of Monteith (1995) and Franks *et al.* (1997) covered a wide range of species and methods, sometimes exposing the whole plant to changes in  $D$  and other times only manipulating the air of a leaf section. Our ability to generalize apparent feedforward responses among species will improve with the use of consistent methods over a larger range of species.

Previous research has shown the significance of hydraulic conductance of different plant tissues as regulators of seasonal changes in the response of stomata to  $D$  (Domec *et al.* 2009). Here, we show the importance of this approach on shorter timescales, as plants respond to diurnal changes in  $D$ . The response of  $g_{\text{wv}}$  to  $D$  is linked to the hydraulic capacity of the leaves, but  $K_{\text{root}}$  influences when plants reach maximum rates of  $E$ . In agricultural systems, the control of water loss in crop plants affects their water use strategy and can affect the seasonal water budget; conservation of water early in the season can leave water for growth during drier parts of the season (Sinclair *et al.* 2007). In natural ecosystems, high  $K_{\text{root}}$  (independent of other root characteristics, Table 2) may

provide a competitive advantage for water uptake in grasses, as these plants would avoid the large midday depression in carbon assimilation that often results from high vapor pressure deficit. Perhaps most importantly, these relationships remain constant across C<sub>3</sub> and C<sub>4</sub> species, simplifying the task of predicting how these two functional groups vary across environments and how they will respond to variability in future climates.

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