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Partitioning hydraulic resistance in *Sorghum bicolor* leaves reveals unique correlations with stomatal conductance during drought

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Abstract. The hydraulic architecture of leaves represents the final path along which liquid water travels through the plant and comprises a significant resistance for water movement, especially for grasses. We partitioned leaf hydraulic resistance of six genotypes of *Sorghum bicolor* L. (Moench) into leaf specific hydraulic resistance within the large longitudinal veins (r^*_{LV}) and outside the large veins (r^*_{OLV}), and correlated these resistances with the response of stomatal conductance (g_s) and photosynthesis (A) to drought. Under well-watered conditions, g_s was tightly correlated with r^*_{OLV} ($r^2 = 0.95$), but as soil moisture decreased, g_s was more closely correlated with r^*_{LV} ($r^2 = 0.97$). These results suggest that r^*_{OLV} limits maximum rates of gas exchange, but the ability to efficiently move water long distances (low r^*_{LV}) becomes more important for the maintenance of cell turgor and gas exchange as soil moisture declines. Hydraulic resistance through the leaf was negatively correlated with evapotranspiration ($P < 0.001$) resulting in more conservative water use in genotypes with large leaf resistance. These results illustrate the functional significance of leaf resistance partitioning to declining soil moisture in a broadly-adapted cereal species.

Additional keywords: drought tolerance, leaf hydraulic conductance, stomatal conductance, plant anatomy.

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Introduction

Leaves provide the direct link between water loss to the atmosphere and carbon gain by plants, but the dynamic resistance to water movement through leaves responds to a range of environmental conditions. The hydraulic resistance of the whole leaf (r^*_{Leaf}) is tightly correlated with maximum rates of gas exchange across a broad range of species (Brodribb *et al*. 2007), but also changes temporally in response to leaf hydration status (Brodribb and Holbrook 2003; Kim and Steudle 2007; Heinen *et al*. 2009) and incident light (Tyree *et al*. 2005; Scoffoni *et al*. 2008). These temporal responses of r^*_{Leaf} can be mediated through cavitation events within the leaf xylem (Nardini *et al*. 2003; Trifilò *et al*. 2003; Johnson *et al*. 2012), cell collapse of the xylem elements (Cochard *et al*. 2004*a*; Brodribb and Holbrook 2005; Blackman *et al*. 2010) or changes in aquaporin regulation after water leaves the vasculature (Cochard *et al*. 2007). Taken together, these results illustrate the varying function of individual components of r^*_{Leaf} on the rates of leaf gas exchange.

Leaf resistance in dicot leaves has been previously partitioned into xylary and extra-xylary components that range widely across species; the relative proportion of these two resistances in leaves range from 26 to 80% of total leaf resistance (Martre *et al*. 2001; Zwieniecki *et al*. 2002; Cochard *et al*. 2004*b*; Sack *et al*. 2004; Nardini and Salleo 2005). Across a range of species and environmental conditions, both xylary and extra-xylary resistance have been linked to maximum rates of gas exchange (Sack *et al*. 2002; Sack and Frole 2006; Brodribb *et al*. 2007), but the role of these two leaf resistances for regulating stomatal responses to decreasing soil moisture has not been investigated. Furthermore, this type of hydraulic partitioning has not been investigated in grasses, in which the majority of the aboveground pathway for water movement occurs within leaves (either the leaf sheath or leaf blade) of most species. Detangling leaf resistances to water movement is vital to a better understanding of hydraulics in this growth form. Long-distance axial transport of water along monocot leaves occurs within the large longitudinal veins, and the local distribution of water to cells occurs via small longitudinal and transverse veins (Altus and Canny 1985; Altus *et al*. 1985). To partition leaf hydraulic resistance in monocots into the two most significant functional components, we classified resistance to long-distance water movement within the large longitudinal veins (r^*_{LV}) and resistance outside the large longitudinal veins (r^*_{OLV}) as water is distributed locally through small longitudinal and transverse veins and leaf tissue outside the vascular bundles.

When component resistances act in series within a network, the largest resistance should be most limiting to the movement of water (Meinzer 2002). When fully hydrated, the type of leaf hydraulic tissue with the greatest resistance varies between species (Sack *et al*. 2005), but for many of the species investigated the major resistance to water movement is outside the xylem (Cochard *et al*. 2004*b*; Gascó *et al*. 2004; Nardini and Salleo 2005; Mott 2007). Coupled measurements of partitioned leaf resistances and g_s are rare, and as such, it is unknown whether maximum g_s correlates with r^*_{OLV} for previously measured plant species, as theory would suggest. A strong correlation has been shown between r^*_{Leaf} and the distance from vascular bundle to stomata (D_m) across a broad range of species (Brodribb *et al*. 2007) and within grass leaves (Kodama *et al*. 2011; Ocheltree *et al*. 2012). D_m is an indirect measure of extra-xylary resistance but it is not clear whether D_m directly affects hydraulic resistance or whether it is indirectly related to another component of leaf hydraulic resistance. Therefore, further investigation is required to relate leaf hydraulic resistance to the control of maximum g_s in grasses.

As soil moisture declines and becomes limiting to plant growth, g_s responds to changes in water potential in the immediate vicinity of the stomata (Buckley 2005) or internal water vapour concentration (Peak and Mott 2011) to maintain hydration of plants cells. Plants often maintain near-maximum rates of g_s until leaf water potential reaches a threshold (Girma and Krieg 1992; Tardieu and Simonneau 1998), at which point g_s is reduced to maintain leaf water potential above the point when cavitation would cause catastrophic loss of hydraulic function while plants are transpiring (Sperry *et al*. 1998). Plants with low hydraulic resistance to long-distance transport should be able to minimise the water potential gradient from the soil matrix to leaf surface and maintain relatively high g_s (Tyree and Zimmerman 2002), which has been identified among woody species (Meinzer and Grantz 1990; Meinzer *et al*. 1995; Hubbard *et al*. 2001) and across developmental stages (Saliendra *et al*. 1995) when soil moisture was limited. The correlation between xylem resistance within leaves (rather than woody tissue) and g_s has not been tested in grass species.

If the tissue with the greatest resistance controls the rate of water movement through a leaf, then maximum stomatal conductance should correlate with that tissue, but the role of hydraulic resistance as a regulator of leaf gas exchange in grasses is under-studied. The goal of this study was to assess the relationship between stomatal conductance and the resistance of different tissues within leaves of six different genotypes of *Sorghum bicolor* that vary in drought tolerance. We hypothesised that: (i) maximum rates of gas exchange will be negatively correlated with r^*_{OLV} under well-watered conditions, but (ii) as water becomes limiting to plant growth, rates of gas exchange will be negatively correlated with r^*_{LV} to minimise the water potential gradient from soil to leaf. As such, genotypes with low resistance within the xylem will be able to maintain higher rates of gas exchange when soil moisture is limiting. Furthermore, we hypothesised that (iii) genotypes with greater r^*_{OLV} will exhibit a more 'water-conservative' growth strategy, having lower initial rates of gas exchange but maintaining these rates further into a drought treatment.

Materials and methods

Plant material

Six genotypes of *Sorghum bicolor* L. (Moench) were selected for this study covering a range of drought tolerance: SC1019, SC1205, SC15, Tx7078, BTx623 and B35. The study was divided into two sections: (1) the first, in which we measured leaf hydraulic resistance for each genotype on one group of plants, and (2) the second in which leaf gas exchange was measured in response to drought on a second group of plants. In both studies, two seeds were planted in 20 L pots filled with potting soil (Metro-Mix 360, Sun-Gro Horticulture Canada Ltd, Vancouver, BC, Canada) and were thinned to one individual per pot after germination. Plants were supplied with a controlled-release fertiliser (Scotts-Sierra Horticultural Products Co., Marysville, OH, USA) and watered daily. When germinating plants for the determination of hydraulic resistance, two replicates for each genotype were planted each week so that plants would develop in stages to allow sampling of replicates at the same developmental stage; hydraulic resistance was measured on the 5th or 6th leaf of each plant. Plants were grown in a greenhouse where maximum daily PAR was between 800 and1200 $\mu mol\ m^{-2}\ s^{-1}$ and daily temperatures were maintained between 22 and 26°C. For the drought response experiment, eight replicates of each genotype were planted at the same time and grown under well-watered conditions until the 5th leaf had fully expanded, then water was withheld to initiate the drought treatment.

Hydraulic resistance

Hydraulic resistance was measured using a hydrostatic gradient to force water through the sample (Sperry *et al*. 1988) with a custom chamber designed for large grass leaves. The chamber was constructed to accommodate the large leaves of sorghum and allowed us to quantify both axial resistance within the large longitudinal veins (r^*_{LV}), and the resistance to water movement locally within the leaf outside the large veins (r^*_{OLV}). The hydraulic chamber consisted of two compartments: the first was a small 'pressurised compartment' where the basal cut section of a leaf was affixed and water forced into the leaf, and the second was a 'collection compartment' where water flowing through the leaf was collected and diverted to a balance through 3.2 mm internal diameter tubing (Bev-a-Line, Thermoplastic Processes, Georgetown, DE, USA). De-ionised and degassed water was forced through the leaf at ~20 kPa and was collected on a balance (±0.0001 g, Pioneer PA214, Ohaus Corporation, Pine Brook, NJ, USA) connected to a datalogger (CR1000, Campbell Scientific Inc., Logan, UT, USA) to monitor the rate of water flow through the system at 5 s intervals. Here we considered 20 kPa likely to be insufficient to displace embolisms, and estimates of resistance likely to include any dysfunction produced by cavitation. We minimised the impact of cavitation on our measurements, however, by ensuring the soil was at pot-holding capacity and the plants were kept in a darkened chamber for ~12 h before the time of the hydraulic measurements. Pressure transducers (Model 68075, Cole-Parmer Instrument Co., Vernon Hills, IL, USA) were placed on both inlet and outlet tubing to measure the pressure gradient across the leaf and a thermocouple measured water temperature

in the collection compartment. Once the flow through the leaf reached steady-state (normally ~15–30 min), 5 min of data was recorded by the datalogger. The background flow was determined by equilibrating the pressure on each side of the leaf so that no pressure gradient existed across the leaf segment and the flow was measured for 5 min (Sperry and Hacke 2002). This was performed before and after each measurement, and the average of the two measurements was subtracted from the pressurised flow and the result multiplied by the pressure gradient to yield hydraulic resistance (MPa s mmol^{-1}) not yet normalised by leaf area or length. The system used to measure hydraulic resistance was cleaned with a mild bleach solution and then rinsed repeatedly with de-ionised water each morning before measurements were initiated.

The most recently matured leaf (5th or 6th leaf) was cut from the plant under water and was placed in the hydraulic chamber so the longitudinal midpoint of the leaf was centred in the 'collection compartment' (as described above). The basal portion of the leaf was re-cut with a razorblade and ~2 cm of the leaf was immediately sealed in the 'pressurised compartment' of the chamber. For determination of leaf hydraulic resistance (r_{Leaf}), ~50 cm^2 of leaf area was enclosed in the 'collection compartment' that was connected to a reservoir on the balance. The leaf area in the 'collection compartment' and the remainder of the leaf distal to the chamber were kept submerged in the water bath during the measurement. In this way, water flowed into the basal end of the leaf and travelled through both xylary and extra-xylary pathways to the collection chamber allowing quantification of total leaf hydraulic resistance, not yet normalised by leaf area (r_{Leaf}, MPa s mmol^{-1}). The collection compartment was illuminated at 2000 µmol m^{-2} s^{-1} PAR using a fibre-optic light source (FL-150, Meiji Techno Co., Saitama, Japan), as r_{Leaf} of the genotypes studied were sensitive to incident light (data not shown). Incident PAR was estimated using a quantum sensor (Li-190, Li-Cor Inc., Lincoln, NE, USA) sealed in an acrylic chamber (made of acrylic identical to the hydraulic chamber) submerged under water and placed at the same level as the leaf.

Following the measurement of r_{Leaf}, the collection compartment was opened and a transverse cut was made across the leaf to remove most of the leaf area and expose the xylem for determination of axial hydraulic resistance. A fresh transverse cut was also made on the basal end of the leaf and then both chambers were re-sealed, leaving a leaf segment ~10 cm long for determination of r_{LV}. These cuts removed the resistance of the smaller longitudinal veins, transverse veins and extra-xylary tissue from the measurement. Flow rate through the xylem proceeded as previously described. To ensure there were no vessel elements longer than our leaf section, we removed water from the 'pressurised chamber' and forced air through the leaf and made sure no air bubbles were passing through the leaf section. Both r_{LV} and r_{Leaf} represent raw resistance values and are not normalised by leaf area or length. Following hydraulic resistance measurements, the leaf area of the entire leaf was measured so that r_{Leaf}, r_{LV} and r_{OLV} could be normalised by leaf area.

We assumed r_{Leaf} consisted of two resistances in series, r_{LV} (MPa s mmol^{-1}) and r_{OLV} (MPa s mmol^{-1}), which allowed r_{OLV} to be calculated based on direct measurements of r_{Leaf} and r_{LV} (Eqn 1) using an electrical analogue approach. Thus, r_{Leaf} and r_{OLV} were normalised by the amount of leaf area (LA, m^2) inside the collection chamber (Eqns 2 and 3 respectively) and r_{LV} was normalised by both the length of the leaf section measured (l_{seg}, m) and the leaf area distal (LA, m^2) to this section (Eqn 4, MPa mmol^{-1} s m):

$$r_{OLV} = r_{Leaf} - r_{LV}, \quad (1)$$

$$r^*_{Leaf} = \frac{r_{Leaf}}{LA}, \quad (2)$$

$$r^*_{OLV} = \frac{r_{OLV}}{LA}, \quad (3)$$

$$r^*_{LV} = \frac{r_{LV}}{LA * l_{seg}}. \quad (4)$$

Symbols including '*' represent data that has been normalised by leaf area. In the case of r^*_{LV}, it has also been normalised by segment length. When normalised in this manner, r^*_{LV} remains constant along leaf blades in a range of grass species (Ocheltree *et al*. 2012).

Anatomy

A small section of each leaf, taken from the longitudinal centre, was placed in a vial of 10% formalin/5% acetic acid/50% ethanol/35% DI H_2O, vacuum infiltrated overnight (Ruzin 1999) and stored at 4°C until the tissue could be embedded with parafilm and stained with toluidine blue for microscopic analysis (Kansas State University Histology Laboratory, College of Veterinary Medicine, Manhattan, KS, USA). Digital images were taken of each leaf, from the midrib to the outer edge of one-half of the leaf (Leica DFC 290; Leica Microsystems GmbH, Wetzlar, Germany) at ×20 magnification using a light microscope (Leica DM1000; Leica Microsystems GmbH). The wide leaves of this species required that multiple images be taken to cover this entire region. Large longitudinal veins were identified by the presence of large meta-xylem vessel elements and the remaining veins were categorised as other smaller veins. Both of these vein classes were counted in the digital images, and used to calculate vein density. We assumed that no veins terminated within 1 mm of the leaf section used for microscope analysis and calculated vein density (mm mm^{-1}) by multiplying the number of veins by 1 mm to get the length of each vein size class and divided this by leaf area. The diffusional distance from vascular bundle to the nearest stomata (D_m) was estimated as described by Ocheltree *et al*. (2012) by calculating the hypotenuse between the vertical and horizontal distances from the edge of the vascular bundle to stomate.

Gas exchange

Gas-exchange measurements were made on the second set of plants during the drought treatment. After plants had five mature leaves, water was completely withheld to simulate a drought. Gas-exchange rates were measured (Li-6400 Li-Cor Inc.) on the centre of the leaf, as rates of gas exchange vary with leaf position in this species (TW Ocheltree, unpubl. data). All measurements were made between 1100 and 1500 hours with clear skies to maximise light availability in the greenhouse and best estimate

maximum rates of gas exchange for that day. Measurements were repeated every 3–4 days to capture the response of leaf-level gas-exchange rates to drying soils. Prior to gas-exchange measurements, the mass of each potted plant was measured (±50 g, model CTB-600, Citizen Scales, Mumbai, India) so we could determine soil water content and rates of evapotranspiration (ET). Gas-exchange measurements were continued until $g_s < 0.05\ mol\ m^{-2}\ s^{-1}$ and the plants were severely wilted, which ranged from 21 to 30 days for the individuals and genotypes measured.

Biomass production

After the drought experiment was complete, plants were harvested and divided into above and belowground components. Soil was washed from the roots with a low-pressure spray nozzle and the dislodged soil was collected in a large bucket to sit overnight, allowing the soil to separate gravimetrically. After ~24 h the majority of the water was siphoned off the top and the soil was dried at 60°C in a shallow pan. Several soil samples were weighed every day during the drying process and it was determined that the soils were >99% dry by the end of 7 days; all subsequent soil samples were dried for 7 days and then weighed (±0.01 g, Pioneer PA3102, Ohaus Corporation, Pine Brook, NJ, USA). Above- and belowground plant biomass was dried at 60°C for 48 h and then weighed (±0.0001 g, Pioneer PA214, Ohaus Corporation). Soil water content (*SWC*) was determined on a mass basis from the measurements of the potted plants during the experiment and the dried soil:

$$SWC = \frac{m_{wet} - m_{dry}}{m_{dry}}, \quad (5)$$

where m_{wet} (kg) is the mass of the potted plant during the experiment and m_{dry} (kg) is the mass of the dry soil. The mass of the plant was included in the measurement of m_{wet}, so changes in *SWC* based on our measurements reflect changes in both soil moisture content and plant biomass. The final plant dry mass was <2% of the total soil dry mass and so changes in *SWC* based on Eqn 5 were mainly driven by changes in soil moisture rather than changes in plant biomass. As previously mentioned, the soil used was a horticultural potting soil, and so *SWC* values presented are much higher than would be expected in field conditions.

Leaf water potential

Leaf water potential (Ψ_{Leaf}) was measured throughout the drought experiment using thermocouple psychrometers (70 series, JRD Merril, Logan, UT, USA) attached to a CR7 datalogger (Campbell Scientific Ltd, Logan, Utah, USA). Leaf water potential measurements were made immediately following gas-exchange measurements by removing a 5 mm leaf disc using a disposable biopsy punch (Integra Miltex, York, PA, USA). The leaf disc was immediately placed in the psychrometer chamber, which was then placed in a 25°C water bath to equilibrate for 1 h before determining Ψ_{Leaf}. The measurement of wet-bulb depression was made according to Comstock (2000) and converted to Ψ_{Leaf} based on the calibration of NaCl standards measured in the same manner as leaf discs.

Statistical analyses

All statistical analyses were performed using the R open-source software package (R Development Core Team 2011). To identify differences in leaf resistances between genotypes, multiple pairwise comparisons were made using the 'Holm' correction for multiple comparisons and significant differences were determined at the $P < 0.05$ level. Standard linear regression was used to identify correlations between leaf resistances, g_s and leaf anatomy. When the relationship between g_s and *SWC* was plotted, three different stages were apparent; constant maximum rates of g_s when soil moisture was high, a linear decrease in g_s as soil moisture decreased, and then a steeper decline in g_s when soil moisture was extremely low. We identified the breakpoints between these three stages using a piecewise regression ('segmented' package in R), and averaged rates of gas exchange within each stage of the $SWC \times g_s$ relationship. A Pearson's correlation was calculated between the leaf resistances measured and leaf anatomy and considered significant at the $P < 0.05$ level. The rate that *SWC* decreased was determined by calculating the slope of the line between *SWC* and sampling date. In order to test for correlations between leaf resistances and ET but account for the effect of increasing plant size on ET, we used a mixed-effects model with 'sampling date' as a random effect in the model.

Results

Hydraulic resistance

We found significant differences among the six genotypes of *Sorghum bicolor* in both r^*_{LV} and r^*_{OLV} (Fig. 1). Results show that r^*_{LV} ranged from 0.21 to 0.43 MPa mmol^{-1} s m across the six genotypes (Fig. 1*a*), which highlights the potential variability within a single species. Further, r^*_{OLV} ranged from 0.020 to 0.036 MPa mmol^{-1} s m^2 (Fig. 1*b*), but few significant differences among genotypes were present owing to the large variability within each genotype, which incorporates the uncertainty associated with measurements of both r_{Leaf} and r_{LV} (Eqn 1). The proportion of r_{Leaf} in the large longitudinal veins ranged between 0.10 and 0.25 for all genotypes (Fig. 1*c*), which means that 75–90% of leaf resistance was outside the large longitudinal veins for the genotypes studied.

Gas exchange

Stomatal conductance correlated with different components of leaf hydraulic resistance as soil moisture declined (Fig. 2). Using data from all the genotypes, we identified three different phases in the relationship between *SWC* and g_s (data not shown); a steady maximum rate of g_s when soil moisture was high (*SWC* >3.2 kg/kg), a linear decline in g_s as soil moisture became limiting to g_s ($1.8 < SWC < 3.2$ kg kg^{-1}), followed by a more rapid decline in g_s when soil moisture was extremely low (*SWC* <1.8 kg kg^{-1}). When *SWC* was high (>3.2 kg kg^{-1}), g_s was inversely related to r^*_{OLV} (Fig. 2*a*, $P < 0.001$) but was not significantly correlated with r^*_{LV} (Fig. 2*b*, $P = 0.42$). As *SWC* decreased to the range of 1.8–3.2 kg kg^{-1}, the g_s of most genotypes declined and was negatively correlated with r^*_{LV}

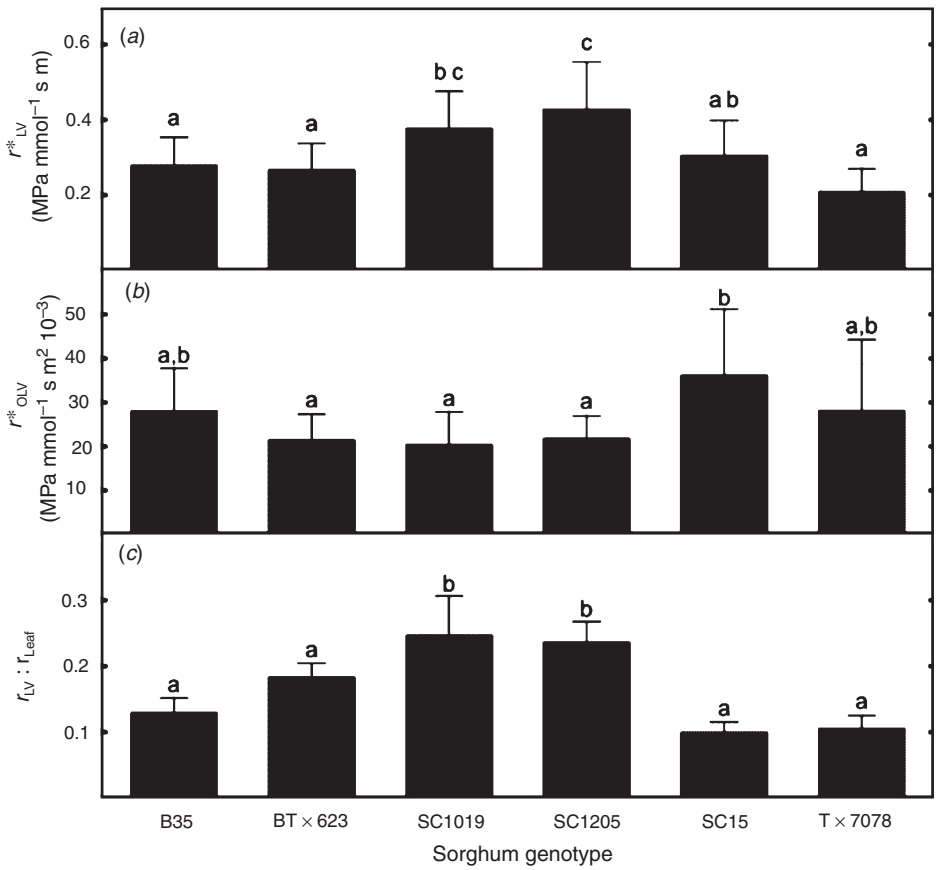

Fig. 1. Hydraulic resistance of different leaf water pathways for six genotypes of *Sorghum bicolor*: eight replicates of each genotype were measured with results expressed as means ±s.e. The resistance in the large longitudinal veins (*a*, r^*_{LV}) and outside the large longitudinal veins (*b*, r^*_{OLV}) are presented normalised by leaf area. The proportion of leaf resistance in the large longitudinal veins (*c*) was calculated based on direct measurements of both r_{Leaf} and r_{LV}, before being normalised by leaf area. Lowercase letters indicate significant differences of pairwise comparisons using a 'Holm' correction for multiple comparisons at the $P < 0.05$ significance level.

(Fig. 2*d*, $P < 0.001$) but not r^*_{OLV} (Fig. 2*c*, $P = 0.51$). When soil moisture dropped below 1.8 kg kg^{-1}, there was no correlation between either r^*_{OLV} or r^*_{LV} (Fig. 2*e*, *f*).

Evapotranspiration (ET) increased throughout the experiment as the demand for water increased with the growing plant canopy (data not shown). To investigate the correlation between r^*_{Leaf} and ET, we first accounted for the increasing ET by including 'sampling date' as a random effect in a mixed-effects model. After accounting for the temporal increase in ET there was a negative correlation between r^*_{Leaf} and ET (Table 1), and genotypes with greater leaf resistance used water more conservatively (ET g day^{-1} = $r_{Leaf} \times 46.6 + 87.0$). A 50% increase in r^*_{Leaf} would only result in a 9% increase in water savings per day, but this amount would be important over the course of a growing season. Data showed r^*_{OLV} was a significant predictor of ET ($P = 0.02$), but the integration of r^*_{OLV} and r^*_{LV} in r^*_{Leaf} provided a better fit for the mixed-effects model explaining ET (Table 1). For example, genotype SC15 had the largest r^*_{Leaf} but had an ET 20% less than the genotype with the lowest leaf resistance (BTx623) despite accumulating more aboveground biomass during the experiment (Table 2).

Soil moisture also decreased more rapidly in plants with low r^*_{Leaf} (Fig. 3). A linear relationship was moderately significant ($P = 0.07$), but the data suggested a non-linear relationship. In order to fit a curve to the data, both r^*_{Leaf} and the rate of *SWC* depletion were scaled between 0 and 1. When scaled in this manner, an exponential decay function fit the data closely (Fig. 3, inset). This supports the ET data, described above, suggesting that genotypes with greater r^*_{Leaf} used water more conservatively.

Leaf water potential

All six genotypes studied had similar Ψ_{Leaf} values throughout the experiment (Table 2), and the average Ψ_{Leaf} was –1.3 MPa across all genotypes and sampling periods. There were no significant difference between genotypes in Ψ_{Leaf} for any of the sampling periods, and there was no significant difference in Ψ_{Leaf} between sampling periods within any genotype. Ψ_{Leaf} was not determined on the last sampling date when $g_s \leq 0.05$ mol m^{-2} s^{-1}. These results suggest that all genotypes regulated g_s to help maintain Ψ_{Leaf} at similar values across a range of soil water availabilities.

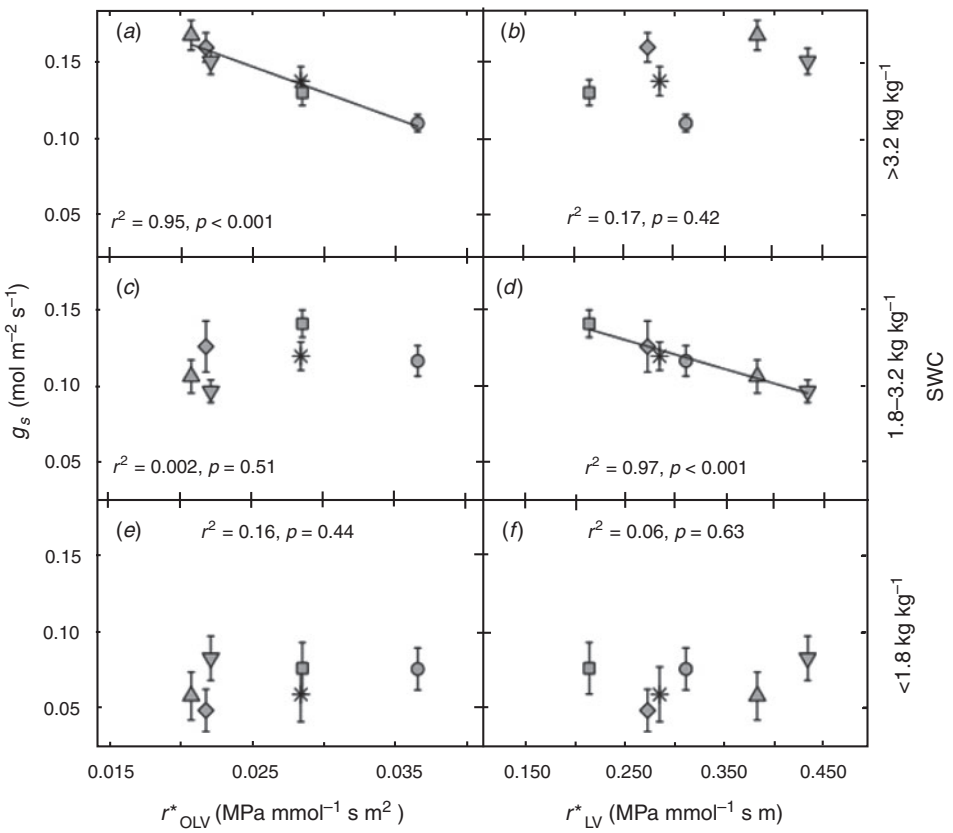

Fig. 2. Relationships between *Sorghum bicolor* stomatal conductance (g_s) and hydraulic resistance in the large longitudinal veins (r^*_{LV}) and outside the large longitudinal veins (r^*_{OLV}). Hydraulic resistance measurements were made on fully-hydrated leaves and plotted against g_s at different levels of soil moisture: (*a*, *c*, *e*) show r^*_{OLV} values (*b*, *d*, *f*) show r^*_{LV}. The level of soil moisture is shown on the right hand side of the figure for each pair of panels. Data are expressed as means ±s.e. Symbols represent the genotypes tested; BTx623 (✳), B35 (◇), SC1019 (△), SC1205 (▽), SC15 (○), TX7078 (□).

Table 1. Correlations between leaf hydraulic resistance, leaf anatomy and evapotranspiration rate (ET)

Pearson correlation coefficients between the different components of leaf resistance and anatomical features are shown, significant correlations are indicated: **, $P < 0.01$. A mixed-effects model was used to investigate the correlations between r^*_{Leaf} and ET, 'sampling date' was included as a random effect to account for changes in ET associated with increases in biomass. The Aikiki's Information Criteria (AIC) is also shown to compare models

	Correlation coefficients				Mixed-effects model to explain ET rates	
Resistance	Large longitudinal vein density	Small longitudinal vein density	Total longitudinal vein density	D_m	*P*-value	AIC
r^*_{Leaf}	–0.14	–0.37	–0.31	0.03	<0.001**	–160.5
r^*_{LV}	–0.94**	–0.26	–0.54	0.77	0.76	–142.6
r^*_{OLV}	0.43	–0.26	–0.09	–0.43	0.02	–147.2

Biomass production

Few differences in final biomass production were found between genotypes when grown under drought (Table 2), but the genotype with the greatest resistance in the leaves (SC15), did have the highest aboveground biomass (Table 2). Aboveground biomass in the control treatment showed greater variability but was not correlated with either r^*_{LV} or r^*_{OLV} (data not shown). Root biomass in the drought experiment varied by genotype (Table 2) and was correlated with r^*_{Leaf} (Fig. 4). Plants with greater resistance in their leaves (and lower g_s) had less root biomass (Fig. 4*a*) and smaller root : shoot ratios (Fig. 4*b*). Root biomass and root : shoot ratio were still highly correlated with r^*_{Leaf} when a Spearman's rank correlation was calculated ($\rho = -0.82$ and –0.77 respectively), suggesting that the genotype with high r^*_{Leaf} was not driving the correlations.

Table 2. Mean (s.e.) values for aboveground biomass, root biomass, and Ψ_{Leaf} for the six genotypes of *Sorghum bicolor*
Aboveground biomass is shown for a 'control' group that was grown under well-watered conditions, and the 'drought' treatment group. Root biomass and Ψ_{Leaf} are shown only for the 'drought' treatment group. The *P*-value for the sampling date in a mixed-effects model is shown for each genotype, indicating that there was no change in Ψ_{Leaf} throughout the drought experiment. Lowercase letters indicate significant differences across genotypes for pairwise comparisons using a 'Holm' correction for multiple comparisons at the $P < 0.05$ significance level

Genotype	Aboveground biomass, control (g)	Aboveground biomass, drought (g)	Root biomass drought (g)	Ψ_{leaf}, drought (MPa)	*P*-value of sampling date
BTx623	78.4 (5.0)a	29.9 (1.6)a,b	12.2 (0.6)a	–1.3 (0.07)a	0.37
B35	117.4 (12.0)b	28.8 (0.9)a	10.9 (0.9)a,b	–1.4 (0.08)a	0.20
SC1019	182.1 (13.3)c	30.5 (1.5)a,b	11.8 (0.8)a	–1.1 (0.08)a	0.10
SC1205	119.6 (10.1)b	29.7 (1.3)a,b	9.4 (0.6)b	–1.4 (0.07)a	0.11
SC15	71.2 (9.2)a	35.5 (1.1)b	6.1 (0.5)c	–1.1 (0.08)a	0.12
Tx7078	76.5 (9.2)a	27.4 (1.9)a	9.4 (0.5)b	–1.4 (0.08)a	0.42

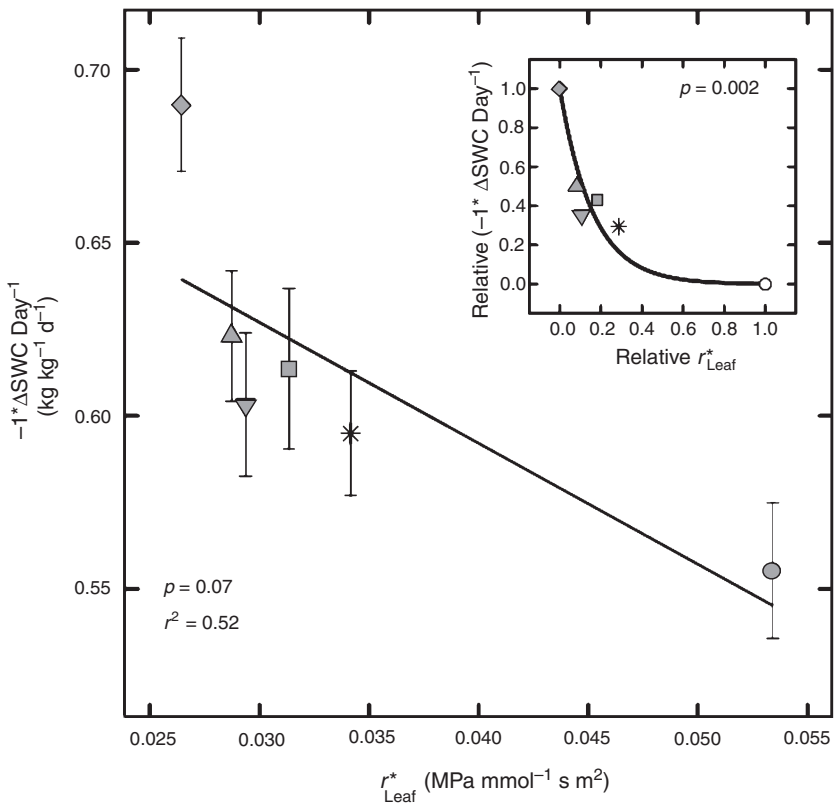

Fig. 3. Relationship between the rate of *SWC* depletion and leaf hydraulic resistance. The relationship appears non-linear, and when both r^*_{Leaf} and the rate of soil moisture depletion values were scaled from 0 to 1, an exponential decay function fit the relationship (inset). Symbols represent the genotypes tested; BTx623 (✳), B35 (◇), SC1019 (△), SC1205 (▽), SC15 (○), TX7078 (□). Data are expressed as means ±s.e.

Anatomy

Large longitudinal vein density was correlated with r^*_{LV} (Fig. 5*a*), suggesting the bulk of axial water movement was in this vein order. Small and total longitudinal vein density did not correlate with any measure of leaf resistance. The distance of water movement from the vascular bundle to stomata (D_m) varied little among genotypes, and only ranged from 39.1–47.9 µm. D_m was not significantly correlated with r^*_{OLV} (Fig. 5*b*), or any other measurement of leaf resistance (Table 1).

Discussion

The partitioning of hydraulic resistance within leaves has important functional consequences for understanding plant responses to declining soil moisture. When resistances were

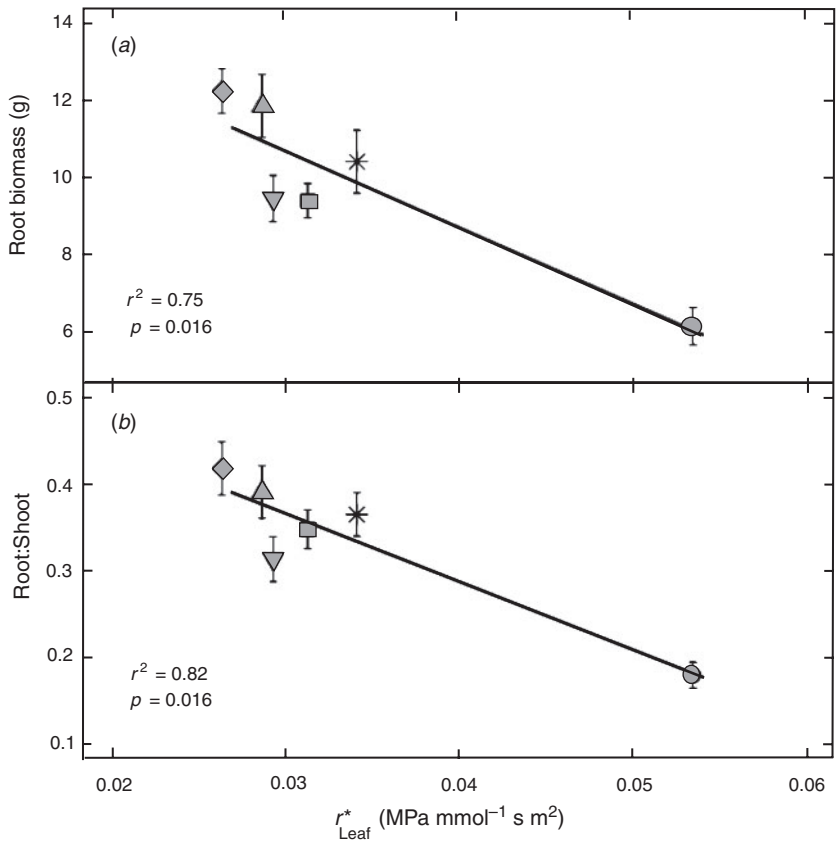

Fig. 4. Correlation between leaf hydraulic resistance and (*a*) root biomass or (*b*) root : shoot ratio for the six genotypes measured. Low r^*_{Leaf} associated with high water use, was correlated with greater root biomass (*a*) and greater allocation of biomass belowground (*b*) for water acquisition. Symbols represent the genotypes tested; BTx623 (✳), B35 (◇), SC1019 (△), SC1205 (▽), SC15 (○), TX7078 (□). Data are expressed as means ±s.e.

calculated for a fully-saturated leaf, the resistance outside the large longitudinal veins (r^*_{OLV}) was tightly correlated with maximum rates of gas exchange when soil moisture was readily available. As soil moisture decreased, the axial resistance within the large longitudinal veins of grass blades (r^*_{LV}) was a better predictor of gas exchange rates. Because these two resistances influenced stomatal conductance under different soil moisture conditions, the integration of these resistances in r^*_{Leaf} was the best predictor of whole-plant water use (estimated by ET) over the course of the experiment.

Previous work partitioning leaf resistances has separated the resistance of water movement within the xylem from the resistance outside the xylem (Sack and Holbrook 2006). The partitioning of resistances done here differs from previous work in that we partitioned r^*_{Leaf} into: long-distance resistance within the large longitudinal veins (r^*_{LV}) from the resistances imposed by other sources of local water distribution (r^*_{OLV}) (Altus and Canny 1985; Altus *et al*. 1985). Measurement of r^*_{OLV} is likely to include the hydraulic resistance within the small longitudinal, transverse veins and the resistance of the mesophyll. However, we used a lower pressure during our r^*_{Leaf} measurements than previous studies, so it is possible that water was not forced through the smallest veins, thus excluding water movement through some veins and the mesophyll during our measurements. Our r^*_{Leaf} values are within values recently reported for grasses (Holloway-Phillips and Brodribb 2011*a*, 2011*b*) and other crop species (Tsuda and Tyree 2000), however, the possibility exists that we overestimated r^*_{Leaf} and r^*_{OLV}. Within a single species we found r^*_{OLV} to vary from 75 to 90% of total leaf resistance, which is within the range reported when partitioning xylary from extra-xylary resistance in leaves (Zwieniecki *et al*. 2002; Cochard *et al*. 2004*b*; Sack *et al*. 2004, 2005; Nardini and Salleo 2005). However, the per cent of whole-leaf resistance in r^*_{OLV} is confined to the high end of the values reported, which is probably because there is less variability within this genotype than among species.

The greatest resistance to water movement occurred outside the large longitudinal veins in all the genotypes studied (Fig. 1*c*), and this source of resistance should be the rate-limiting step in the movement of water through these plants when water was not limiting. Consistent with this reasoning, we found r^*_{OLV} to be tightly correlated with g_s when soil moisture was readily available (Fig. 2*a*). Previous work has shown indirect estimates of resistance outside the xylem correlated with maximum rates of gas exchange, as the distance from vascular bundle to stomata (D_m) was correlated with g_s within

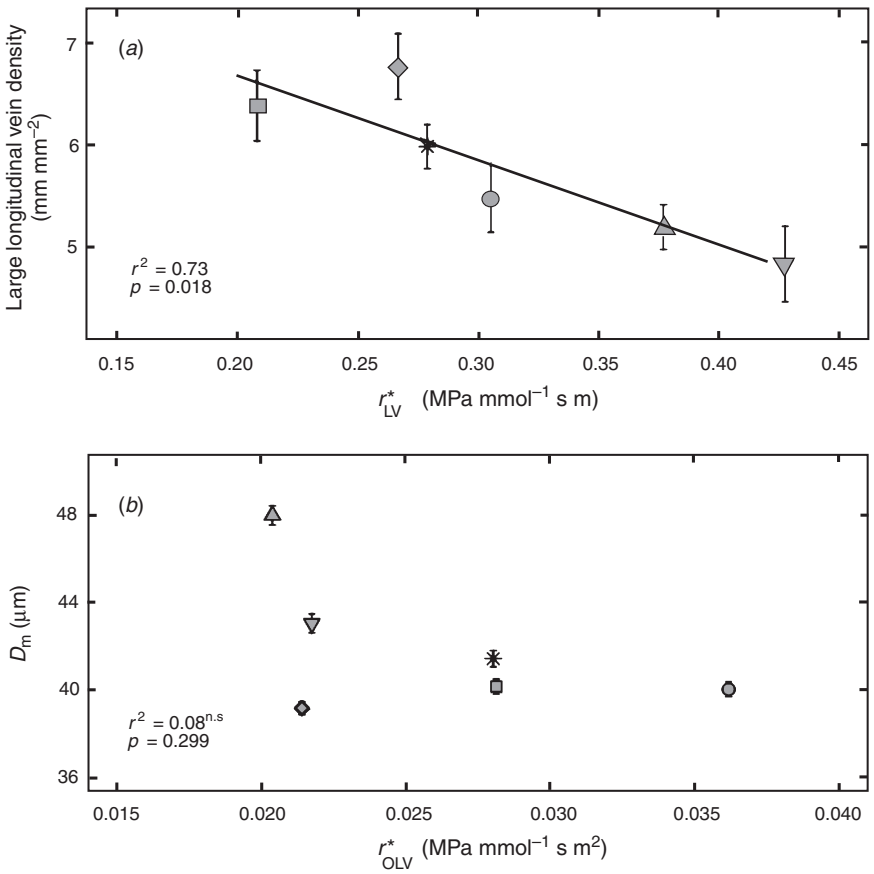

Fig. 5. Relationship between leaf structure and hydraulic resistance in leaves of *Sorghum bicolor*. The density of large longitudinal veins correlated with axial hydraulic resistance in the xylem (*a*). The distance water travels from vascular bundle to stomata (D_m) was not significantly correlated with the resistance outside the large longitudinal veins (*b*). Symbols represent the genotypes tested; BTx623 (✳), B35 (♢), SC1019 (△), SC1205 (▽), SC15 (○), TX7078 (□). Data are expressed as means ±s.e.

grass blades (Ocheltree *et al.* 2012) and among dicot leaves (Brodribb *et al*. 2007). We found no correlation between D_m and g_s in this study (Fig. 4*b*) and other recent work also failed to find this relationship among closely related species (Nardini *et al*. 2012). The range of D_m values among the six genotypes of *Sorghum bicolor* studied here was small (39.1–47.9 µm) compared with previous work (~100–1200 µm, Brodribb *et al*. 2007). Therefore, it is possible that D_m is indirectly related to leaf resistance and captures the variability across large gradients in leaf structure, but among species with similar leaf structure other mechanisms may control maximum hydraulic conductance through the leaf (Nardini *et al*. 2005; Sadok and Sinclair 2010).

As water becomes limiting to plant growth and g_s is reduced in order to maintain the hydration of plant cells, the ability to efficiently transport water long distances should minimise pressure gradients within plants. All six genotypes included in this study maintained midday Ψ_{Leaf} at approximately –1.3 MPa as soil moisture declined (Table 2), which falls within the range of values previously reported for this species (Ackerson *et al*. 1977; Jones and Turner 1978). This relatively constant Ψ_{Leaf} throughout the drought treatment suggests that g_s decreased to maintain the water status of the leaves. At similar Ψ_{Leaf} values among genotypes, we expected genotypes with low r^*_{LV} to minimise the pressure gradients along the leaves and maintain higher rates of g_s at comparable levels of soil moisture and vapour pressure deficit. Our data support this idea (Fig. 2*d*), as there was a tight correlation between g_s and r^*_{LV} when soil moisture was limiting that did not exist under well-watered conditions (Fig. 2*b*). Stomatal conductance of *Helianthus annuus* cv. Margot has also been shown to correlate with the resistance in the leaf xylem when plants were grown at different levels of soil moisture availability (Nardini and Salleo 2005). These results emphasise the importance of leaf xylem resistance in controlling rates of leaf gas exchange during periods of water limitation.

It should be noted that our measurements of leaf hydraulic resistance were made on well-watered plants, which were then related to gas-exchange measurements during a drought treatment. Leaf hydraulic resistance responds to leaf water status through, at least, two mechanisms: reduced conductance due to xylem dysfunction (Trifilò *et al*. 2003; Cochard *et al*. 2004*a*; Marenco *et al*. 2006), or changes in aquaporin transport as water moves through the leaf mesophyll (Kim and Steudle

2007). Hydraulic resistance through the xylem changes on a diurnal basis as vessel elements cavitate and refill in response to changes in leaf hydration status (Marenco *et al*. 2006) and has recently been shown to correlate closely with changes in r^*_{Leaf} (Johnson *et al*. 2012). The leaf water potential of the genotypes in our study did not change significantly in response to the drought treatment (Table 2); instead g_s decreased keeping the leaf water potential relatively constant during our experiment. This suggests that diurnal patterns of cavitation events or changes in aquaporin regulation due to changes in hydration status would have had consistent affects on leaf hydraulic resistance throughout most of our experiment. As such, the resistance partitioning performed on well-watered plants should have remained relevant to leaf function as the plants experienced drought.

We found significant correlations between leaf structure and hydraulic resistance within the xylem of the genotypes we studied. The number of large longitudinal veins correlated strongly with r^*_{LV} across these six genotypes (Fig. 5*a*). The dimensions and number of vessel elements in the large longitudinal veins were similar across genotypes (data not shown), so differences in r^*_{LV} between genotypes resulted from the absolute number of large longitudinal veins present in the leaf (Fig. 5*a*). This matches model simulations of leaves where increasing major vein density led to a linear decrease in xylem resistance (McKown *et al*. 2010). Decreasing r^*_{LV} through changes in the number of vascular bundles rather than the size of vessel elements is expensive to plants as cost of xylem construction is fairly high (McCulloh *et al*. 2003). We found no significant correlations between any anatomical characteristics (D_m or vein density) and r^*_{OLV}, which suggests that the hydraulic resistance outside the large longitudinal veins may have been controlled by other mechanisms. For example, we did not account of the density of transverse veins, which could also account for additional variation in r^*_{OLV}.

It has been suggested that plants with high r^*_{Leaf} exhibit a conservative water-use strategy (Sinclair *et al*. 2008), leaving more water for biomass production later in the growing season. Consistent with this idea, we found r^*_{Leaf} was correlated with ET throughout the experiment among the genotypes we studied (Table 1). This relationship was likely driven by differences in the transpiration component of ET, as evaporation from the soil surface was unlikely to differ much between individuals due to similarity in canopy structure above the soil surface. Differences in ET could also have resulted from differences in biomass between genotypes, as larger plants with greater leaf area used more water independent of r^*_{Leaf}. This explanation is unlikely though, as genotypes with the lowest ET rates also had the greatest aboveground biomass at the end of the experiment (e.g. genotype SC15). The best explanation is that slower ET rates were directly related to low r^*_{Leaf}, as genotypes with the greatest leaf resistance had the slowest soil moisture depletion rates (Fig. 3).

If a water conservation growth strategy leaves more water for biomass production later into a drought, we would have expected the genotypes with high r^*_{Leaf} to have higher final biomass production; this was not the case for the genotypes in this study. This may have been because our experimental design, as we were not simulating field conditions but exposing plants to an extreme drought over a relatively short period of time while confining roots to relatively small volumes of soil. Comparing these genotypes *in situ* would help determine if a 'water conserving' strategy resulting from high r^*_{Leaf} is advantageous to biomass production. The lower demand for water with increasing r^*_{Leaf} did correlate with differences in biomass partitioning between genotypes. Across all six genotypes the root : shoot ratio was significantly correlated with the resistance of leaves (Fig. 4*b*). High resistance in the leaf resulted in less demand for water with greater resource allocation aboveground. Because we only measured biomass at the end of the experiment, we cannot be certain that the biomass partitioning was a response to the drought treatment or whether this represents inherent differences among genotypes. In either scenario, the strong correlation between r^*_{Leaf} and root : shoot ratios suggests a link between hydraulic architecture and plant growth strategy that demands further *in situ* investigations.

In natural ecosystems it is unclear when a 'water conserving' strategy would be competitively advantageous for plants; dominant species are often inefficient users of water (DeLucia and Schlesinger 1991), so large r^*_{OLV} that limits water use may not be a successful competitive strategy in many systems. In mesic systems where drought is typically moderate, minimising r^*_{OLV} and maximising the efficiency of water transport through the xylem to maintain hydrated cells should be advantageous. Indeed, within many ecosystems fast-growing species often have the highest stem hydraulic conductance (Brodribb and Feild 2000; Markesteijn *et al*. 2011). In xeric systems where competition for water may not be as important as tolerating long periods of drought, conserving water may be advantageous to growth and survival (Fernández and Reynolds 2000). Under this scenario, high r^*_{OLV} in leaves may facilitate whole-plant water conservation despite the hydraulic architecture of the remainder of the plant. The ability to tolerate low soil moisture and low leaf water potentials would be more important in xeric systems, and likely reflects characteristics of the individual vessel elements that limit cavitation (Wheeler *et al*. 2005; Hacke *et al*. 2006; Blackman *et al*. 2010) and major vein density (Scoffoni *et al*. 2011). Investigating how leaf resistances are partitioned in a widely distributed group of grasses would provide new insights into how leaf resistances contribute to plant distributions across the landscape.

Our results show the correlation between leaf hydraulic resistance and the response of leaf-level gas exchange to drying soils in *S. bicolor*. High resistance outside the large longitudinal veins correlated with low rates of gas exchange when soil moisture was readily available to plants, but these genotypes of *S. bicolor* conserved water and were able to maintain higher rates of gas exchange further into the drought treatment. Furthermore, the efficient transport of water within the large longitudinal veins sustained leaf-level gas exchange under moderate drought. Future work should focus on understanding leaf function under severe drought, and measure leaf resistance partitioning across a broader range of species. Our results suggest that the partitioning of hydraulic resistance within leaves provides a framework to assess mechanisms of plant growth under a range of soil moisture conditions.

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