Hydraulic conductance of *Acacia* phyllodes (foliage) is driven by primary nerve (vein) conductance and density

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ABSTRACT

We determined effects of venation traits on hydraulic conductance of phyllodes (foliage), using an array of *Acacia* s.str. species with diverse phyllode morphologies as the source of variation. Measurements were made on phyllodes from 44 species, grown in common gardens but originating from different positions along a precipitation gradient. $K_{phyllode}$ varied 18-fold and was positively correlated with primary nerve hydraulic conductance, and with primary nerve (vein) density but not with minor nerve density, in contrast with previous studies of true leaves in other dicotyledons. Phyllodes with higher primary nerve density also had greater mass per area (PMA) and larger bundle sheath extensions (BSEs) from their minor nerves. We suggest that higher primary nerve conductivity and density may decrease the distance travelled in the high-resistance extra-xylem pathways of the phyllode. Further, larger BSEs may increase the area available for dispersion of water from the xylem to the extra-xylem tissue. High PMA phyllodes were more common in acacias from areas receiving lower annual precipitation. Maximizing efficient water movement through phyllodes may be more important where rainfall is meagre and infrequent, explaining relationships between nerve patterns and the climates of origin in Australian phyllodinous *Acacia*.

Key-words: density; phyllode; water.

INTRODUCTION

The plant hydraulic pathway is a critical determinant of maximum rates of gas exchange, whole plant growth and drought responses (Nardini, Salleo & Raimondo 2003; Sack *et al*. 2003; Brodribb *et al*. 2005; Sack & Holbrook 2006; Brodribb, Feild & Jordan 2007; Brodribb & Jordan 2008). One of the greatest sites of resistance to water flow in plants is the leaf or foliage (Sack & Holbrook 2006). This is so even when the bulk of the transport path-length is through woody stems and roots (Tyree & Zimmermann 2002). Despite the importance of foliage in plant water relations, the impact of variations in foliage form and structure on flow of water through the major photosynthetic organ is

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only now beginning to be resolved [reviewed in Sack & Holbrook (2006) and Brodribb (2009)]. Further, little is known of the determinants of hydraulic conductance for special cases of 'leaves', such as phyllodes. Phyllodes perform the same photosynthetic and respiratory functions as true leaves but are developmentally derived from a combination of the rachis, petiole and leaf (Gardner *et al*. 2005, 2008). Unlike true leaves, phyllodes possess two distinct layers of veins, a layer of water storage tissue (phyllodes lack spongy mesophyll) separating two photosynthetic layers and relatively equal numbers of stomata at each of the abaxial and adaxial surfaces (Lemesle 1963; Lange *et al*. 1987; Gardner *et al*. 2008). In *Acacia* s.str., for example, the morphology of phyllodes varies strongly across the 917 species that are differentially distributed in diverse terrestrial ecosystems including forests, woodlands, heathlands, deserts, coastal dunes and shrublands (Specht, Roe & Boughton 1974; Chapman *et al*. 2001a,b; Maslin, Miller & Seigler 2003; Murphy *et al*. 2003). The aim of our study was to establish the influence of variation in the unique anatomy of *Acacia* phyllodes on their hydraulic conductance.

The hydraulic pathway of the true leaf includes xylem and extra-xylem components. Water enters a leaf through xylem vessels that traverse the petiole and branch in the lamina into a system of veins in which water is distributed from larger to progressively smaller veins (Canny 1990). Water then moves from veins into the surrounding leaf tissue before evaporating into intercellular gas spaces and diffusing out of the leaf via stomatal pores (Holbrook & Zwieniecki 2008). The anatomy of the vein system influences the resistance of the xylem component of the pathway; greater vessel diameter and higher density (length per leaf area) of the smallest veins can increase flow (Sack & Frole 2006). The Hagen–Poiseuille equation predicts that flow rate increases with the fourth power of vessel diameter (Tyree & Zimmermann 2002). As such, leaves with larger vessel diameters would have higher area-based flow rates for a given water potential driving force, that is, a higher leaf hydraulic conductance (K_{leaf}) (Aasamaa, Sober & Rahi 2001; Sack & Frole 2006; McKown, Cochard & Sack 2010). Similarly, increased density of major and minor veins may increase the xylem flow pathways in parallel, and also increase the area available for dispersion of water from the vascular system to the tissues that lie beyond, leading to a

higher K_{leaf} (Sack & Frole 2006; McKown *et al*. 2010). Thus, where the vascular pathway dominates resistance, leaf vein density and vessel dimensions are paramount in maintaining adequate water supply for leaf function (Sack & Holbrook 2006). In an analogous manner, we determined correlations of $K_{phyllode}$ with venation traits, using an array of *Acacia* species with diverse phyllode morphologies as the source of variation.

The path of water beyond the veins, the extra-xylem pathway, remains uncertain. Symplastic, apoplastic and transcellular routes have been suggested (Canny 1990; Sack & Holbrook 2006). In the present study, we explored the potential role of different extra-vascular structures in diminishing or expediting water flow through *Acacia* phyllodes. Extra-vascular tissues with more points of contact may provide greater hydraulic conductance. The epidermis and spongy mesophyll have more extensive lateral connections between cells than the palisade mesophyll (Wylie 1952; Esau 1977). Thus, leaves with a greater ratio of palisade to spongy mesophyll may require a closer spacing of minor veins (a greater minor vein density) to ensure adequate water distribution throughout the leaf (Wylie 1952; Philpott 1953; Sack & Frole 2006). Similarly, the flow of water to photosynthetic tissues may be facilitated by bundle sheath extensions (BSEs) (Zwieniecki, Brodribb & Holbrook 2007; Scoffoni *et al*. 2008) that form a hydraulic extension from minor veins to the epidermis (Armacost 1944; Wylie 1952).

Notably, other tissues in the leaf may also correlate with K_{leaf}, leading to a coordination of 'water flux-related traits' that relate to gas exchange per leaf area (Sack *et al*. 2003; Franks & Brodribb 2005). For example, stomatal pore area has been found to correlate with K_{leaf}, as has stomatal conductance and photosynthetic rate per leaf area (Sack *et al*. 2003; Sack & Tyree 2005; Brodribb *et al*. 2007; Boyce *et al*. 2009). Thus, variation in the structure of the venation and extra-xylem tissues may affect the conductance of the leaf blade to both liquid water and water vapour.

Our study focused on phyllodes in the wide-ranging, evergreen angiosperm genus, *Acacia*. We tested hypotheses for the coordination of phyllode hydraulic conductance ($K_{phyllode}$) with venation architecture and other phyllode traits. We hypothesized that $K_{phyllode}$ would correlate positively with minor nerve (vein) density, nerve conductance, presence of BSEs and stomatal pore area, and negatively with extra-xylem path-length and the ratio of palisade mesophyll to water storage tissue. We also hypothesized correlations of phyllode traits with the climate of origin of studied plants. We hypothesized that phyllodes with greater vessel diameters and numbers and thus higher $K_{phyllode}$ would be more common in species from areas receiving higher annual precipitation, just as, in stems of diverse species, vessel diameters tend to be larger for species of moist areas that face lower risk of drought-induced cavitation (Carlquist 2001; Wheeler *et al*. 2005). Greater foliage mass per area (PMA) has been associated with lower rates of water loss and greater longevity in leaves and phyllodes (Wright *et al*. 2005; Hao *et al*. 2008). Thus, we hypothesized

that phyllodes with higher PMA would be more common in species from lower rainfall areas.

MATERIALS AND METHODS

Plant material and experimental design

Material was collected from three irrigated common gardens: the Australian National Botanic Gardens [35 °17′ S, 149 °6′ E; 571–677 m a.s.l., mean annual precipitation (MAP) = 664 mm, mean annual temperature (MAT) = 19.7 °C], the Mount Annan Botanic Gardens (34 °03′ S, 150 °46′ E; 118 m a.s.l., MAP = 829 mm, MAT = 23.3 °C) and the CSIRO Tropical Forest Research Centre Arboretum (17 °16′ S., 145 °29′, alt. 752 m a.s.l., MAP = 1379 mm, MAT = 25.6 °C). Plants of 44 mature phyllodinous *Acacia* species were selected and sampled (Supporting Information Table S1) based on their geographic origin. Previous studies have found four main regions of *Acacia* diversity within Australia (Hnatiuk & Maslin 1988, Maslin, 1997); species were selected to represent the regional diversity. For example, as 34% of *Acacia* species are found in the central Australian region, 14 acacias (34% of the 44 species in the study) were accordingly from the central region.

Each species was represented by one plant and species were not replicated across the three garden sites. Each garden had species from all regions of diversity. When data from each of the three garden sites were compared, response variables were not significantly different at $P = 0.05$ between sites. For example, there was no significant difference ($P = 0.121$, $F = 2.24$) in mean $K_{phyllode}$ between each of the three garden sites. Equally, analysis conducted at each garden site gave the same direction in results as analysis conducted at all sites; the same trends were present in the data regardless of any differences in growing conditions or species present. As there was no significant difference in the direction or mean value of measured responses, data from all three garden sites are combined for the analysis contained herein.

Phyllode hydraulic conductance

Branches were sampled from robust, healthy acacias between September 2006 and May 2007 during their growing season such that newly mature phyllodes were available for measurement. Branches with sun-exposed phyllodes were cut under water pre-dawn. Cut stems remained in water with foliage bagged in the dark for transport to the laboratory for measurements the same day. Phyllodes were re-cut under water from branches and hydraulic conductance was measured immediately.

The hydraulic conductance of the phyllode lamina ($K_{phyllode}$) was determined for two phyllodes from each plant using the evaporative flux method (Sack *et al*. 2002). Water uptake for illuminated individual excised phyllodes was measured every 10 s using a balance (Mettler Toledo AX205 Delta range $\pm$ 0.01 mg, Columbus, OH, USA).

Phyllodes received an incident quantum flux density of 1000 μmol m^{-2} s^{-1} photosynthetically active radiation [measured using a Li-Cor 1000 quantum sensor (Lincoln, NE, USA) with a ME-531 digital multimeter attached (Soar Corporation, Hanishina, Japan)]. Air temperature adjacent to phyllodes was measured with a Microcomputer thermometer (Model 7001CH, Jenco, Grand Prairie, TX, USA) during water uptake measurement. Within 10 min, the phyllode flow rate stabilized to within 0.1 mg s^{-1} and the phyllode flow rate was then recorded for at least another 15 min during which time flow rate was determined as the average of measurements made every 10 s for a 10 min period. Our use of a 15 min period of stable flow compares favourably with other studies of diverse leaves in which calculations of K_{leaf} were based on measurements of stable flow made over periods of 3 min (Brodribb & Holbrook 2006, 2007), 3 to 5 min (Scoffoni *et al.* 2008) and 10 min (Sack *et al.* 2002; Pasquet-Kok, Creese & Sack 2010). Phyllodes were then bagged and water potential was measured using a pressure chamber (Model 3005, Soil Moisture Equipment Corp., Santa Barbara, CA, USA). $K_{phyllode}$ was calculated as flow rate divided by water potential driving force, normalized by the lamina area (LI 3000A portable area meter with a PAM196 head, Li-Cor). Calculated flow rates were adjusted to a common temperature of 25 °C to standardize for changes in the viscosity of water (Korson, Drost-Hansen & Millero 1969).

The stabilization of both flow rate and water potential is important for accurate determination of K_{leaf} and $K_{phyllode}$. While flow stability can be assessed directly during measurement, the stability of water potential cannot be determined for each leaf and might be expected to lag behind the stabilization of flow rate due to water storage capacitance. However, for a range of species differing strongly in leaf bulk water storage capacitance, Blackman & Brodribb (2011) showed good correspondence between measurements of K_{leaf} made using the steady-state evaporative flux method with 3–5 min stabilization time and a dynamic method based on the rapid phase of rehydration kinetics. Their results imply that exchange of water between leaf tissues and the transpiration stream occurs over time scales of seconds, thereby buffering against rapid variation in leaf water potential during fluctuations in transpiration rates. Consistent with that principle, recent studies found that for leaves varying widely in bulk capacitance, the K_{leaf} of hydrated leaves was independent of measurement time when the flow rate had stabilized (Scoffoni *et al.* 2008) and that K_{leaf} of dehydrated leaves was similar using rehydration times of 30 and 300 s (Blackman, Brodribb & Jordan 2010). To address whether the issue of capacitance might be more important in phyllodes given their water storage tissue, we analysed data of Pasquet-Kok *et al.* (2010) for phyllodes of a Hawaiian species, *Acacia koa*. We found no relationships when $K_{phyllode}$ was plotted against either measurement time or time during which flow was stable ($n = 25$; $R^2 = 0.004$ and 0.009, respectively; $P = 0.65$–0.75). Notably, *A. koa* had extensive water storage tissue, equivalent in thickness to the median for arid species of Australian phyllodinous acacias

(Boughton 1986). Indeed, the effect of phyllode water storage tissue on absolute capacitance is not necessarily large. For *A. koa*, the relative capacitance from the pressure volume curve was moderately high at 0.121 MPa^{-1} but the absolute capacitance (= relative capacitance × mass of water per leaf area) was moderate at 23.1 g m^{-2} MPa^{-1} (cf. comparative values of 0.053–0.113 MPa^{-1} and 14–29 g m^{-2} MPa^{-1}, respectively, for the six species in Scoffoni *et al.* 2008). The mass of water per leaf area tends to be a major contributor to the absolute bulk capacitance, and this is high in thick leaves regardless of whether or not they have water storage tissue (Sack *et al.* 2003). It follows that if the water storage time constant is assessed as absolute bulk capacitance / K_{leaf} (Brodribb & Holbrook 2003), then the time constant may be moderate for phyllodes; for the *A. koa* phyllode it was <1.5 min, explaining why water potential and $K_{phyllode}$ have ample time to stabilize during the measurement. Phyllodes with even higher bulk capacitances would thus also be expected to achieve stable $K_{phyllode}$ values using the evaporative flux method.

Phyllodes were oven dried at 80 °C for 48 h for dry mass determination. Phyllode PMA was calculated as phyllode dry mass divided by area.

Phyllode anatomy

Stomatal imprints were obtained with nail varnish applied to both surfaces of a phyllode adjacent to that measured for hydraulic conductance. Stomatal density and guard cell length were measured from the imprints (×100 and ×200; Olympus BHB light microscope; Tokyo, Japan). An index of stomatal pore area per leaf area (SPI) was calculated as stomatal density × guard cell length^2, averaging across both surfaces (Sack *et al.* 2003).

The length and width at widest point of two phyllodes from each plant were measured and then phyllodes were sectioned by hand for cross-sectional anatomy at their midpoint, fixed in formalin–acetic acid–alcohol–water solution (10, 5, 70, 15 by volume), washed in 70% ethanol and then stored in an ethanol–water–glycerol solution (75:20:5). Sections were stained with 0.01% Safranin O and imaged at ×40, ×100 and ×200 with a Rhodamine 20 filter (Carl Zeiss Axioplan 2 epifluorescence microscope; Göttingen, Germany). We determined conduit number, maximum lumen diameter of each conduit, BSE length, perpendicular distance from the nerve to the epidermis, distance between adjacent nerves and the thickness of the palisade (adaxial and abaxial layers summed) and water storage tissue. A hydraulically weighted mean conduit lumen diameter was calculated as $d_h = \Sigma\, d^5 / \Sigma\, d^4$ (Lewis & Boose 1995). We calculated a phyllode-area normalized theoretical total primary nerve (where primary nerves are those that are present at the base of the phyllode and extend to the tip of the phyllode) conductance as

$$K_{phyllode \cdot primary} = \frac{((K_{conduit} \times conduit \cdot number)/phyllode \cdot length) \times primary \cdot nerve \cdot number}{phyllode \cdot area}$$

(units mmol m^{-2} s^{-1} MPa^{-1}) with conduit conductivity $(K_{conduit}) = \dfrac{r^4 \pi}{8\eta}$, where r is conduit radius and η is the viscosity of water at 25 °C. We calculated the mean theoretical hydraulic resistance of minor and primary nerves as the inverse of $K_{conduit}$ using five minor nerves and the middle primary nerves in these calculations. An index of extra-xylem path-length was calculated as the product of the perpendicular distance from the minor nerve to the epidermis and the distance between adjacent minor nerves (related to the Dm index of Brodribb *et al.* 2007). We also estimated mean primary nerve size as estimated by nerve area in transverse section (μm^2) for primary nerves present.

To quantify venation architecture, a further two phyllodes from each plant were cleared with 15% w/v sodium hydroxide in ethanol for 2 weeks, then washed in water, bleached and stored in lactic acid. Cleared phyllodes were stained with 0.01% Safranin O and imaged at ×1, ×16, ×32 and ×64 magnification using a Wild M400 photomacroscope with Spot Flex CCD camera (Leica-Wild Heerbrugg, Heerbrugg, Switzerland). Primary (first order) and minor nerve density (third order and above if present, both adaxial and abaxial sides summed) were measured using ImageJ software (Rasband 2009). Primary nerve density was calculated as the number of nerves running from the base to the tip of the lamina divided by phyllode width at the widest point. Minor nerve density was calculated as length of minor nerve per total lamina area. Minor nerve density per photosynthetic lamina area (the surface area excluding that occupied by nerves) was also calculated. Total nerve density was determined as the sum of minor and primary nerve densities.

Six *Acacia* species with a variety of phyllode nervation patterns (*Acacia aneura* var. *microcarpa*, *Acacia cyclops*, *Acacia melanoxylon*, *Acacia pendula*, *Acacia pycnantha* and *Acacia salicina*) were selected for study of the transpiration pathway according to O'Dowd & Canny (1993). We fed 0.1% sulphorhodamine (SR) in water to two phyllodes of each plant that were transpiring under 1000 μmol m^{-2} s^{-1} photosynthetically active radiation for 30 min. We then examined paradermal sections at ×100 magnification (Olympus BHB light microscope) to locate areas where SR, which cannot easily pass with water through cell membranes, had been left behind when water moved into the extra-vascular tissue (Canon Powershot S3 IS camera with a Martin Microscope Company S/N: 3092 MM99 Adapter and a Canon Conversion Lens Adapter LA-DC58E, Tokyo, Japan).

Climate analysis

We calculated climate envelopes for each individual *Acacia* from the locations from which they had been collected prior to common garden cultivation using ANUCLIM v. 5.1 (Houlder *et al.* 2003). MAP, precipitation of the wettest month, precipitation of the driest month, precipitation range (wet–dry month), precipitation of the wettest quarter and precipitation of the driest quarter were determined.

The precipitation variables showed strong intercorrelation ($r^2 = 0.89$–0.99, $P < 0.001$ to 0.002) and hence we present results only for MAP. Temperature and radiation estimates were also calculated but showed no directional relationship with $K_{phyllode}$.

Statistical analysis

Ordinary linear regressions and Spearman rank correlation coefficients were calculated using GenStat v. 12.1 (Payne *et al.* 2009). When data were non-normally distributed, they were log transformed before analysis. We tested for autocorrelations between area-based variables by dividing or multiplying those variables by phyllode area, thereby removing their area dependence. Where multiple variables were intercorrelated, we used partial correlation analyses to resolve underlying relationships (Sokal & Rohlf 1995).

RESULTS

Xylem pathway

$K_{phyllode}$ varied 18-fold (Table 1) and correlated strongly and positively with primary nerve density (Fig. 1), which itself varied by 120-fold. Primary nerve density was negatively correlated with minor nerve density (Fig. 2, Table 1) which resulted in a negative relationship between minor nerve density and $K_{phyllode}$ (Table 1). This was so even when minor nerve density was expressed on a photosynthetic lamina area basis rather than based on total lamina area ($K_{phyllode} = -0.03$ minor nerve density$_{photosynthetic area}$ + 0.87, $r^2 = 0.11$, $P = 0.023$). Total nerve density was strongly driven by minor nerve density (total nerve density = 0.89 minor nerve density + 3.63, $r^2 = 0.94$, $P \le 0.001$). Thus, it was no surprise that total nerve density showed no relationship with $K_{phyllode}$ ($r^2 = 0.04$, $P = 0.122$).

Given the intriguing negative relationships between primary and minor nerve densities, we explored differences in vessel diameter between nerve orders, based on our hypothesis that reduced distance travelled in the high resistance pathways of minor nerves may explain observed relationships with $K_{phyllode}$. When considered across all acacias, minor nerve vessels were on average 107 times more resistant to flow than the vessels of primary nerves. Our dye study showed that minor nerves and their BSE were the only sites of water movement out of the xylem (Fig. 3), confirming that water must flow through the high-resistance small vessels of the minor nerves in order to exit the xylem network.

When total theoretical phyllode primary nerve conductance ($K_{phyllode\ primary}$) was examined, accounting for differences in phyllode length, number of primary nerves and phyllode area serviced by the vascular network, $K_{phyllode\ primary}$ showed 44-fold variation and a strong positive correlation with measured $K_{phyllode}$ (Fig. 1, Table 1).

Primary nerve size as estimated by nerve area in transverse section varied some 7.8-fold across studied acacias and bore no relationship to primary nerve density [$P = 0.727$,

Table 1. Relationships between phyllode traits and mean annual precipitation

	$K_{\text{phyllode log}}$	$K_{\text{phyllode primary}}$	Primary nerve density$_{\text{log}}$	Minor nerve density	Minor nerve BSE	PMA$_{\text{log}}$	NE	NE:BN	Palisade: water storage	MAP$_{\text{log}}$	Data range (min–max)
$K_{\text{phyllode log}}$		*0.33*	*0.28*	*0.09*	*0.26*	*0.25*	*0.16*	*0.14*	*0.03*	*0.15*	1.070–19.679
$K_{\text{phyllode primary}}$	**0.529**		*0.35*	*0.27*	*0.33*	*0.42*	*0.28*	*0.38*	*0.00*	*0.37*	9.459–414.568
Primary nerve density$_{\text{log}}$	**0.545**	**0.594**		*0.27*	*0.47*	*0.51*	*0.30*	*0.54*	*0.06*	*0.36*	0.064–7.359
Minor nerve density	0.407	−0.509	−0.560		*0.28*	*0.40*	*0.17*	*0.26*	*a*	*0.27*	5.272–30.876[b]
Minor nerve BSE	0.407	0.378	0.634	−0.514		*0.36*	*0.30*	*0.52*	*0.11*	*0.24*	17.782–272.50
PMA$_{\text{log}}$	0.471	**0.578**	**0.708**	−0.634	**0.474**		*0.60*	*0.64*	*0.09*	*0.55*	72.413–641.188
NE	0.374	**0.537**	**0.535**	−0.483	0.367	**0.783**		*0.797*	*0.16*	*0.33*	51.280–277.331
NE:BN	0.277	**0.596**	**0.677**	−0.556	0.489	**0.816**	**0.797**		*0.10*	*0.50*	0.304–2.443
Palisade: water storage	−0.242	−0.136	−0.331	0.035	−0.307	−0.378	−0.453	−0.355		*0.03*	0.240–2.876
MAP $_{\text{log}}$	−0.398	**−0.608**	**−0.637**	0.584	−0.410	**−0.762**	**−0.599**	**−0.739**	0.237		202–2560

[a]Residual variance exceeds variance of response variate.

[b]*Acacia* phyllodes are isobilateral and thus have nerves running on both sides of the phyllode. These values could be halved for comparison with vein densities of 'normal' leaves.

Values in normal font are Spearman rank-correlation coefficients. Italicized values are percentage variation explained by lines fitted using bivariate regression analyses. Boldfaced values are significant at $P \leq 0.001$, $n = 39$.

K_{phyllode}, phyllode hydraulic conductance (mmol m^{-2} s^{-1} MPa^{-1}); $K_{\text{phyllode primary}}$, calculated total primary nerve conductance (mmol m^{-2} s^{-1} MPa^{-1}); Primary nerve density, calculated as number of primary nerves per phyllode width (mm^{-1}); Minor nerve density calculated as length of minor nerves per area of phyllode (mm mm^{-2}); Minor nerve BSE, mean length of the bundle sheath extension of the minor nerves (μm); PMA, phyllode mass per area (g m^{-2}); NE, mean distance from the nerve to the epidermis (μm); NE:BN, the ratio of the distance from the nerve to the epidermis and the distance between adjacent nerves; Palisade: water storage, ratio of thickness of palisade mesophyll (μm) and water storage (μm) layers; MAP, mean annual precipitation (mm).

$R^2 = 0.00$, primary nerve area (μm^2) = −558 primary nerve density + 32 025]. Within a phyllode, the central primary nerves most often appeared larger; however, most primary nerves were relatively homogenous in size.

Extra-xylem pathway

As the BSEs can provide a hydraulic link between the xylem and the epidermis, we expected presence of BSEs to expedite water flow through the phyllode. Indeed, all acacias had BSEs with the principal apparent difference being the length of the BSE that extended from the nerve to the epidermis (Fig. 4). K_{phyllode} correlated positively with BSE length (Table 1). Given the intercorrelation between variables, we undertook partial correlation analysis to resolve underlying relationships determining K_{phyllode}. After partialling out primary nerve density, the length of the BSE of the minor nerve showed a smaller correlation with K_{phyllode} (partial correlation coefficient = 0.17, $P > 0.1$; partial correlation of K_{phyllode} and primary nerve density when BSE was taken out = 0.34, $P < 0.05$).

One would expect that smaller distances between adjacent nerves and between nerves and the epidermis would result in shorter distances travelled in the extra-xylem pathway and thus lower extra-xylem resistance and a higher K_{phyllode}. However, extra-xylem path-length showed a weak non-significant positive relationship with K_{phyllode} ($K_{\text{phyllode}} = 7.56 \times 10^{-6}$ extra-xylem path-length + 0.49, $r^2 = 0.05$, $P = 0.09$). Notably, the distance between adjacent nerves correlated negatively with the perpendicular distance from nerve to the epidermis (Fig. 4); and thus, acacias with nerves closer together tended to have deeper nerve placement within the phyllode (a greater NE:BN, Table 1). Thus, the overall distance travelled in the extra-xylem pathway was conservative.

We saw no relationship between the ratio of palisade mesophyll to water storage layer thickness and either nerve density or K_{phyllode} (Table 1), suggesting that the more extensive area of contact between adjacent water storage cells (as compared with palisade cells) does not play a substantial role in water movement even when nerve densities are low.

Stomatal anatomy did not relate to K_{phyllode}. Neither stomatal density nor guard cell length showed significant relationships with K_{phyllode} ($r^2 = 0.02$, $P = 0.214$ and $r^2 = 0.02$, $P = 0.196$, respectively). We found a negative correlation of stomatal size (i.e. shorter guard cell length) with stomatal density (guard cell length = −0.85 stomatal density + 3.48, $r^2 = 0.29$, $P = < 0.001$). The SPI varied only 4.4-fold across all acacias and showed no relationship with K_{phyllode} ($r^2 = 0.02$, $P = 0.311$).

Climate and drought adaptive traits

We hypothesized that phyllodes with greater d_{h} would be more common in species from areas receiving higher annual precipitation. Indeed, a positive relationship between d_{h} and MAP was found but MAP explained very little of the variation in d_{h} ($d_{\text{h}} = 6.20$ MAP + 0.46, $r^2 = 0.09$,
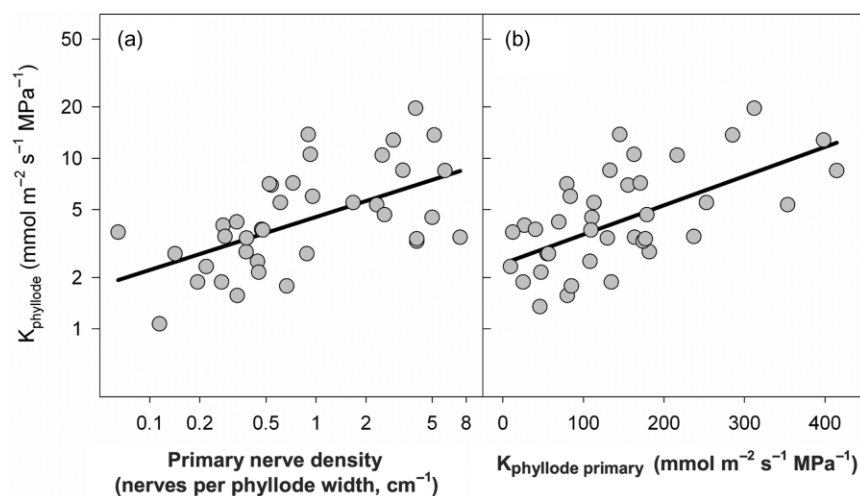
Figure 1. Increasing phyllode hydraulic conductance ($K_{phyllode}$; log scale) with (a) phyllode primary nerve density (log scale) in the genus *Acacia* ($r^2 = 0.28$, $P \leq 0.001$), (b) total theoretical phyllode primary conductance ($K_{phyllode\ primary}$, $r^2 = 0.33$, $P \leq 0.001$). Each value was obtained from a different plant. Lines drawn by simple linear regression.

$P = 0.031$). Phyllode PMA was positively related to $K_{phyllode}$ (Table 1). As phyllodes with higher PMA generally had greater primary nerve density, we undertook a partial correlation analysis, holding primary nerve density constant, to determine the strength of the relationship between PMA and $K_{phyllode}$. Unsurprisingly, the relationship between PMA and $K_{phyllode}$ was much reduced when the effect of primary nerve density was held constant (partial correlation coefficient = 0.22, $P > 0.1$; partial correlation of $K_{phyllode}$ and primary nerve density when PMA was taken out = 0.29, $P < 0.05$), suggesting that primary nerve density was the driver of this relationship.
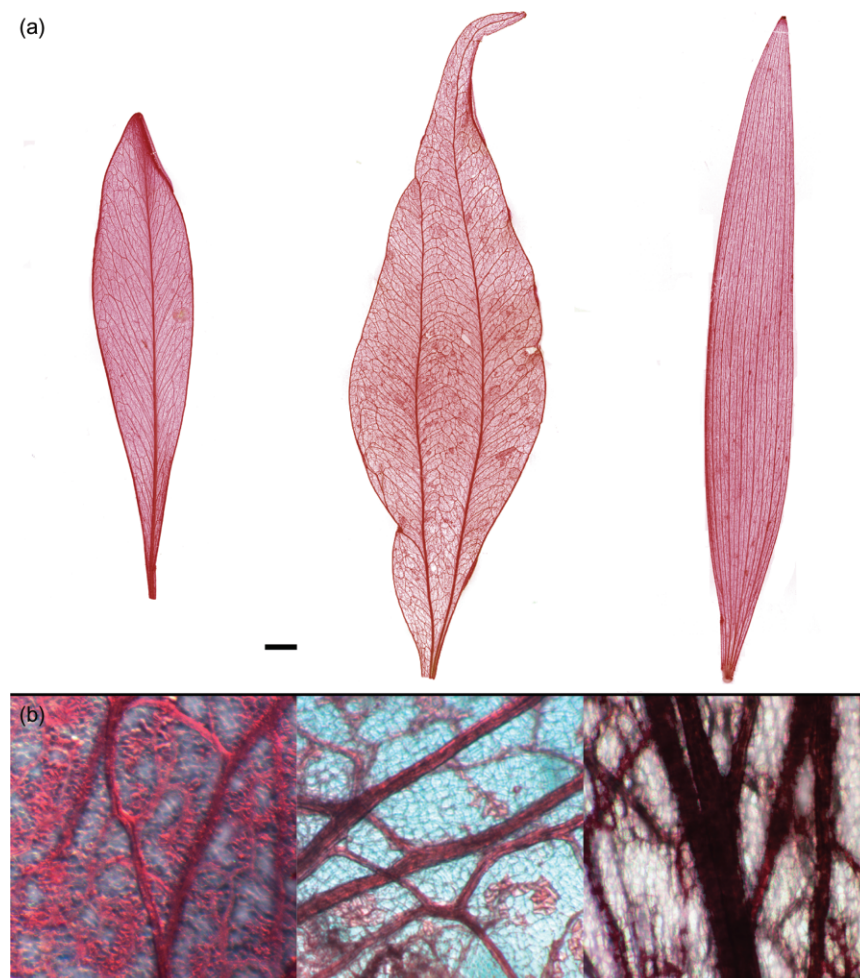
Figure 2. Nerve density patterns in *Acacia* phyllodes of (left–right) *Acacia penninervis, Acacia binervata* and *Acacia hylanoma*. (a) Low to high primary nerve density (left to right), scale bar equal to 1 cm; (b) high to low minor nerve density (left to right), each square is 1 mm². Minor nerve images show two layers of nerves as is typical in phyllodes.
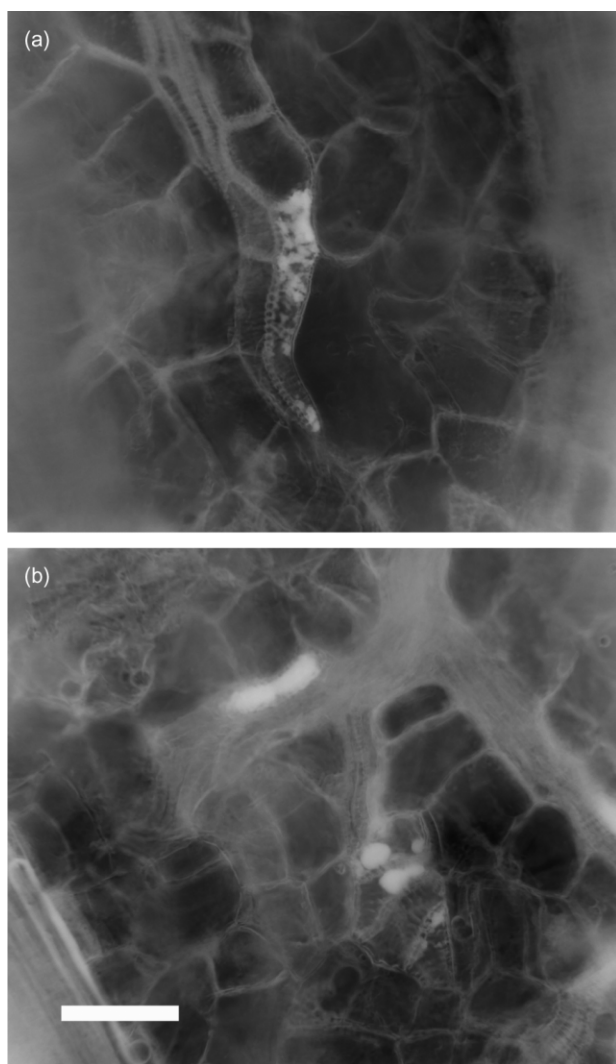
Figure 3. Paradermal sections of *Acacia* phyllodes showing accumulation of the dye, sulphorhodamine at high concentration (as shown in white) in 'sumps' in the minor nerves and their associated bundle sheath extension of (a) *Acacia melanoxylon* and (b) *Acacia pycnantha*. Scale bar equal to 100 μm.

We observed a strong positive relationship between PMA and MAP (Table 1). High PMA phyllodes were more common in plants that originated in drier areas, as predicted (Table 1). Contrary to our expectations, phyllodes with higher $K_{phyllode}$, those with a greater primary nerve density, were generally more common in plants that originated in drier areas (Table 1).

SPI did not vary with changes in MAP ($r^2 = 0.027$, $P = 0.340$).

DISCUSSION

The results of the present study revealed two major relationships between hydraulic conductance and morphological variation in *Acacia* phyllodes. Firstly, $K_{phyllode}$ in *Acacia* increased with primary nerve density. This novel finding

contrasts with studies on true leaves, in which hydraulic conductance generally increases with the density of minor veins (Sack & Frole 2006; Sack & Holbrook 2006; Brodribb *et al*. 2007). However, this finding is consistent with the leaf model simulations of McKown *et al*. (2010) where major vein density was found to drive higher K_{leaf}, all else being equal. Secondly, phyllodes with high hydraulic conductance occurred mainly in *Acacia* from areas with low rainfall. This unexpected finding merits further study, as it indicates a potentially important role of hydraulics in enabling rapid exploitation of ephemeral water resources in environments with unpredictable rainfall. As discussed next, these novel results give functional significance to morphological diversity in *Acacia* phyllodes.

Hydraulic conductance and phyllode structure

Unlike true leaves, there was no relationship between $K_{phyllode}$ and minor vein density. These results were surprising because our SR dye studies showed that minor nerves and their BSE were the only sites of water dispersion out of the vascular network. Minor nerves were on average 107 times more resistant to flow than primary nerve vessels. Both minor nerves, with their smaller vessel diameters, and extra-vascular tissues may offer high resistance to the flow of water (Trifilo *et al*. 2003; Cochard, Nardini & Coll 2004; Nardini, Gortan & Salleo 2005; Sack & Tyree 2005). Hence, overcoming this resistance would require compensatory flow pathways through the phyllode. This was apparently achieved through complementary variation in at least two factors: the density of primary nerves and the length of BSEs from minor nerves.

In contrast to minor nerves, primary nerves may act as super highways for the transport of water across a phyllode. The positive correlation between measured $K_{phyllode}$ and theoretical conductance of the primary nerves supported our hypothesis that density of the primary nerves plays a critical role in 'driving' $K_{phyllode}$. Increasing density of primary nerves would decrease the distance travelled in the higher resistance pathways relative to primary nerves, thereby enhancing $K_{phyllode}$. However, a full realization of this benefit requires a coordinated change in the hydraulic conductance of extra-vascular pathways.

The path of water through the extra-vascular tissues remains the subject of some conjecture (Canny 1990; Sack & Holbrook 2006; Ye, Holbrook & Zwieniecki 2008; Heinen, Ye & Chaumont 2009); we examined several structures that may influence this pathway, such as water storage tissue and BSEs. We found no relationship between the ratio of palisade to water storage tissue and $K_{phyllode}$ like that reported between the palisade: spongy ratio and K_{leaf} for diverse dicot leaves (Sack & Frole 2006). This suggests that the water storage tissue does not play a pivotal role in the flow of transpired water in *Acacia* phyllodes.

BSEs may offer an alternative, less tortuous path for water out of the vascular network. Several studies have suggested that water may move rapidly through the connected cells of the BSEs and into the epidermis (Wylie
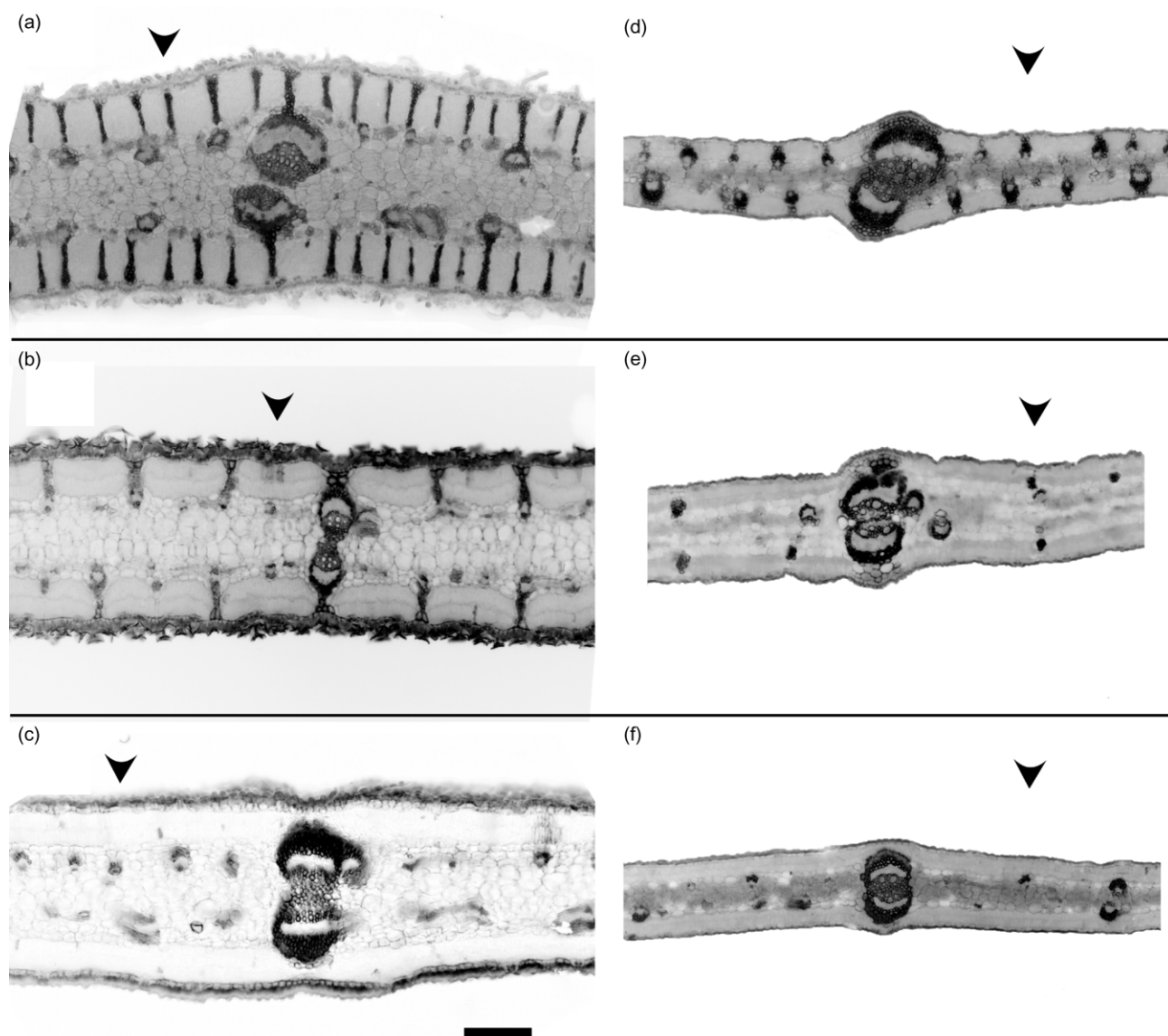
Figure 4. Variation in the length of the bundle sheath extensions (BSEs) of the minor nerves in *Acacia* phyllodes whose origins were distributed differentially along a gradient of mean annual precipitation (MAP). (a) *Acacia calcicola*, MAP = 252 mm; (b) *Acacia ayersiana*, MAP = 455 mm; (c) *Acacia anceps*, MAP = 502 mm; (d) *Acacia melanoxylon*, MAP = 1653 mm; (e) *Acacia cincinnata*, MAP = 1775 mm; (f) *Acacia hylanoma*, MAP = 2650 mm. (a) and (d) bundle sheaths extending from the minor nerves to the epidermis (b) and (e) bundle sheaths extensions of varying lengths (c) and (f) short BSEs. Scale bar is equal to 200 μm. Arrowheads are placed above BSEs. Sections stained with Safranin O and imaged using a Zeiss Axioplan 2 epifluorescence microscope with a Rhodamine 20 filter.

1943, 1952). In these cases, the greater connection between the xylem vessels and the stomata in the epidermis may allow the leaf to operate close to critical xylem water pressures, maximizing opportunities for carbon gain (Zwieniecki *et al*. 2007). *Acacia* phyllodes showed a 15-fold variation in length of the BSE. These bundle sheaths did not always connect to the epidermis, suggesting that BSEs in *Acacia* phyllodes may have an additional role in water movement beyond simply connecting the vascular network to the epidermis. Indeed, $K_{phyllode}$ was significantly correlated with the length of BSEs from minor nerves.

In summary, our results show that $K_{phyllode}$ was correlated with conductance and density of primary nerves and the length of BSEs from minor nerves. We argue that these factors contribute to greater $K_{phyllode}$ by reducing the potential impact of the high resistance pathway through minor nerves. Specifically, we suggest that when minor nerve density is low, high primary nerve conductance increases the efficiency of flow in the major nerve system; high primary nerve density reduces the distance travelled by water in minor nerves while greater length of BSE from minor nerves provides additional pathways for water dispersion. Together, these mechanisms would increase the importance of primary nerve density (rather than minor nerve density) in driving $K_{phyllode}$.

Hydraulic conductance and climate

We found no relationship between SPI and $K_{phyllode}$, possibly reflecting adaptations of the plants to very different climatic conditions (Sack, Tyree & Holbrook 2005). Presumably an *Acacia* adapted to function at a higher vapour pressure difference or lower soil water potential would need a higher $K_{phyllode}$ relative to its SPI, if it were to operate at a given phyllode water potential. However, further work comparing the relationship between SPI and $K_{phyllode}$ in acacias from similar and different environments is required.

$K_{phyllode}$ increased with increasing PMA. While this relationship was driven by increased primary nerve density, high PMA phyllodes were more common in areas receiving lower annual precipitation. We anticipated a relationship between the moisture availability and plant water transport capacity; however, the direction of the observed relationship was the opposite of that hypothesized. A greater maximum capacity for water flow in *Acacia* phyllodes from areas where water is scarce may offer advantages in surviving meagre and unpredictable precipitation.

Thus, a high phyllode conductance may allow a plant to better maintain water transport and mesophyll hydration during transpiration when soil water potential declines. A highly conductive (low resistance) path through the phyllode may prevent large drops in phyllode water potential. This may in turn prevent xylem cavitation and allow continued carbon gain. Bhaskar, Valiente-Banuet & Ackerly (2007) proposed a similar hypothesis to explain why stem hydraulic conductance adjusted for leaf area was higher in plants from areas receiving low summer rainfall relative to related species from areas that received higher summer rainfall. *Acacia* plants with higher $K_{phyllode}$ in drier areas may be able to more swiftly respond to unpredictable rainfall or otherwise maintain water transport in dry periods.

Greater primary vein density also confers increased tolerance of disruption in the venation network. Sack *et al.* (2008) demonstrated that severing the mid-vein of leaves with multiple first-order veins led to only a small drop in leaf hydraulic conductance relative to leaves with only one first-order vein. This suggests that leaves with multiple primary veins would better sustain leaf function following mechanical or herbivore damage. Such an ability would be particularly advantageous in long-lived leaves that are, by chance alone, more likely to encounter damaging forces throughout their life. Westoby *et al.* (2002) showed that foliage with a higher PMA from 218 species (including acacias) across different habitats and continents lived longer. Indeed, in the Glopnet database established by Wright and co-workers (2004), eight *Acacia* species are recorded; their PMA ranging from 105 to 412 g m^{-2} and associated, respectively, with phyllode lifespans ranging from 10 to 37 months. Thus, the increased primary nerve density observed in high PMA phyllodes from drier areas in the present study might also contribute greater tolerance of hydraulic disruption than mesic species.

CONCLUSION

Australian phyllodinous *Acacia* are highly diverse in nerve arrangement and afforded a unique opportunity to test the role of nerve patterns in affecting resistance to water flow across the phyllode lamina. As for true leaves, the hydraulic conductance of the primary nerves was strongly correlated with the overall phyllode hydraulic conductance. However, unlike in leaves, the hydraulic conductance of phyllodes showed a strong correlation with the density of primary nerves but not with the density of minor nerves, possibly because of the conductive role of the BSEs superseding that of minor nerves themselves. Phyllodes with higher hydraulic conductance had higher PMA and were more common in plants originating from drier areas.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. List of studied *Acacia* species and their region of origin according to Hnatiuk & Maslin (1988).

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