

Turning over a new ‘leaf’: multiple functional significances of leaves versus phyllodes in Hawaiian *Acacia koa*

JESSICA PASQUET-KOK^{1,2}, CHRISTINE CREESE¹ & LAWREN SACK¹

¹Department of Ecology and Evolutionary Biology, University of California, Los Angeles, 621 Charles E. Young Drive South, Los Angeles, CA, 90095-1606, USA and ²Department of Biology, University of Bordeaux, UMR BIOGECO, Av. des Facultés, 33405 Talence, France

ABSTRACT

Hawaiian endemic tree *Acacia koa* is a model for heteroblasty with bipinnately compound leaves and phyllodes. Previous studies suggested three hypotheses for their functional differentiation: an advantage of leaves for early growth or shade tolerance, and an advantage of phyllodes for drought tolerance. We tested the ability of these hypotheses to explain differences between leaf types for potted plants in 104 physiological and morphological traits, including gas exchange, structure and composition, hydraulic conductance, and responses to varying light, intercellular CO₂, vapour pressure deficit (VPD) and drought. Leaf types were similar in numerous traits including stomatal pore area per leaf area, leaf area-based gas exchange rates and cuticular conductance. Each hypothesis was directly supported by key differences in function. Leaves had higher mass-based gas exchange rates, while the water storage tissue in phyllodes contributed to greater capacitance per area; phyllodes also showed stronger stomatal closure at high VPD, and higher maximum hydraulic conductance per area, with stronger decline during desiccation and recovery with rehydration. While no single hypothesis completely explained the differences between leaf types, together the three hypotheses explained 91% of differences. These findings indicate that the heteroblasty confers multiple benefits, realized across different developmental stages and environmental contexts.

Key-words: drought; heterophylly; hydraulics; leaf traits; legume; light-response curves; pressure-volume curves; stomata; water storage; xylem.

INTRODUCTION

Numerous plant species exhibit heteroblasty, i.e. distinct juvenile and adult leaf forms, and species of *Acacia* are a model for this phenomenon (Lambers, Chapin & Pons 1998; Taiz & Zeiger 2006). We assessed the functional consequences of heteroblasty in *Acacia koa* (koa; Fig. 1), an ecologically, economically and culturally important endemic species that dominates native forests across the Hawaiian

Islands, from 0–2000 m elevation and 1850–5000 mm annual rainfall (Harrington *et al.* 1995; Anderson *et al.* 2002; Wilkinson & Elevitch 2003; Baker, Scowcroft & Ewel 2009; Baker *et al.* 2009), and is a target for conservation (Goldstein *et al.* 2006; Pejchar & Press 2006; Daily *et al.* 2009). Within 1–2 years, koa seedlings produce bipinnate compound leaves, then transitional forms, followed by phyllodes formed of flattened petiole and rachis (Walters & Bartholomew 1984). The leaflet contains palisade and spongy mesophyll, whereas in the phyllode, the palisade surrounds layers of large cells without chloroplasts (Fig. 1c,f), previously interpreted as ‘spongy mesophyll’ (Walters & Bartholomew 1984; Hansen 1986), although studies of other *Acacias* indicated a water storage function (Boughton 1986).

Previous studies proposed three hypotheses for the functional significance of heteroblasty in *A. koa*: (H1) true leaves are beneficial in early establishment for rapid growth and (H2) for shade tolerance, whereas (H3) phyllodes are better adapted to drought (Walters & Bartholomew 1984; Hansen 1986, 1996; Walters & Bartholomew 1990; Hansen & Steig 1993). Those studies and work on other *Acacias* tested leaf traits to provide support for one or more of these hypotheses (reviewed in *Discussion*). For a first explicit assessment of all three hypotheses, we quantified 104 traits, including traits directly related to plant-scale performance, such as the dynamics of gas exchange, and additional traits relating to structure and composition, with 92 traits in common for both leaf types, of which only 26 traits had been determined in previous studies. A particularly novel focus of our study was on the dynamics during drought and recovery of leaf hydraulic conductance (K_{leaf}) and gas exchange (Brodribb & Holbrook 2006; Sack & Holbrook 2006). We tested 123 expectations for trait differences from the three hypotheses. We followed in the tradition of earlier leaf trait studies (e.g. Hansen 1986, 1996; Givnish 1987; Brodribb & Hill 1993; Ackerly 2004; Brodribb & Feild 2008; Dunbar-Co, Sporck & Sack 2009) by developing explicit expectations for how traits should differ based on previous studies of acclimation of given species or variation among species (Tables 1–3).

The growth hypothesis (H1) generated 33 expectations related to gas exchange and allocation. To promote relative growth rate, leaves would have higher area-, mass- and nitrogen-based gas exchange and electron transport rates,

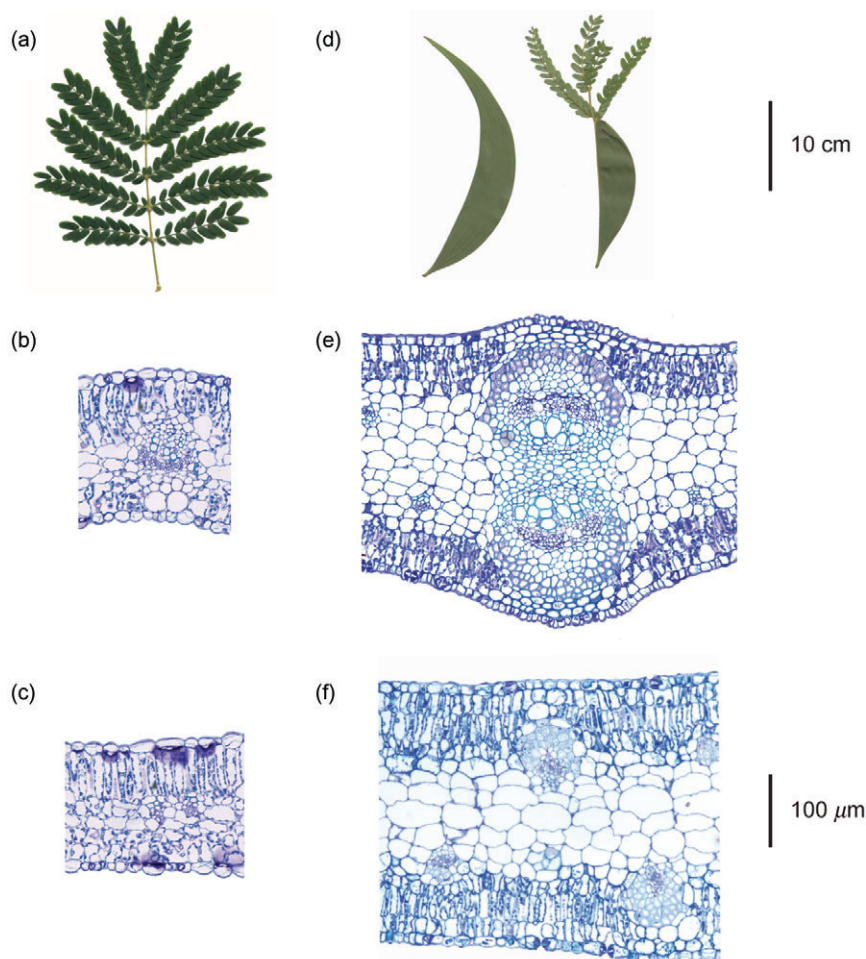


Figure 1. Contrasting morphology and anatomy of true leaves and phyllodes of *A. koa*: a, whole leaf; d, phyllode and transition form; b and e, cross section through the midrib of a leaflet and the central vein of a phyllode, respectively; c and f, cross-section through lamina of leaflet and phyllode. In c and f, note the small veins in cross section, and the achlorophyllous central parenchyma in the phyllode.

and higher saturation and light compensation irradiances (Smith *et al.* 1997; Evans 1998; Pattison, Goldstein & Ares 1998; Walters & Reich 1999; Evans *et al.* 2000; Wright *et al.* 2004; Coste *et al.* 2005; Marino, Aqil & Shipley 2010). Leaves would have higher stomatal pore area and conductance, as well as higher hydraulic capacity (Sack *et al.* 2003a; Dunbar-Co *et al.* 2009). Leaves would be thinner, with lower mass per area (*LMA*), lower C concentration per area and higher saturated water content per mass (Garnier & Laurent 1994; Lambers *et al.* 1998). All else being equal, to promote faster growth, leaves would have palisade rich mesophyll, with higher nutrient concentrations per area and mass, and thus lower C per mass and higher *N/C* (Field & Mooney 1986; Penuelas & Estiarte 1997; Wright & Westoby 2001). Leaves would also have relatively higher allocation to P- than N-containing molecules for faster growth (i.e. lower *N/P*; Elser *et al.* 2000).

The shade hypothesis (H2) generated 35 expectations related to light capture efficiency and reduced tissue costs. For advantage in shade, leaves would have lower compensation and saturation irradiances, lower gas exchange and electron transport rates, lower stomatal density, pore area and conductance, and lower hydraulic capacity (Givnish 1988; Terashima & Evans 1988; Rosati *et al.* 1999; Walters & Reich 1999; Sack *et al.* 2003a; Coste *et al.* 2005, 2009, 2010;

Sack, Tyree & Holbrook 2005; Valladares & Niinemets 2008; Dunbar-Co *et al.* 2009; Shipley *et al.* 2010). As typical for shade foliage, leaves would be hypostomatous, with greater spongy: palisade mesophyll ratio, and thinner epidermis and cuticle (Wylie 1951; Givnish 1988; Smith *et al.* 1997). Leaves would be larger in area and mass but thinner, with lower C per area and *LMA*, lower N per area and per mass and, therefore, higher C per mass (Givnish 1988; Sack, Grubb & Marañón 2003b).

The drought hypothesis (H3) generated 55 expectations. Phyllodes would maintain function at low leaf water potentials, with gas exchange and K_{leaf} able to resist decline and to recover with rehydration (Brodribb & Holbrook 2006). Phyllodes would be smaller and thicker, with thicker tissues and higher *LMA* and nutrient concentrations per area (Smith *et al.* 1997). Phyllodes would have smaller chlorenchyma and epidermal cells and lower cuticular conductance (Cutler, Rains & Loomis 1977; Smith *et al.* 1997; Sack *et al.* 2003a). Phyllodes would be amphistomatic, with higher stomatal density, for effective CO_2 capture across the mesophyll for thick leaves and for effective cooling when water is available (Mott, Gibson & O'Leary 1982; Dunbar-Co *et al.* 2009; Franks, Drake & Beerling 2009). Given their large-celled water storage tissue, phyllodes would have lower density, elastic modulus

Table 1. Traits measured for *Acacia koa* leaf types relating to morphology, composition and stomatal, mesophyll and xylem anatomy; symbols; units; and expectations from each of three hypotheses for given traits, based on the rationale and references in the *Introduction*

Traits	Symbols	Units	H1: Leaves benefit relative growth rate	H2: Leaves contribute shade tolerance	H3: Phyllodes contribute drought tolerance	Leaves/ phyllodes higher? (L/P/ns)
Leaf morphology						
Thickness		mm	P •	P •	P •	P
Density		g cm ⁻³			L •	L
Area and mass ^a		cm ² and g		L •	L •	L
Leaf mass per area	<i>LMA</i>	g m ⁻²	P •	P •	P •	P
Saturated water content	<i>SWC</i>	g g ⁻¹	L			ns
Leaf composition						
Water mass per area		g m ⁻²			P •	P
Nitrogen per area	<i>N_{area}</i>	g m ⁻²	L	P •	P •	P
Nitrogen per mass	<i>N_{mass}</i>	%	L	P •	P •	P
Phosphorus per area	<i>P_{area}</i>	g m ⁻²	P			ns
Phosphorus per mass	<i>P_{mass}</i>	%	L •			L
Carbon per area	<i>C_{area}</i>	g m ⁻²	P •	P •	P •	P
Carbon per mass	<i>C_{mass}</i>	%	P •	L	L	P
Nitrogen: phosphorus ratio	<i>N/P</i>		P •			P
Nitrogen: carbon ratio	<i>N/C</i>		L		P •	P
Carbon isotope ratio	$\delta^{13}\text{C}$	‰			P •	P
Stomatal traits						
% stomatal density adaxial	<i>%SD_{ad}</i>	mm ⁻²		P •	P •	P
Stomatal density, total	<i>SD_{tot}</i>	mm ⁻²		P	P	ns
Guard cell lengths and widths ^{ab}	<i>GCL</i>	μm			L	ns
Pore length, adaxial	<i>PL_{ad}</i>	μm			L •	L
Pore length, abaxial	<i>PL_{ab}</i>	μm			L	ns
% adaxial stomatal pore index	<i>%SPI_{ad}</i>			P •	P •	P
Stomatal pore index, total	<i>SPI_{tot}</i>		L	P		ns
Theoretical stomatal conductance	<i>g_{th}</i>	mol m ⁻² s ⁻¹	L	L		ns
Mesophyll anatomy traits						
Cuticle thickness		μm		P •	P •	P
Epidermal thickness		μm		P	P	ns
Palisade mesophyll thickness		μm	L	P	P	ns
Spongy mesophyll thickness		μm		L •		L
Water storage tissue thickness		μm			P •	P
Cell dimensions						
Epidermal cell width		μm			L •	L
Palisade cell length		μm			L •	L
Palisade cell width		μm			L •	L
Xylem traits						
Theoretical primary vein hydraulic conductivity normalized by leaf area and length	<i>K_t'</i>	mmol m ⁻² s ⁻¹ MPa ⁻¹	L •	P		L
Theoretical minor vein hydraulic conductivity	<i>K_t</i>	mmol m s ⁻¹ MPa ⁻¹	L	P		ns

Under each hypothesis, the expectation of a higher value for leaves or phyllodes is indicated by L or P, respectively. The final column contains the actual significant differences found (see Table S1–S10), with ns indicating no significant difference at $P < 0.05$. The • symbol indicates that the expectation from the given hypothesis was supported for that trait.

^aMultiple traits considered as single traits because of intrinsic correlation.

^bAdaxial and abaxial faces.

and relative water content at turgor loss, and higher water mass per area, capacitance, and osmotic potential; these traits contribute to drought tolerance in soft or succulent-leaved dry area plants (Walter 1985; Schulte 1992; Sack & Tyree 2005). Phyllodes would have higher water use efficiency, associated with higher N (and lower C) per mass, higher carbon isotope ratio, and stronger and more

rapid stomatal closure under high vapour pressure deficit (Hansen & Steig 1993; Franks & Farquhar 1999; Wright, Reich & Westoby 2001; Dunbar-Co *et al.* 2009).

We quantified the ability of the growth, shade and drought hypotheses singly and in combination to account for trait differences between the leaf types.

Table 2. Traits measured for *Acacia koa* leaf types relating to drought tolerance; symbols; units; and expectations from each of three hypotheses for given traits, based on the rationale and references in the *Introduction*

Traits	Symbols	Units	H1: Leaves benefit relative growth rate	H2: Leaves contribute shade tolerance	H3: Phyllodes contribute drought tolerance	Leaves/phyllodes higher? (L/P/ns)
Cuticular conductance	g_{\min}	$\text{mmol m}^{-2} \text{s}^{-1}$		L		ns
Pressure volume parameters						
Osmotic potential full turgor	π_{ft}	MPa		P		ns
Turgor loss point	π_{tlp}	MPa		L		ns
Elastic modulus	ϵ	MPa		L•		L
Relative capacitance at full turgor	C_{ft}	MPa^{-1}		P•		P
Relative capacitance at turgor loss	C_{tlp}	MPa^{-1}		P		ns
Absolute capacitance per leaf area, at full turgor	C_{ft}^*	$\text{g m}^{-2} \text{MPa}^{-1}$		P•		P
Absolute capacitance per leaf area, at turgor loss	C_{tlp}^*	$\text{g m}^{-2} \text{MPa}^{-1}$		P•		P
Relative water content at turgor loss point	RWC_{tlp}	%		L•		L
Parameters of drought responses						
Leaf hydraulic conductance at full turgor	$K_{\text{leaf,max}}$	$\text{mmol m}^{-2} \text{s}^{-1} \text{MPa}$	L	P•		P
Leaf water potential for transpiring leaf, moist soil	$\psi_{\text{leaf, moist soil}}$	MPa		L		ns
Leaf hydraulic conductance at $\psi_{\text{leaf, moist soil}}$	$K_{\text{leaf, moist soil}}$	$\text{mmol m}^{-2} \text{s}^{-1} \text{MPa}$	L	P•		P
ψ at 20% loss of $K_{\text{leaf, moist soil}}$		MPa			L	ns
ψ at 50% loss of $K_{\text{leaf, moist soil}}$		MPa			L	P
ψ at 80% loss of $K_{\text{leaf, moist soil}}$		MPa			L	P
Stomatal conductance						
ψ at 20% loss of g_{\max}		MPa		L		P
ψ at 50% loss of g_{\max}		MPa		L		ns
ψ at 80% loss of g_{\max}		MPa		L		ns
Photosynthetic carbon assimilation rate						
ψ at 20% loss of maximum light-saturated rate		MPa		L		P
ψ at 50% loss of maximum light-saturated rate		MPa		L		ns
ψ at 80% loss of maximum light-saturated rate		MPa		L		ns
Recovery after rehydration						
Recovery in water potential for rehydrated shoots		MPa		P		ns
Recovery of leaf hydraulic conductance for rehydrated shoots		%		P•		P
Recovery of leaf hydraulic conductance for rehydrated plants		%		P•		P
Recovery of stomatal conductance for rehydrated plants		%		P		ns
Recovery of photosynthetic rate for rehydrated plants		%		P		ns

Under each hypothesis, the expectation of a higher value for leaves or phyllodes is indicated by L or P, respectively. The final column contains the actual significant differences found (see Table S1–S10), with ns indicating no significant difference at $P < 0.05$. The • symbol indicates that the expectation from the given hypothesis was supported for that trait.

Table 3. Traits measured for *Acacia koa* leaf types relating to gas exchange, and its response to irradiance, CO₂ and vapour pressure deficit; symbols; units, and expectations from each of three hypotheses for given traits, based on the rationale and references in the *Introduction*. Under each hypothesis, the expectation of a higher value for leaves or phyllodes is indicated by L or P, respectively

Traits	Symbols	Units	H1: Leaves benefit relative growth rate	H2: Leaves contribute shade tolerance	H3: Phyllodes contribute drought tolerance	Leaves/phyllodes higher? (L/P/ns)
Maximum gas exchange and light response parameters						
Quantum efficiency per area	QE_{area}	mol CO ₂ (mol photons) ⁻¹	L	L		ns
Quantum efficiency per mass	QE_{mass}	mmol CO ₂ g ⁻¹ (mol photons m ⁻²) ⁻¹	L •	L •		L
Respiration rate per area	R_{area}	μmol m ⁻² s ⁻¹	L	P		ns
Respiration rate per mass	R_{mass}	nmol CO ₂ g ⁻¹ s ⁻¹	L	P		ns ^a
Light saturated assimilation per area	A_{area}	μmol m ⁻² s ⁻¹	L	P		ns
Light saturated assimilation per mass	A_{mass}	μmol g ⁻¹ s ⁻¹	L •	P		L
Light saturated assimilation per nitrogen	A/N	mmol (mol N) ⁻¹ s ⁻¹	L •	P		L
Saturation irradiance	I_s	μmol photons m ⁻² s ⁻¹	L	P		ns ^b
Light compensation point	I_c	μmol photons m ⁻² s ⁻¹	L	P		ns ^b
Maximum stomatal conductance	g_{max}	mol m ⁻² s ⁻¹	L	P		ns
Instantaneous water use efficiency	WUE	mmol H ₂ O (mol CO ₂) ⁻¹	L	P	P	ns
A-c, parameters						
Maximum carboxylation velocity per area	$V_{c,\text{max}}$	μmol m ⁻² s ⁻¹	L •	P		L
Electron transport capacity	J_{max}	μmol e ⁻ m ⁻² s ⁻¹	L •	P		L
Quantum efficiency of photosystem II	Φ_{PSII}		L	L		ns ^b
Maximum carboxylation velocity per area per nitrogen	$V_{c,\text{max}}/N$	μmol CO ₂ (mol N) ⁻¹ s ⁻¹	L •	P		L
Electron transport capacity per nitrogen	J_{max}/N	mmol e ⁻ (mol N) ⁻¹ s ⁻¹	L •	P		L
VPD parameters						
Stomatal conductance ratio	g_2/g_1				L •	L
Photosynthetic rate ratio	A_2/A_1				L	ns
Transpiration rate ratio	E_2/E_1			L		ns
Time for 50% stomatal closure	$t_{50\%}$	min		L		ns
Time for 95% stomatal closure	$t_{95\%}$	min		L		ns
Time of 'wrong-way response'	t_{WWR}	min		L		ns

The final column contains the actual significant differences found (see Table S1–S10), with ns indicating no significant difference at $P < 0.05$. The • symbol indicates that the expectation from the given hypothesis was supported for that trait.

^aValues were significantly higher for leaflets than for phyllodes, and empirically but not significantly higher for the whole-leaf than the phyllode (see *Results*).

^bComparison made between the value for the leaflet and that for the phyllode, rather than that for the whole leaf and that for the phyllode.

METHODS AND MATERIALS (FOR ADDITIONAL DETAILS, SEE APPENDIX S1)

Plant material

Plants were grown from seed collected in the forest in Hawai'i Volcanoes National Park, Island of Hawai'i, in November 2007 (precipitation > 1000 mm year⁻¹). At the time of measurements, September 2008 to July 2009, plants ranged 1–2 m in height and 1–2 cm in diameter at 1 cm from the base. Leaf types were generally compared on the same plants, except for vapour pressure deficit responses, and for hydraulic and drought responses that required numerous shoots.

Leaf morphology

We determined thickness, area, mass and mass per area for leaf rachis, leaflets and phyllodes, averaging for one to two leaves and phyllodes for each of seven to eight plants. For leaflet and rachis area fractions, we divided their area by the total leaf area; we calculated mass fractions similarly using mass values. We calculated density as mass per area divided by thickness.

Leaf composition

For dried leaflet rachis and lamina and phyllodes we determined carbon, nitrogen, and phosphorus per mass, and carbon isotope ratio (C_{mass} , N_{mass} , P_{mass} and $\delta^{13}\text{C}$), N/P and N/C ratios, and concentrations per area (C_{area} , N_{area} and P_{area}), as mass-based concentrations \times mass per area.

Anatomical traits: stomatal traits

One leaf and phyllode from each of six plants were measured for stomatal traits using microscopy of impressions from nail varnish peels. We determined adaxial and abaxial stomatal densities, guard cell and pore lengths, guard cell complex widths, and an index of stomatal pore area per leaf area ($SPI = \text{stomatal density} \times \text{pore length}^2$; Sack *et al.* 2003a, 2006), and theoretical stomatal conductance (g_{th} ; after Franks & Farquhar 2007).

Anatomical traits: mesophyll and xylem traits

One leaf and phyllode from each of five plants were measured for cross-sectional thickness of the lamina, epidermises, and cuticle, and of palisade, spongy and water storage parenchyma. We also measured cell widths and heights for epidermis and palisade parenchyma, and cell diameters for spongy mesophyll and water storage cells. We averaged three measurements of each type for each cross-section. Additionally, we measured xylem cross-sectional traits for a primary vein (the leaflet midrib and the central phyllode primary vein, which includes two isobilateral bundles; Fig. 1b,e) and for a minor vein (Fig. 1c,f). We determined vessel number and mean maximum diameter, theoretical

vein hydraulic conductivity (K_i ; mmol m s⁻¹ MPa⁻¹), and, for the midrib, area- and length-normalized theoretical hydraulic conductivity (K'_i ; mmol m⁻² s⁻¹ MPa⁻¹).

Gas exchange: light response curves

Photosynthetic light response curves were measured for leaves and phyllodes on 13 plants, between 0900 and 1600 (using a LI-6400 XT; Li-Cor, Lincoln, NE, USA). We determined stomatal conductance (g_{max}), and maximum photosynthetic rate, dark respiration and quantum efficiency per area (A_{area} , R_{area} and QE_{area}) and per mass (A_{mass} , R_{mass} and QE_{mass}); these measures were calculated as the area-based values divided by leaf mass per area), light compensation point (I_c), saturation irradiance (I_s), assimilation rate per nitrogen (A/N) and water use efficiency ($WUE = A_{\text{area}}/\text{transpiration per area}$).

Gas exchange: photosynthetic assimilation – c_i response curves

For both leaf types on 11 plants we determined photosynthetic CO₂ responses between 0900 and 1600 (using a LI-6400 XT with 6400-40 Leaf Chamber Fluorometer), including maximum carboxylation rate ($V_{c,\text{max}}$), electron transport capacity (J_{max}), and $V_{c,\text{max}}/N$ and J_{max}/N . We also determined the quantum yield of photosystem II (Φ_{PSII}) at chamber [CO₂] of 400 ppm.

Gas exchange: responses to vapour pressure deficit

In July 2009 we compared leaf and phyllode responses to vapour pressure deficit for leaves and phyllodes from five plants (Franks & Farquhar 1999; LI-6400 XT). Measurements were made from 0730–1630 h, at 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetically active radiation, 25 °C, and 400 ppm CO₂; we logged gas exchange values each minute. Leaves and phyllodes stabilized in stomatal conductance (g), photosynthetic rate (A) and transpiration rate (E) after 1–2 h at vapour pressure deficit (VPD) of 1 kPa and then the infrared gas analysers (IRGAs) were matched and g , E and A for VPD of 1 kPa (g_1 , E_1 and A_1) were determined as the average of five stable values and the VPD was switched to 2 kPa. In the typical response curve, when VPD was changed to 2 kPa, stomata opened transiently (the 'wrong-way response'; cf. Powles *et al.* 2006), before declining progressively and stabilizing. We determined g_2 , A_2 and E_2 by averaging 10 stable readings; our criteria for stability were 10 min with a coefficient of variation <5% (in two cases, 6 and 9%), and without a directional trend (i.e. an R^2 value for the plot against time not significant at $P = 0.05$). Our measure of VPD responsiveness is thus a characterization of the first stomatal closure response; after this period, the stomata behaved unpredictably, sometimes trending upward or downward, or fluctuating over the following hour. The sensitivity of g , E and A were calculated as g_2/g_1 ,

E_2/E_1 and A_2/A_1 , respectively; lower ratio values indicate greater sensitivity. We also quantified the timing of stomatal responses, as the duration of the wrong-way response (t_{WWR}), and the times taken for stomata to close by 50 and 95% of their total response from the time of the change in VPD ($t_{50\%}$ and $t_{95\%}$).

Drought tolerance traits: cuticular conductance

Cuticular conductance (g_{min} *sensu* Kerstiens 1996) was determined for one leaf and one phyllode from each of 13 saplings, by intermittently weighing during drying.

Drought tolerance traits: pressure volume curves

Pressure-volume curve parameters were determined for leaves and phyllodes from five to seven plants, using the bench-drying method (Koide *et al.* 2000; Sack *et al.* 2003a). We determined the saturated water content (SWC), osmotic potential at full turgor and at the turgor loss point (π_{ft} and π_{tip}), relative water content at turgor loss point (RWC_{tip}), elastic modulus (ϵ), relative capacitances ($\Delta\text{RWC}/\Delta\Psi_{\text{leaf}}$) above and below turgor loss point (C_{ft} and C_{tip}), and leaf area-specific absolute capacitances (C_{ft}^* and C_{tip}^*).

Leaf hydraulic conductance, vulnerability and recovery

We measured leaf and phyllode hydraulic conductance (K_{leaf}) using the evaporative flux method (Sack *et al.* 2002). K_{leaf} was calculated for excised leaves as the steady-state transpirational flow rate (E , $\text{mmol m}^{-2} \text{s}^{-1}$) divided by the water potential driving force ($\Delta\Psi_{\text{leaf}} = -\Psi_{\text{leaf}}$; MPa), further normalized by leaf area (Li-Cor 3100 leaf area meter). We constructed hydraulic vulnerability curves for K_{leaf} , from bench-dried shoots and droughted plants, with leaves and phyllodes ranging from full turgor to strong desiccation; we plotted K_{leaf} against Ψ and fitted a sigmoidal curve.

Seven shoots desiccated below turgor loss point were used to test for recovery of K_{leaf} with rehydration (after Trifilo *et al.* 2003). From shoots with six leaves or phyllodes, two were excised and measured for 'dehydrated' Ψ_{leaf} , and the rest of the shoot was excised under distilled water, covered with plastic to rehydrate 1 h, then removed for 30 min equilibration in a plastic bag. Two leaves were excised and measured for the rehydrated Ψ_{leaf} , and the remaining two re-cut under distilled water for K_{leaf} measurement.

Responses to drought and recovery in leaf hydraulic conductance and gas exchange

We determined the drought responses of K_{leaf} ($n = 51$ – 52), g ($n = 51$ – 74) and A_{area} ($n = 24$ – 29). Groups of plants were droughted 3–5 d until severe wilting; control plants were

watered each day. Each day between 0900 and 1800 h, for one to three leaves and phyllodes on droughted and control plants, we measured g (both surfaces; Delta-T Devices porometer, Cambridge, UK) and, for different plants, A_{area} (LI-6400 XT, with $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$, RH of 60–80%, 25 °C and $[\text{CO}_2]$ of 400 ppm). For each measured plant, two leaves of each type were collected in plastic bags for Ψ_{leaf} determination. Additionally, for hydraulic responses, shoots of three leaves of a given type were excised and bagged and two were measured for Ψ_{leaf} , the third for K_{leaf} . We also estimated Ψ_{soil} , by placing plastic bags on two leaves and two phyllodes, and allowing the plant and soil to equilibrate in a plastic bag at least 1 h. We plotted the Ψ_{leaf} against Ψ_{soil} for leaves and phyllodes during drought; extrapolating to the y axis indicated the Ψ_{leaf} for transpiring plants in moist soil ($\Psi_{\text{leaf, moist soil}}$).

For K_{leaf} and g , data were binned into Ψ_{leaf} intervals of 0.25 to 0.50 MPa. We plotted sigmoidal responses of K_{leaf} , g and A_{area} to Ψ_{leaf} and determined maximum values, as well as the Ψ_{leaf} at which K_{leaf} , g and A_{area} declined by 20, 50 and 80% of their values at $\Psi_{\text{leaf, moist soil}}$. We used $\Psi_{\text{leaf, moist soil}}$ as the basis for describing responses because K_{leaf} for the phyllodes was very high at full turgor (see *Results*).

When both leaf types were severely wilted, plants were watered to field capacity each day for 5 d, and then measured for the recovery of Ψ_{leaf} , K_{leaf} , g and A_{area} .

Statistical analyses

Differences between leaf types were determined using *t*-tests (Sokal & Rohlf 1995), paired when replicate leaf types were compared on the same plants (Table 1; Minitab Release 15, State College, PA, USA). Functions were fitted to data using SigmaPlot 10 (Systat; San Jose, CA, USA).

We measured 104 traits, 92 in common for leaf types (Tables S1–S10); of these, several were intrinsically related (e.g. leaf mass and area; adaxial and abaxial guard cell dimensions) and some were used to determine higher-level traits (adaxial and abaxial stomatal densities and pore areas were added to determine total values; xylem conduit sizes and numbers were used to determine conductivities), and thus 81 traits were used to test hypothesized expectations for which leaf type would have the higher value (Tables 1–3). For each hypothesis, we generated expectations based on the previous literature (see *Introduction*).

This comprehensive analysis involved multiple significance tests. Because we only tested *a priori* hypotheses rather than mining data, we maintained a 5% significance level in our tests. However, we tested whether the overall proportion of significant differences was greater than the 5% expected from chance to confirm non-random trait differences between leaf types overall (Waite & Sack 2010). We used a simple multivariate procedure for examining the relative success of three hypotheses in accounting for differences between two leaf types, using probability theory (Tijms 2007). Each hypothesis led to expectations of a significantly higher trait value for one leaf type. For each hypothesis, we quantified proportions of the expectations

that were supported, and tested if these were greater than would arise from chance (proportion tests, Minitab Release 15). For each hypothesis, we calculated the ‘specific predictive power’ as the percentage of its trait expectations that were supported (from Tables 1–3), and the ‘overall predictive power’ as the percentage of *all* the tested traits for which that hypothesis made a correct prediction. We calculated the predictive power of the three hypotheses combined as the percentage of traits for which at least one hypothesis made a correct prediction. The predictive power, being based on expectations of trait differences, would be reduced by traits being similar between leaf types. We additionally quantified the ‘explanatory power’ of each hypothesis in the same way as for predictive power, but based only on traits with significant differences. We tested our proportions against ‘null models’ for the percentage of trait expectations that would be supported by chance (see *Supplementary Materials*). Note that we follow previous studies of *Acacia* leaf types in focusing on the potential function of traits, rather than their evolution. We cannot discover in our data evolutionary explanations for the origin of the heteroblasty; *A. koa* derives from a Pacific Island or Australian species that already had leaves and phyllodes (Baker *et al.* 2009). Rather, we focused on the extent to which leaf type differences in *A. koa*, a model for heteroblasty, supported the hypotheses for the differential function of the leaf types.

RESULTS

Leaf morphology

Leaves and phyllodes differed strongly in morphology. Leaves were four- to fivefold greater in mass and area (Table S1; Fig. 1). For leaves, 84% of the area and 62% of mass were in leaflets, with the remainder in rachis, which made up *c.* 40% of the *LMA*. Phyllodes had more than double the thickness and *LMA* of the leaflets, and 23% higher *LMA* than whole leaves.

Mesophyll, stomatal and xylem anatomy of leaflets and phyllodes

Leaf types showed major anatomical differences (Fig. 1). The most obvious difference was the eight-cell-layer, 155 μm thick parenchyma lacking chloroplasts in the centre of the phyllode, accounting for half its thickness, with cell diameters 56% greater than those in leaflet spongy mesophyll (Table 4). The phyllode had two palisade layers surrounding the water storage tissue, whereas leaflets had a five-cell, 72 μm thick spongy mesophyll layer beneath the palisade. The leaflets had palisade cells 23% wider and 68% longer than those of phyllodes, but the palisade tissues were statistically similar in thickness. The epidermal cells were 63–72% wider for abaxial and adaxial epidermis in leaflets but similar in height (thus epidermal thickness) for leaflets

Table 4. Assessing three hypotheses (H1, H2 and H3) for predicting and explaining the trait differences between *Acacia koa* heteroblastic leaf types

	H1: Leaves benefit relative growth rate	H2: Leaves contribute shade tolerance	H3: Phyllodes contribute drought tolerance	All three hypotheses ^a
Number of traits for which expectations were generated	33	35	55	81
Number of traits for which expectations were generated and that differed significantly between leaf types	19	21	31	44
Number of traits with expectations supported (i.e., that differed significantly between leaf types in the way expected)	14	13	26	40
Hypothesis specific predictive power (%) ^b	42**	37**	47**	
Hypothesis specific explanatory power (%) ^c	74*	62 ^{ns}	84**	
Hypothesis overall predictive power (%) ^d	17	16	32	49**
Hypothesis overall explanatory power (%) ^e	32	30	59	91**

Trait expectations generated, numbers of traits that differed significantly between leaf types, and those that differed in the ways expected, and predictive power and explanatory power ^{a–e}, calculated based on expectations and findings summarized in Tables 1–3. Proportions were tested against null models for chance effects^f: ^{ns}, $P > 0.05$; * $P < 0.05$; ** $P < 0.001$.

^aPredictive and explanatory power were calculated for all three hypotheses combined by counting success if at least one hypothesis provided a correct expectation.

^bSpecific predictive power is the number of trait expectations supported as a proportion of the number of trait expectations generated from that hypothesis.

^cSpecific explanatory power is the number of trait expectations supported as a proportion of the number of trait expectations generated from that hypothesis, for only those traits that differed significantly between leaf types.

^dOverall predictive power is the number of trait expectations supported divided by 81, the total number of traits for which expectations were generated.

^eOverall explanatory power is the number of traits with expectations supported divided by 44, the total number of traits for which expectations were generated and that differed significantly between leaf types.

^fThe null model for predictive power was 0.025 for individual hypotheses and 0.038 for all hypotheses combined. The null model for explanatory power was 0.5 for individual hypotheses and 0.64 for all hypotheses combined (see Methods and *Supplementary Material*).

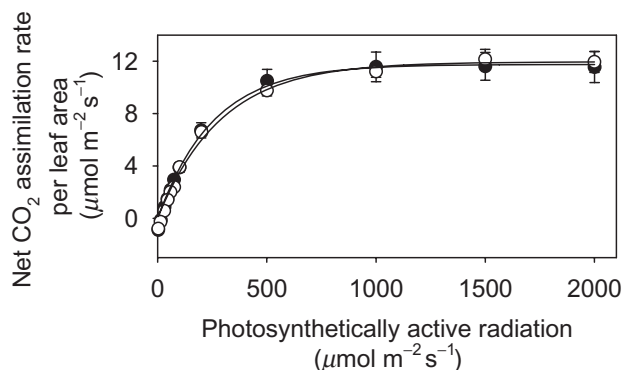


Figure 2. Photosynthetic light response curves for *A. koa* leaflets (closed symbols) and phyllodes (open symbols); exponential curves fitted, $y = a \times (1 - e^{(-b \times x)})$; for leaflets, $a = 11.8$, $b = 0.0039$; $R^2 = 0.995$; $P < 0.001$; for phyllodes, $a = 11.9$, $b = 0.0035$; $R^2 = 0.995$; $P < 0.001$, each point an average for 13 plants.

and phyllodes. The phyllode had a two to threefold thicker adaxial and abaxial cuticle than the leaflet (Table S4).

Leaves and phyllodes differed strongly in stomatal distribution (Table S3). The phyllodes were amphistomatic and the leaves virtually hypostomatic, with twice the abaxial stomatal density as phyllodes. Thus, the leaf types were statistically similar in total stomatal density, stomatal pore index and theoretical stomatal conductance.

The phyllode midrib had 39-fold higher K_t than that of the leaflet, due to a fivefold higher conduit number, with these on average c. twofold greater in maximum diameter. However, when K_t was normalized by leaf area and length and number of primary veins (four to five in the phyllodes), the leaflet midrib had 13-fold higher K'_t . The minor veins of the two leaf types did not differ significantly in conduit numbers, size, or K_t (Table S5).

Leaf composition

Leaves and phyllodes differed strongly in composition. Consistent with their greater thickness, phyllodes were 24% and 49% higher in C_{area} and N_{area} than leaves, though the leaf types were statistically similar in P_{area} (Table S2). Phyllodes allocated strongly to water storage, and had lower mass-based nutrient concentrations than leaflets. The rachis, and thus whole leaves had lower N_{mass} and C_{mass} , and high P_{mass} relative to phyllodes. Stoichiometry differed among leaf types; phyllodes had higher N/P than leaflets and whole leaves. Leaflets and phyllodes were similar in N/C but due to low rachis N values, leaves had lower N/C .

Leaves and phyllodes were similar in saturated water content, but phyllodes had 34% higher water mass per leaf area and 1.1% higher $\delta^{13}C$ than leaves.

Drought tolerance traits: pressure volume curve parameters and cuticular conductance

The leaf types had similarly low values for g_{min} , 3.6–4.0 $mmol\ m^{-2}\ s^{-1}$, and moderate π_{ft} of -1.2 to -1.3 MPa and

π_{tip} of -1.4 to -1.5 MPa. Consistent with their having large-celled water storage tissue, phyllodes had 48% lower ϵ , 39–102% higher C_{ft} , C_{tip} , C_{ft}^* , and C_{tip}^* , and 9% lower RWC_{tip} (Table S6).

Gas exchange responses to light, CO₂ and vapour pressure deficit

Leaflets and phyllodes had virtually identical light response curves considered per area (Fig. 2; Table S8), and were similar in QE_{area} , R_{area} , A_{area} , g_{max} , WUE , I_c and I_s . Whole leaves would also be similar to phyllodes, whether the rachis had area-based gas exchange rates like those of the leaflets or much lower; when we assumed negligible gas exchange by the rachis we also found statistically similar values for whole leaves as phyllodes. By contrast, due to their lower LMA , leaflets had higher mass-based gas exchange parameters; A_{mass} , QE_{mass} , and R_{mass} were 2.4- to 2.9- fold higher, and leaflets had 2.6-fold higher A/N . Whole leaves also had significantly higher A_{mass} , QE_{mass} and A/N than phyllodes, even when estimated conservatively assuming the rachis to have negligible gas exchange; the whole leaf R_{mass} was empirically but not significantly higher than phyllode in this estimation. Further, in their CO₂ responses, leaves showed two- to sevenfold higher $V_{c,max}$, J_{max} , Φ_{PSII} , $V_{c,max}/N$ and J_{max}/N than phyllodes (Table S9). In response to VPD, the phyllodes showed stronger closure, with mean \pm SE for g_2/g_1 of 0.50 ± 0.078 by comparison with 0.72 ± 0.068 for leaves (Fig. 3; Table S10). Leaves and phyllodes did not differ significantly in the other indices of gas exchange responses to VPD (i.e. A_2/A_1 and E_2/E_1) or the timing of these responses,

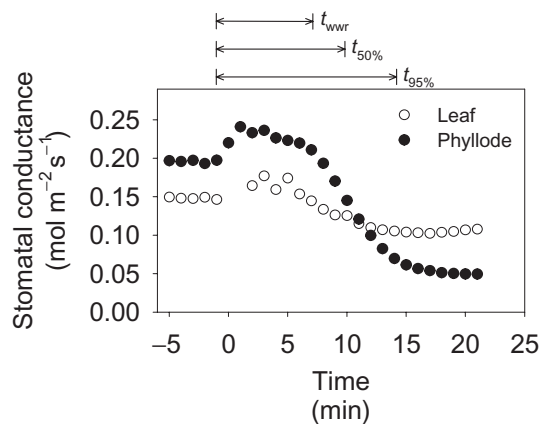


Figure 3. A typical trajectory of the response of stomatal conductance to vapour pressure deficit (VPD) for *A. koa* leaflets (open symbols) and phyllode (closed symbols). Zero on the x-axis is the time at which VPD was stable at 1 kPa and switched to 2 kPa, and the arrows above the graph illustrate the intervals used for the phyllode to determine the duration of the wrong-way response (t_{wWR}), the time taken for stomata to close by 50% of their response ($t_{50\%}$) and by 95% of their response ($t_{95\%}$). The missing points in the trajectory for the leaflets reflect off-scale outlier points at the beginning of the wrong-way response.

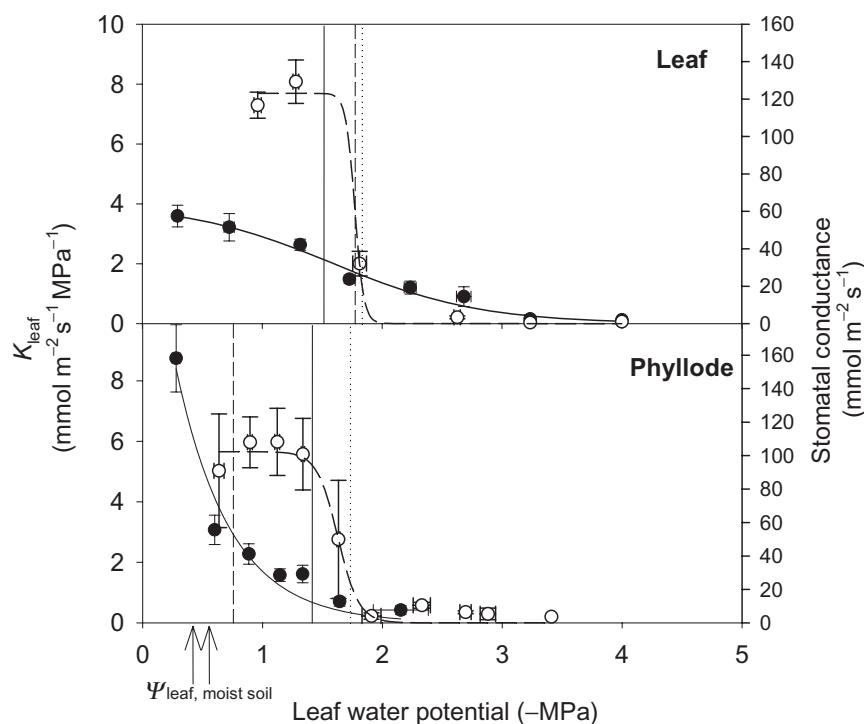


Figure 4. The response of leaf hydraulic conductance (K_{leaf}) and stomatal conductance (g) to declining leaf water potential (Ψ_{leaf}) for leaves and phyllodes of *A. koa*, during drought, including control plants, with fitted sigmoidal curve K_{leaf} or $g = \frac{a}{1 + e^{-(\Psi_{\text{leaf}} - x_0)/b}}$; for K_{leaf} , a , b and x_0 were 4.01, -0.614 , and 1.59 , respectively, for leaves ($R^2 = 0.97$; $P = 0.0002$, 155 measurements in 0.5 MPa bins) and 790 , -0.449 , and -1.76 for phyllodes ($R^2 = 0.96$; $P = 0.002$, 120 measurements in 0.25 MPa bins as data covered a narrower range); for g , a , b and x_0 were 13.0 , -0.0376 , and 1.77 for leaves ($R^2 = 0.995$; $P = 0.0004$, 51 measurements) and 102 , -0.0877 and 1.63 for phyllodes ($R^2 = 0.93$; $P < 0.0001$, 74 measurements). Left and right arrows indicate the Ψ_{leaf} typical for transpiring phyllodes and leaves, respectively. The solid line represents Ψ_{leaf} at turgor loss point, the long dashes at 50% loss of K_{leaf} , and the dotted line at 80% stomatal closure.

being statistically similar in t_{WWR} (overall mean \pm SE 8.4 ± 1.6 min), $t_{50\%}$ (13 ± 1.8 min), and $t_{95\%}$ (20 ± 4.3 min).

Leaf hydraulic conductance and the drought response of hydraulics and gas exchange

Leaves and phyllodes differed strongly in K_{leaf} and its response to desiccation. The K_{leaf} vulnerability curves for leaves and phyllodes were combined (Fig. 4) because they were similar for bench dried shoots and droughted plants; a three-parameter sigmoidal function fitted the data for leaves and phyllodes ($R^2 = 0.93\text{--}0.996$, $P = 0.0007\text{--}0.017$), and the parameters did not differ significantly for bench-dried versus droughted plants for either leaf type ($P = 0.48\text{--}0.998$; t -tests). This similarity in the curves indicated that when shoots were cut from droughted plants, any additional embolism introduced into long vessels did not result in a reduced K_{leaf} relative to bench dried shoots without potentially introduced embolism. While the responses of leaves and phyllodes were both fitted by a sigmoid function, the phyllode showed an initially exponential or linear decline. The leaves and phyllodes had, respectively, maximum K_{leaf} (i.e. at full hydration) of 3.73 and 15.5 $\text{mmol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$, with 50% declines at Ψ_{leaf} of -1.78 and -0.74 MPa (Fig. 4).

The leaf types operated at similar Ψ_{leaf} during transpiration in moist soil. Extrapolating the y -intercept of the $-\Psi_{\text{leaf}}$ versus $-\Psi_{\text{soil}}$ plots for the drought experiments allowed estimation of ' $\Psi_{\text{leaf, moist soil}}$ ', -0.43 and -0.55 MPa (for leaves, $n = 72$ measurements in nine 0.5 MPa bins; for phyllodes, $n = 54$ in 11, 0.25 MPa bins; $R^2 = 0.81\text{--}0.99$, $P \leq 0.0001$).

The leaf types both maintained stomata open for gas exchange after strong declines in K_{leaf} . The K_{leaf} of

leaves and phyllodes declined by greater than 50% before g and A_{area} declined. The g and A_{area} showed sigmoidal responses to declining Ψ_{leaf} for both leaf types (Figs 4 & 5), remaining stable until Ψ_{leaf} was -1.4 to -1.6 MPa, and

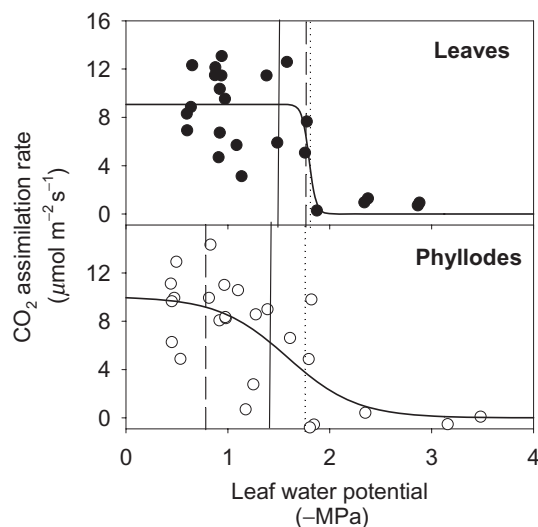


Figure 5. The response of light-saturated carbon assimilation rate to declining leaf water potential (Ψ_{leaf}) for leaves and phyllodes of *A. koa*, during drought, and including control plants, and sigmoidal curves fitted as in Fig. 3; for leaflets (closed symbols), a , b and x_0 were 9.08 , -0.0341 , and 1.80 , respectively ($R^2 = 0.61$; $P < 0.001$, $n = 24$), and phyllodes (open symbols) 10.0 , -0.340 , and 1.58 ($R^2 = 0.64$; $P < 0.001$, $n = 29$ points including points at low water potential off the scale). The solid line represents Ψ_{leaf} at turgor loss point, the long dashes at 50% loss of K_{leaf} , and the dotted line at 80% stomatal closure.

then declining by 80% within 0.3–0.5 MPa below π_{tip} (Table S7).

The impacts of the progressive drought treatments were verified against four well-watered control plants measured on the same days; K_{leaf} , g and A_{area} were independent of drought treatment time ($n = 8\text{--}19$, $R^2 = 0.006\text{--}0.14$, $P = 0.18\text{--}0.76$).

Functional recovery of desiccated leaves and of rewatered plants after drought

We found a limited ability of strongly desiccated leaves and phyllodes to recover after rehydration in experiments on excised shoots and droughted plants. For desiccated shoots with leaf and phyllode Ψ_{leaf} of on average -1.9 and -2.2 MPa, placed with cut stem ends in water for 1 h, Ψ_{leaf} recovered by 0.5–0.6 MPa. The K_{leaf} increased only 4.7% in phyllodes, consistent with its vulnerability curve, whereas for leaves, K_{leaf} did not recover significantly (Fig. 6a,b and Table S7). For plants that were droughted until leaves and phyllodes were severely wilted, and rewatered to field capacity for five days, plants recovered partially or completely to the level of control leaves in Ψ_{leaf} . As in the experiments on excised shoots, for phyllodes the K_{leaf} recovered marginally, whereas for leaves K_{leaf} did not recover at all (Fig. 6c,d). However, g recovered in both leaf types by 79–87%, and A_{area} recovered to the levels expected from their trajectories against Ψ_{leaf} during the drought (Fig. 6e–h and Table S7).

Testing trait differences with the growth, shade and drought hypotheses

We found significant differences between leaf types for 54% of the 81 traits used to test hypotheses (more than the 5% expected due to chance; $P < 0.001$). The growth, shade and drought hypotheses applied singly had specific and overall predictive powers of, respectively, 37–47% and 16–32%; applying all three hypotheses was successful for predicting 49% of all of the leaf type differences (overall predictive power). These predictive powers were reduced because of the numerous trait similarities between the leaf types (Table 4). Focusing only on trait differences, the three hypotheses applied individually had specific and overall explanatory power of, respectively, 62–84% and 30–59%; applying the three hypotheses together was successful for explaining 91% of the leaf type differences (overall explanatory power; $P < 0.001$).

DISCUSSION

A. koa leaf types showed strong similarities and differences. We relate these findings to previous work on *Acacia* species, highlighting the novel findings and their significance. Additionally, we show how the growth, shade and drought hypotheses were successful in predicting and explaining trait differences.

Similarities in structure and function between leaf types

The leaf types were similar in 46% of traits (i.e. 44/92 traits measured in common; 37/81 used to test expectations), remarkable given the differences in gross form between leaf types and their development from different tissues (Boke 1940), suggesting important constraints. Presumably, selection would be parallel for several traits in open-grown plants, as leaf types would face similar canopy microclimates and carbon demand per area. We found no significant difference between leaf types in Ψ_{leaf} , moist soil, A_{area} , g_{max} , WUE or photosynthetic light response parameters. Previous studies of *A. koa* and other species also reported leaf types to have similar g_{max} and A_{area} , and light response curves, or relatively minor differences, for example, g_{max} 16–19% higher for leaves, or A_{area} 12% higher for phyllodes (Walters & Bartholomew 1984; Hansen 1986, 1996; Hansen & Steig 1993). Studies of other heteroblastic *Acacia* species showed that leaf types can be similar or different in gas exchange per area; for *A. mangium*, A_{area} , g_{max} and QE_{area} were similar across leaf types but phyllodes had higher I_c and R_{area} (Yu & Li 2007), whereas for *A. melanoxylon* A_{area} was 33% higher for phyllodes (Brodribb & Hill 1993). This variation across species in the degree that gas exchange per area is coupled across leaf types merits further investigation.

Additionally, we found leaf types to be similar in several anatomical and composition traits. Consistent with their g and A_{area} , the leaf types were similar in epidermal thickness, total stomatal density, stomatal size and stomatal pore area (SPI_{tot} and g_{th}). The g_{th} values were very high, indicating that stomata likely open partially and/or heterogeneously. The leaf types had similar SWC (see also Hansen 1986) and P_{area} . The minor veins of leaflets and phyllodes did not differ in conduit numbers or sizes, or in vein conductivity.

Leaves and phyllodes were also similar in several drought tolerance traits. Both leaf types showed a degree of hydraulic redundancy (Brodribb & Holbrook 2004; Ewers *et al.* 2007; Pratt *et al.* 2008; Sack *et al.* 2008); g and A_{area} remained high during desiccation while K_{leaf} declined substantially. The leaf types were similar in the Ψ_{leaf} at which g and A_{area} declined by 50 and 80%, and at which K_{leaf} declined by 20%, and in their recovery in g and A_{area} after rehydration. The leaf types were also similar in their timing of stomatal closure under high VPD. Leaves and phyllodes were also similar in g_{min} (see also Walters & Bartholomew 1984); although the phyllodes had thicker cuticle, g_{min} is determined by cuticle composition and the leakiness of closed stomata (Kerstiens 1996). Leaves and phyllodes were similar in π_{ti} and π_{tip} (see also Hansen 1986), and in C_{tip} .

Divergences between leaf types: support for the growth hypothesis

We found strong differences in external structure and internal anatomy of leaves that supported expectations from the growth hypothesis. In particular, leaves had higher gas exchange rates per dry mass, per carbon and per nitrogen

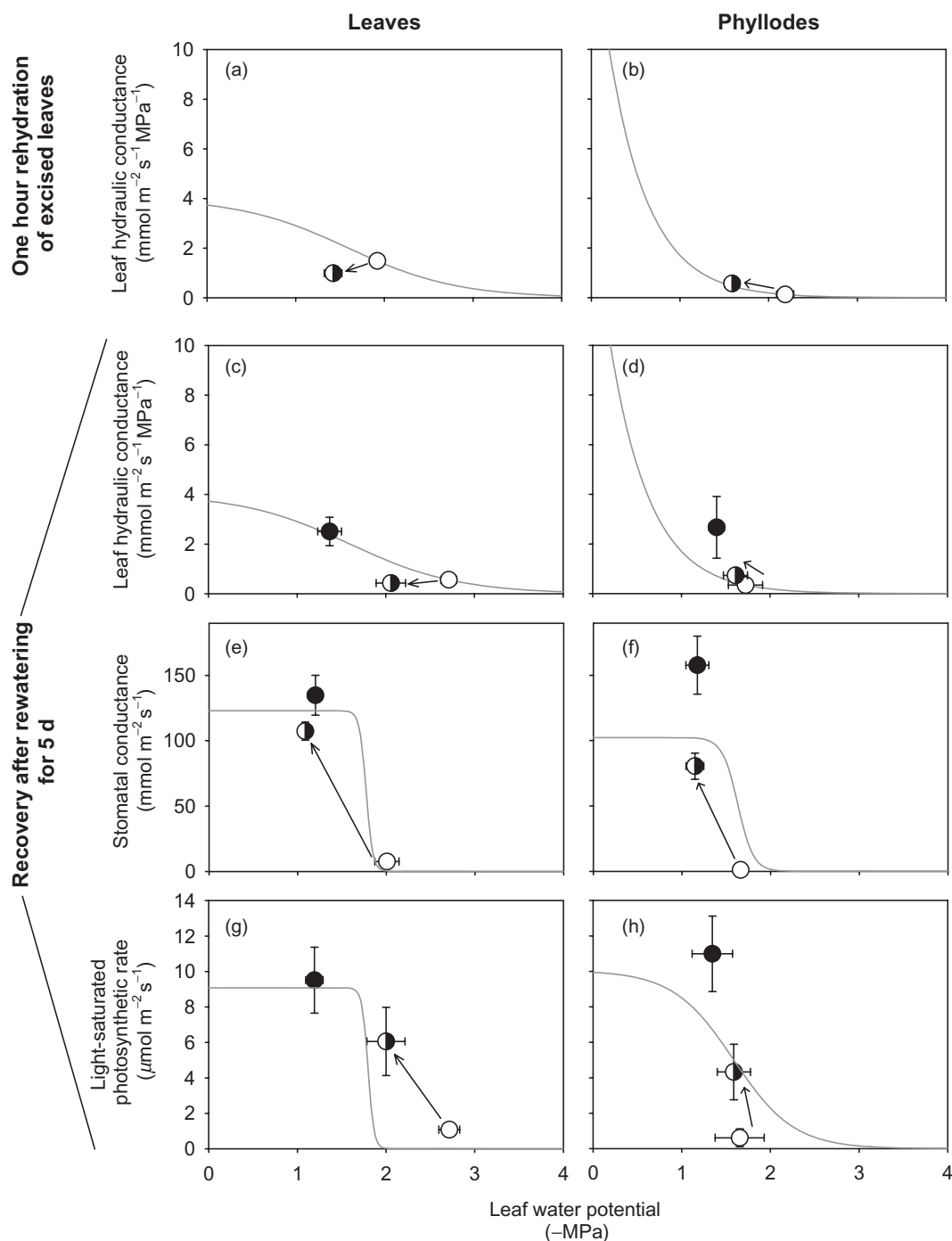


Figure 6. Tests for recovery of leaves and phyllodes of *A. koa* with rehydration after desiccation. a and b, the rapid recovery of leaf hydraulic conductance (K_{leaf}) for desiccated shoots rehydrated 1 h in water; c–h, the recovery of K_{leaf} , stomatal conductance and photosynthetic rate for plants droughted to below turgor loss point and rewatered to field capacity for five days. Symbols represent mean values \pm SE, open symbols for desiccated shoots (a and b) or plants (c–h), mixed symbols after rehydration, and closed symbols well watered control plants on measurement days (n -values: a, 6; b, 8; c, 7–8; d, 6–8; e, 4–8; f, 6–8; g, 4–6 and h, 3–4). Different plants randomly chosen from the same pool were used to determine the responses shown in c/d, e/f and g/h. Grey lines indicate sigmoidal functions fitted to the responses of the y-axis variable to water potential in desiccation experiments (Figs 4 & 5).

investment, at all irradiances, which would contribute to faster relative growth rate (Coste *et al.* 2005; Kruger & Volin 2006; Quero *et al.* 2006). Considering the traits for which it had expectations (see *Introduction*) the growth hypothesis

had 42 and 74% specific predictive and explanatory power. Leaves had lower thickness and *LMA* than phyllodes, as previously reported for *A. koa* (Walters & Bartholomew 1984; Hansen 1986), and for other *Acacia* species (Evans

et al. 2000; Yu & Li 2007). The greater phyllode thickness and the allocation to rachis within leaves explained many composition differences supporting the growth hypothesis. Being thinner, leaves had lower N_{area} and C_{area} than phyllodes, as reported for other Acacias (Evans et al. 2000; Yu & Li 2007). Consistent with their lower LMA , leaflets were higher in N_{mass} (see also Hansen 1986), P_{mass} , and C_{mass} . However, including the leaf rachis, with its low nutrient concentration, the whole leaves had lower N_{mass} , C_{mass} , N/C and N/P than phyllodes, though P_{mass} remained higher for leaves than phyllodes. Consistent with these differences in composition and anatomy, leaves had higher QE_{mass} , A_{mass} , A/N , $V_{c,\text{max}}$, J_{max} , $V_{c,\text{max}}/N$ and J_{max}/N . The leaves having higher $V_{c,\text{max}}$ and J_{max} than phyllodes, despite the similarity of leaf types in A_{area} , was counter-intuitive because these parameters are typically correlated; however, the leaves also had larger values for mitochondrial respiration in high irradiance (R_d), another parameter of the $A-c_i$ curve (-10.6 ± 1.14 versus $-4.35 \pm 0.347 \mu\text{mol m}^{-2} \text{s}^{-1}$; $P < 0.001$; data not shown). Consequently, leaves and phyllodes had similar $A-c_i$ curves at ambient CO_2 levels below 400 ppm (which corresponded to a mean $c_i \pm \text{SE}$ value of 203 ± 9.3 ppm; leaf types did not differ at $P > 0.05$; t -test). At higher c_i values the curves diverged with the leaves having higher values, accounting for their difference in $V_{c,\text{max}}$. We note that a similar pattern has been reported several times in comparisons of varieties, intrageneric hybrids, and juvenile and adult leaves of given species (Wu & Campbell 2006; Kubien, Jaya & Clemens 2007; Chang et al. 2008). Overall, these differences observed between leaves and phyllodes extend previous reports of leaves having higher A_{mass} and A/N in *A. koa* (Hansen 1996), and higher A_{mass} , A/N , $V_{c,\text{max}}/N$ and J_{max}/N in four other Acacias (Brodrick & Hill 1993; Evans et al. 2000; Yu & Li 2007).

Contrary to the growth hypothesis, the phyllodes had higher maximum K_{leaf} than leaves. However, K_{leaf} declined rapidly with desiccation, and at Ψ_{leaf} of -1 MPa the leaf types had similar K_{leaf} , matching their similar gas exchange rates per area. We also found strong differences in xylem anatomy. The phyllode primary vein had higher conductivity than the leaflet midrib, but, once normalized by leaf size, length and primary vein number, the leaflet primary vein K'_i was 13-fold higher. This feature would have contributed to a higher K_{leaf} (McKown, Cochard & Sack 2010), as expected from the growth hypothesis, though K_{leaf} was actually higher for phyllodes. This disparity may be explained in part by the fact that the leaflet is supplied by the petiole and two orders of rachis; the additional resistance of this upstream xylem would lower the overall K_{leaf} .

Previous work on whole seedlings indicates that these leaf traits conducive to faster growth should scale up to plant performance during early establishment. In a study of 10 Australian *Acacia* species grown for 3 months, those that produced phyllodes very early had substantially slower relative growth rates weeks than those producing phyllodes after 1–2 months of growth or those with only leaves (our analysis of data in Atkin et al. 1998; $n = 3\text{--}4$ per type; ANOVA; $P = 0.004$).

Divergences between leaf types: support for the shade hypothesis

Many trait differences were found as expected from the shade hypothesis. Considering the traits for which it had expectations (see *Introduction*), the shade hypothesis had 37 and 62% specific predictive and explanatory power. Leaves did not have higher A_{area} , but they had greater A_{mass} at all irradiances, and greater QE_{mass} , J_{max} , $V_{c,\text{max}}$, A/N , J_{max}/N and $V_{c,\text{max}}/N$. Lacking the water storage tissue of phyllodes, and with lower LMA , N_{area} and N_{mass} , thinner cuticle, and lower maximum hydraulic capacity, leaves allocated more N and C towards productive photosynthetic tissues, and had less costly tissues per area and/or per mass. Such higher productivity and lower tissue costs would improve efficiency in light capture and carbon gain, for leaves that only last 1–2 years, and thus benefit shade tolerance (Givnish 1988; Terashima & Evans 1988; Rosati et al. 1999; Walters & Reich 1999; Coste et al. 2005, 2009, 2010; Janse-ten Klooster, Thomas & Sterck 2007; Lusk et al. 2008).

Additionally, the larger size of leaves than phyllodes would contribute to light capture relative to stem support (Givnish 1988). Leaves also had spongy mesophyll, which would benefit diffuse light capture. Notably, a previous study reported higher total chlorophyll concentration per mass in leaves, also consistent with investment in greater light capture (Walters & Bartholomew 1984). Leaf hypostomy may also confer shade tolerance by reducing adaxial light obstruction (L. Sack & M. J. Sporck, unpublished data).

Traits that contribute to leaf performance in shade would scale up to whole plant performance. *A. koa* is a light requiring species establishing from long-lived seeds typically in open areas (Baker et al. 2009), and shade tolerance traits would provide benefits under sparse overstorey. Indeed, *A. koa* maintains leaves longer when grown in shade, and when high irradiance plants were transferred to shade they reverted to producing only leaves (Walters & Bartholomew 1990). In *A. implexa*, low light delayed the transition to phyllodes (Forster & Bonser 2009a,b).

Divergences in between leaf types: support for the drought hypothesis

Many differences in morphology, anatomy and composition were found as expected from the drought hypothesis. In general, the drought tolerance of phyllodes related to their greater water storage capacity, rather than to an ability to maintain physiological function at lower leaf water status. Considering the traits for which it had expectations (see *Introduction*), the drought hypothesis had 47 and 84% specific predictive and explanatory powers. In the phyllodes, half the mesophyll thickness was composed of the large-celled water storage tissue. This tissue was associated with greater water mass per area, C_{ft} , C_{ft}^* and C_{tip}^* , and lower RWC_{tip} and would delay mesophyll desiccation after stomatal closure. As hypothesized, the phyllode also had thicker cuticle, and higher N_{area} and C_{area} , and the phyllodes

were amphistomatic. The *A. koa* phyllode showed a more rapid complete stomatal response to VPD. This greater stomatal sensitivity was consistent with earlier findings of diurnal g to be more responsive in *A. koa* phyllodes than leaves to light, temperature, VPD and Ψ_{leaf} (Hansen 1986), and of stronger responsiveness of g to VPD in phyllodes than leaves of *A. melanoxylon* (Brodribb & Hill 1993).

Phyllodes also had smaller area, mass and epidermal cell sizes consistent with drought adaptation (Cutler *et al.* 1977; Givnish 1987). Additionally, consistent with drought adaptation, phyllodes had higher N_{mass} and N/C than leaves, due to the low N_{mass} in leaf rachis, and higher $\delta^{13}\text{C}$ (see also Hansen & Steig 1993; Hansen 1996; Wright *et al.* 2001). The higher $\delta^{13}\text{C}$ in phyllodes may reflect greater resistance to internal CO_2 diffusion (cf. Dawson *et al.* 2002), rather than a higher integrated WUE ; the leaf types had similar instantaneous WUE , as previously reported for *A. melanoxylon* (Brodribb & Hill 1993). While a previous study did find 11–15% higher WUE for phyllodes, that difference was smaller than expected from the $\delta^{13}\text{C}$ values (Hansen & Steig 1993).

The stronger reduction of $K_{\text{leaf},g}$ and A_{area} with decline of Ψ_{leaf} in phyllodes departed from the drought hypothesis. For *A. melanoxylon*, phyllodes also showed stronger reduction of g as Ψ_{leaf} declined (Brodribb & Hill 1993). The *A. koa* leaf types showed the contrasting shapes of K_{leaf} decline described previously for different species, which arise due to differences in mesophyll desiccation response and/or xylem cavitation (Brodribb & Holbrook 2006); the leaf showed a sigmoidal decline, and the phyllodes an initial exponential or linear decline. The pronounced K_{leaf} decline in phyllodes may correspond to shrinkage of water storage tissue, and/or xylem more prone to cavitation, potentially associated with its larger primary vein conduit diameters. The leaf types also diverged in response to rehydration. The K_{leaf} recovered slightly in phyllodes, but not in leaves. A previous study reported that desiccated sunflower leaves recovered in K_{leaf} after 15 min with petioles in water (Trifilo *et al.* 2003). Recovery of K_{leaf} is evidently species-specific and, as found here, can vary between heteroblastic leaf types within a species.

Phyllode water storage is potentially important in drought tolerance across the genus *Acacia*. For 144 phyllocladous Australian Acacias, the ratio of water storage: palisade tissue correlated with habitat aridity (Boughton 1986). Our ratio for *A. koa* was just above the reported average for Australian arid species, higher than that for 90% of humid species. This water storage may enable longer survival after stomatal closure during drought (Sack *et al.* 2003b). For example, if the time to dehydrate to Ψ_{leaf} of -3 MPa is calculated from the stored water (determined from C_{fit}^* and C_{tip}^*) divided by $g_{\text{min}} \times \text{VPD}$ of 1 kPa, we find 1.3 and 2 d for leaves and phyllodes, respectively. The phyllode water may last longer still if their vertical position results in cooler temperatures (and lower VPD) than the horizontally positioned leaves.

Phyllode traits may scale up to considerable plant level drought tolerance. Drought tolerance has been a major

evolutionary explanation for phyllodes in Australian *Acacia* species (Boughton 1986). Consistent with that idea, in a seedling common garden experiment, phyllodes replaced leaves earlier for species from semi-arid versus mesic sites (our analysis of data of Atkin *et al.* 1998; t -test, $n = 3-4$; $P = 0.044$). The same pattern was found for seedlings of *A. melanoxylon* populations from drier versus mesic sites (Farrell & Ashton 1978).

Support for all three hypotheses, overall functional implications and future work

We found strong support for each hypothesis in key leaf traits related to plant function. However, none of the hypotheses singly could completely predict or explain the trait variation between leaf types. The three hypotheses combined explained 91% of total differences. We conclude that *A. koa* heteroblasty relates to multiple functional specializations, i.e. benefits for growth, shade- and drought-tolerance.

We note that while our functional survey might be novel in breadth, it is based on a traditional approach, testing expectations for individual traits established by previous studies of the functional significance of these traits in other species. We acknowledge there is some degree of uncertainty, because the trait expectations may not be in all cases equally valid for *A. koa*, and some differences between leaf types may relate to other functions; for example, the thick cuticle in phyllodes might also provide a longer lifespan. Further studies, for example, using mutants, would be necessary as conclusive evidence. However, one advantage of testing numerous expectations for each hypothesis is that the key finding, i.e. that multiple hypotheses are needed to explain the differences between leaf types, will be robust to the removal of some traits from the analysis if those are later found to be inappropriate. This approach can be improved when there is knowledge of the relative importance of individual traits in functional specialization, such that trait differences can be weighted for support of hypotheses. Most ideally, when a model for estimating plant performance from leaf traits becomes available one could determine how the combinations of traits scale up to plant growth, shade- and drought tolerance.

We note that advantages for growth, shade tolerance and drought tolerance would apply most importantly at different life stages (Grubb 1998). A growth and/or shade-tolerance advantage for leaves might be more important for small plants whereas the benefit of phyllodes in drought tolerance may be especially strong for larger plants, with greater root limitation (Woodruff, Meinzer & Lachenbruch 2008). Such changing benefits with plant size might be amplified by the differences in orientation of the leaf types; the leaves spread horizontally, and would capture more irradiance within small canopies, whereas phyllodes hang vertically and allow light penetration throughout a large canopy (Walters & Bartholomew 1984; Hansen 1986, 1996). Another important area for study is the plasticity of leaf types. There can be substantial plasticity and ecotypic

variation in size, *LMA* and composition *within* each *A. koa* leaf type across different elevations, water supplies and forest types (Ares & Fownes 1999; Daehler *et al.* 1999). Combined with this plasticity, heteroblastic leaf types would lead to a very wide range of variation for advantage under different growth conditions and life stages.

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SUPPORTING INFORMATION

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

Table S1. Leaf morphology traits, symbols and units, and mean values \pm standard error for leaves and phyllodes of *Acacia koa* grown under controlled conditions, and significance of paired *t*-tests for comparisons of leaf types on the same individual plants.

Table S2. Leaf composition traits, symbols and units, and mean values \pm standard error for leaves and phyllodes of *Acacia koa* grown under controlled conditions, and

significance of paired *t*-tests (except †, unpaired due to inclusion of additional, unmatched replicates) for comparisons of leaf types on the same individual plants.

Table S3. Leaf stomatal traits, symbols and units, and mean values \pm standard error for leaves and phyllodes of *Acacia koa* grown under controlled conditions, and significance of *t*-tests for comparisons of leaf types unpaired due to inclusion of additional, unmatched replicates.

Table S4. Leaf mesophyll anatomy traits, symbols and units, and mean values \pm standard error for leaves and phyllodes of *Acacia koa* grown under controlled conditions, and significance of paired *t*-tests for comparisons of leaf types. For traits in which leaves had adaxial and abaxial values, both were tested for difference with phyllode values.

Table S5. Leaf xylem anatomy traits, symbols and units, and mean values \pm standard error for leaves and phyllodes of *Acacia koa* grown under controlled conditions, and significance of paired *t*-tests for comparisons of leaf types.

Table S6. Leaf cuticular conductance and pressure volume curve parameters, symbols and units, and mean values \pm standard error for leaves and phyllodes of *A. koa* grown under controlled conditions, and significance of *t*-tests for comparisons of leaf types, unpaired due to inclusion of additional, unmatched replicates (except paired for cuticular conductance).

Table S7. Parameters of leaf hydraulics, stomatal conductance and photosynthesis drought response and recovery after rehydration of shoots and whole plants, symbols and units, and mean values \pm standard error for leaves and phyllodes of *A. koa* grown under controlled conditions, and significance of *t*-tests for comparisons of leaf types.

Table S8. Leaf photosynthetic light response traits, symbols and units, and mean values \pm standard error for leaflets, leaves and phyllodes of *A. koa* grown under controlled conditions, and significance of *t*-tests for comparisons of leaf types (leaflet versus phyllode and whole leaf versus phyllode, respectively).

Table S9. Parameters of the leaf photosynthetic response to intercellular CO₂ concentration (*c*_i), symbols and units, and mean values \pm standard error for leaflets, leaves and phyllodes of *A. koa* grown under controlled conditions, and significance of *t*-tests for comparisons of leaf types (leaflet versus phyllode and whole leaf versus phyllode, respectively).

Table S10. Leaf traits measured relating to vapour pressure deficit, symbols and units, and mean values \pm standard error for leaflets, leaves and phyllodes of *A. koa* grown under controlled conditions, and significance of one-tailed *t*-tests; *n* = 5 for all parameters.

Appendix S1. Supplementary Methods.

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