Supplementary Materials for:

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

Jessica Pasquet-Kok1,2, 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*

*2Department of Biology, University of Bordeaux, UMR BIOGECO, Av. des Facultés, 33405 Talence, France*

Supplementary Methods, and Supplementary Tables S1-S10**Supplementary Methods**

*Plant material*

Seeds were germinated in potting soil (1:1:3:1:3 of medium size vermiculite: perlite: peat moss: Redondo Beach sandy loam) in trays in March 2008 and grown on greenhouse benches at the UCLA Plant Growth Center (average minimum, mean and maximum values for temperature: 21.1, 23.2 and 26.0oC; for humidity: 44, 51 and 59%), and transferred to 3.6 L pots as seedlings with several true leaves. Plants were irrigated every two days, with fertilizer (200-250 ppm of 20:20:20 N:P:K). The light availability measured at mid-day on a sunny day was up to 550 µmol photon · m-2 · s-1, and on average 300 µmol photon · m-2 · s-1 (LI-250 light meter; LI-COR Biosciences, Lincoln, Nebraska, USA). Experiments were conducted September 2008 to July 2009.

*Leaf morphology*

We measured leaf lamina and phyllode thickness using electronic digital calipers (Fisher Scientific, Pennsylvania, USA), averaging four measurements at the center of each phyllode and for six leaflets per leaf, midway between midrib and margin, and scanned leaves and phyllodes for determination of leaf area (Scanner Canon CanoScan LiDE90; images analyzed using ImageJ; Rasband, 1997-2008, National Institutes of Health, Bethesda*,* Maryland*,* USA). Leaflets were removed from rachis, and counted and scanned separately for determination of total leaflet area and rachis area. Components were dried in an oven at > 70oC for more than 48 h before dry mass determination for leaf rachis and leaflets, and phyllodes.

*Leaf composition*

Dried leaves and phyllodes were ground and analyzed for concentrations of nitrogen, phosphorus and carbon per mass (*N*mass, *P*mass and *C*mass, respectively), and for carbon isotope ratio (δ13C) using high temperature combustion in an elemental analyzer (Costech ECS 4010; Valencia, California, USA), with effluent passed into a continuous flow isotope ratio mass spectrometer (ThermoFinnigan Delta V Advantage with a Conflo III interface; ThermoFisher Scientific; Walthan, Massachusetts, USA; Fry *et al.*, 1996). Samples were dry ashed in glass vials (Miller, 1998), dissolved in 1N HCL and analyzed for *P*mass using inductively-coupled plasma-optical emission spectrometry (Varian Vista MPX Instrument, Varian Inc., Palo Alto, California USA; Porder, Paytan & Vitousek, 2005).

*Anatomical traits: stomatal traits*

We quantified stomatal densities, guard cell lengths, stomatal pore lengths and the width of the guard cell complex on adaxial and abaxial surfaces, using microscopic measurement of impressions from abaxial nail varnish peels taken centrally, midway between midrib and margin. Photographs were made for two locations per peel at 20 and 40× magnification, and images were analyzed using ImageJ; measurements of each type were averaged for the two locations per peel. We determined a theoretical stomatal conductance (*g*th; Franks & Farquhar, 2007):

, eqn 1

where *d* is the diffusivity of water in air (0.249 × 10-4 m2 · s-1 at 20oC and 101.325 kPa), υ is the molar volume of air (24.05523 × 10-3 · m3 · mol-1), and *l* is the depth of the stomatal pore (m, approximated as half the width of the guard cell complex).

*Anatomical traits: mesophyll and xylem traits*

Leaves and phyllodes were preserved in formalin acetic acid (FAA; 37% formaldehyde, glacial acidic acid, 95% ethanol, and deionized water in a 10:5:50:35 mixture). For five leaflets and phyllodes we measured the cross-sectional anatomy using sections cut halfway along the length, 0.5 µm thick, stained with toluidine blue and imaged at 200–400× using ImageJ.

For determining vein theoretical hydraulic conductivities, we treated the xylem conduits as ellipses and measured long and short axes for each conduit to obtain a theoretical hydraulic conductivity (*K*t) for the primary and the minor vein, as

 eqn 2

where *a* and *b* are the long and short axes and η is the viscosity of water at 25oC (mmol · m · s-1 MPa-1; Lewis & Boose, 1995; Cochard, Nardini & Coll, 2004; Sack & Frole, 2006). To compare leaf and phyllodes for primary vein xylem supply capacity per leaf area, we calculated an area- and length-normalized theoretical hydraulic conductivity by dividing *K*t by leaf area and leaf length, and, for the phyllode, multiplying by the number of the large axial primary veins (*K*t’; mmol · m-2 · s-1 · MPa-1); all else being equal, if we assume that the midrib delivers water similarly along its length, a leaf of half the length should require half the conductance.

*Gas exchange: light response curves*

In March 2009, photosynthetic light response curves were measured using a LI-6400 XT portable photosynthesis system (LI-COR, Lincoln, Nebraska, USA). Leaves were acclimated at a photosynthetically active radiation (PAR) of 1500 µmol · m2 · s-1. Throughout measurements, reference CO2 was set at 400 µmol · mol-1, and cuvette temperature was (mean ± SE) 24 ± 0.33°C, relative humidity was 61.2 ± 1.5%, and VPD was 1.07 ± 0.04 kPa. Leaves were measured for net CO2 assimilation (*A*) at PAR steps of 2000, 1500, 1000, 500, 200, 100, 75, 55, 45, 30, 15 and 0 µmol · m-2 · sec-1, with 120- 200 s stabilization at each irradiance (Givnish, Montgomery & Goldstein, 2004). Leaves were then harvested, scanned for leaf area, dried at 70°C for 48 h, and weighed to determine leaf mass per unit area (LMA) for the leaf area in the chamber. For leaflets and phyllodes, we determined light-response parameters from plots of CO2 assimilation rate versus PAR. The dark respiration rate per area (*R*area), was determined as the negative *A* at zero PAR, the light compensation point (*I*c) as the *x*-intercept, the quantum efficiency (*QE*area) as the initial slope and the saturation irradiance (*I*s) as the irradiance at which 95% of the light-saturated carbon assimilation rate per area (*A*area) was achieved, where *A*area was determined as the average of values for PAR of 1000, 1500 and 2000 µmol · m-2 · s-1 (Fig. 2) . Values for maximum stomatal conductance (*g*max) were determined as the average of the values at the three highest PAR levels. All area-based measurements for leaflets were corrected for the leaf area in the chamber. Values for mass-based dark respiration, quantum efficiency and photosynthetic rate (*R*mass, *QE*mass and *A*mass) were determined for the measured leaflets and phyllodes by dividing by their values for mass per area. The assimilation rate per nitrogen concentration (*A/N*) was determined by dividing *A*mass by mass-based N concentration (*N*mass), for leaflets and for phyllodes (Table S2).

*Gas exchange: photosynthetic assimilation – ci response curves*

In March 2009, photosynthetic CO2 response curves were measured for leaves and phyllodes on 11 plants, between 0900 and 1600 alternating leaves and phyllodes from the same plants (using a LI-6400 XT with 6400-40 Leaf Chamber Fluorometer; Givnish *et al.*, 2004). We conducted all measurements at 1000 µmol · photon · m-2 · s-1, a saturating light level for this species (Fig. 3), and average cuvette temperature was (mean ± SE) 25 ± 0.02°C, relative humidity was 66.7 ± 1.2%, and VPD was 1.09 ± 0.04 kPa. Leaves were acclimated at 50 ppm chamber [CO2] to induce stomatal opening, and the CO2 response curves were determined at steps of 0, 50, 100, 200, 400, 600, 1000, 1500 and 2200 ppm chamber [CO2], with three minutes equilibration time at each step. Plots of photosynthesis (*A*) vs. leaf intercellular [CO2] (*c*i) were used to solve for *V*c,max and *J*max corrected to 25°C ( Farquhar, Caemmerer & Berry, 1980, Bernacchi *et al.*, 2001, Bernacchi, Pimentel & Long, 2003, Long & Bernacchi, 2003) ), and *V*c,max/N and *J*max/N by dividing by mass per area for the leaf in the chamber and then dividing by *N*mass (Table S2). We determined the quantum yield of photosystem II (ФPSII) at 400 ppm CO2.

*Gas exchange: estimated values for whole leaves*

To allow comparisons of leaves with phyllodes, gas exchange parameters for whole leaves were estimated by multiplying the area-based and mass-based values for leaflets by respectively by leaflet area fraction and leaflet mass fraction. This estimation assumed the photosynthesis of the rachis to be negligible relative to that of the leaflet, in keeping with their low *N*mass (see below). These estimates provided a lower-bound for the whole-leaf values, while the leaflet values provided a higher bound for the whole-leaf values (i.e., assuming the rachis to be equivalent in gas exchange per area or mass to the leaflet). Thus, phyllode values were compared with both leaflet and whole-leaf values, and if the phyllode value was found to be similar or different from both, as was generally the case, the interpretation of similarity or difference between leaf types would be robust.

*Drought tolerance traits: cuticular conductance*

For measurement of cuticular conductance (= ‘minimum conductance’, *g*min *sensu* Kerstiens, 1996), hydrated leaves and phyllodes were dried on a laboratory bench, at PAR < 10 mmol photons · m-2 · s-1, for 3–4 h to drive stomatal closure, and then weighed for at least eight intervals of 10–30 min, during which the slope of water loss versus time was highly linear (*R*2 > 0.995), and thus taken to represent cuticular transpiration. The *g*min was calculated as cuticular transpiration/ mole fraction gradient in water vapour from the leaf to air, assuming the leaf internal air to be fully saturated (Pearcy, Schulze & Zimmermann, 2000); values were averaged for the last four intervals for each leaf. Ambient temperature and relative humidity fluctuated minimally during the measurements (up to ± 0.4°C and ± 1.5%); mean values were 21.6°C and 19.5%.

*Drought tolerance traits: pressure volume curves*

Pressure-volume curve parameters were determined by progressively drying leaves and phyllodes on a laboratory bench, and measuring at intervals by equilibrating for 10 min in a Whirpak bag (Whirl-Pak, Nasco, Fort Atkinson, Wisconsin, USA) before weighing and determining leaf water potential (Ψleaf) with a pressure chamber (Plant Moisture Stress, Model 1000, Albany, Oregon, USA). Subsequently dry mass was determined after more than 72 h at 70˚C. Pressure-volume curve parameters were calculated (Koide *et al.*, 2000; Sack *et al.*, 2003): osmotic potential at full turgor and at the turgor loss point (πft and πtlp), relative water content at turgor loss point (RWCtlp), elastic modulus at full turgor (ε), and relative capacitance at full turgor (*C*ft; ΔRWC/ΔΨleaf, between full turgor and turgor loss point) and at zero turgor (*C*tlp; ΔRWC/ΔΨleaf, below turgor loss point). ‘Plateau effects’ associated with leaf airspace infiltration were found for some of the leaves, and corrected (Kubiske & Abrams, 1991). For parameters calculated from slopes of two ‘dependent variables’, i.e. for ε, *C*ft and *C*tlp, standard major axes were used (Sokal & Rohlf, 1995, Sack *et al.*, 2003). The saturated water content (SWC) was determined as the *y*-intercept of the least-squares line fitted to data for water mass versus Ψleaf for leaves at the beginning of dehydration, divided by dry mass. The water mass per leaf area was determined as SWC multiplied by LMA. The leaf area specific capacitance above turgor loss point (*C*ft\*) was calculated as *C*ft multiplied by water mass per leaf area and the leaf area specific capacitance below turgor loss point (*C*tlp\*) was calculated as *C*tlp multiplied by water mass per leaf area and by RWCtlp.

*Leaf hydraulic conductance, vulnerability and recovery*

We measured hydraulic conductance for leaves and phyllodes using the evaporative flux method (EFM; Sack *et al.*, 2002). The leaf was cut from the shoot with a fresh razor blade under distilled water, the petiole was wrapped in parafilm and was then rapidly connected to tubing containing distilled and degassed water (degassed for at least 8 h with a vacuum pump; GAST manufacturing, Inc, Michigan, USA) underwater to prevent air entering the system. The tubing connected the leaf to a cylinder of water on a balance (Mettler Toledo, XS205 DualRange, ± 0.01/0.1 mg), which logged data every 30 s to a computer for the calculation of flow rate into the petiole (*E*). Leaves were held adaxial surface upwards using a wood frame strung with fishing line, which held the leaf horizontal and immobile above a large box fan (Lakewood Engineering & Manufacturing Company, Chicago, Illinois, USA). Leaves were illuminated by a light source (1000-1500 μmol photons · m-2 · s-1; model 73828 1000 W, “UV filter”; Sears, Roebuck, Hoffman Estates, Illinois, USA) suspended above a Pyrex glass container (Corning Incorporated, Corning, New York, USA) filled with 5-6 cm of water above the leaf producing >1000 mmol · m-2 · s-1 PAR at the leaf surface. The leaf temperature was maintained between 23 and 28°C during the experiments. Leaves were allowed to transpire on the fan apparatus for at least 30 min and until flow rate stabilized with a coefficient of variation <5% for at least 3-5 min. A 30 min period was chosen to ensure that leaves had sufficient time to acclimate to light which previous studies have shown to enhance *K*leaf by several-fold for certain species (Sack et al., 2002; Tyree *et al.*, 2005, Cochard *et al.*, 2007, Scoffoni *et al.*, 2008). When flow rate was very low (< 8 µg · s-1) and did not stabilize with that criterion, the measurement was continued until the running average of the last ten 30 s flow measurements stabilized with a coefficient of variation <5% for at least 5 min. Additionally, flow rate was plotted against time to ensure stability. Measurements were discontinued if the flow suddenly changed, either due to leakage from the system or apparent blockage by particles or air bubbles. Leaf temperature was recorded with a thermocouple thermometer (Cole-Parmer Instrument Company, Vernon Hills, Illinois, USA) and the final 2.5 min of flow rate were averaged. The leaf was quickly removed from the tubing, the petiole dabbed dry, and the leaf placed into a Whirlpak bag, which had been previously exhaled in. Following at least 20 min equilibration, the final leaf water potential (Ψf) was measured using a pressure chamber. To correct for changes in *K*leaf induced by the temperature dependence of water viscosity, *K*leaf values were standardized to 25°C (Weast, 1974, Sack *et al.*, 2003).

We constructed hydraulic vulnerability curves for *K*leaf, by measuring desiccated leaves and phyllodes. Shoots were excised into segments with three leaves each under distilled water and left to desiccate with a fan for different periods of time to reach different Ψleaf. Following desiccation, plastic bags were placed over the leaves on the shoot (Whirl-Pak, Nasco, Fort Atkinson, Wisconsin, USA). The shoots were then placed into a larger zipper plastic bag (Ziploc, SC Johnson, Racine, Wisconsin, USA), and equilibrated for at least 20 min. After the equilibration period, two leaves from the shoot were excised and measured for Ψleaf. Shoots for which both leaves had Ψleaf within 0.2 MPa were used for EFM, with the average of these two values used for the initial water potential (Ψo). For very desiccated shoots, this range was extended to 0.5 MPa. The third leaf was used to determine *K*leaf using the EFM. In our calculation of the response of *K*leaf to Ψleaf, the lower value was taken from the original Ψleaf for sampled leaves, and the Ψleaf at the end of the EFM measurement (i.e., the Ψo and Ψf). This lowest value (= “Ψlowest”) would reflect the maximum desiccation stress experienced by the leaf that may affect *K*leaf. The Ψo and Ψf were closely correlated (for leaves *R*2 = 0.96, *P* = 0.0002, 155 measurements in 0.5 MPa bins for the bench-drying and drought experiment combined; for phyllodes *R*2 = 0.78, *P* = 0.002, 120 measurements in 0.25 MPa bins as data covered a narrower range), and the Ψo tended to be lower because of recovery of Ψleaf during measurement (that was the case for 92% at 73% of measurements for leaves and phyllodes respectively). We removed outliers different at *P* < 0.05 from the binned data (Sokal & Rohlf, 1995); 1 and 7 outliers were removed from 155 leaf and 120 phyllode points respectively in the combined vulnerability curves for desiccating shoots and droughted plants. The desiccation response of *K*leaf was obtained by plotting *K*leaf against Ψlowest and fitting the sigmoidal curve:

 eqn 3

*Statistical analyses*

For each hypothesis we calculated the proportion of expectations of trait differences between leaf types that should be predicted or explained by chance. These were “null models” that we tested the actual proportions against.

For *predictive power*, the null model was for no significant difference between leaf types for any trait at *P* = 0.05. Thus, the chance probability of a single hypothesis predicting the right leaf type to have a significantly higher value was 0.025 (i.e., the 0.05 probability of significant difference, by chance alone multiplied by the 0.5 probability of the expected leaf type to have the higher value). Multiple hypotheses increase the chance probability of prediction. Thus for the predictive power of all three hypotheses combined a weighted chance probability was calculated accounting for the number of hypotheses that applied for each trait; while the chance probability of a single hypothesis being supported was 0.025, that for two hypotheses was 1-(1-0.025)2 = 0.049375, and that for three hypotheses was 1-(1-0.025)3 = 0.073141 (“complement rule”; Tijms, 2007). We multiplied these probabilities by the proportion of traits with expectations from one, two or three hypotheses respectively to determine the overall probability of predicting the trait expectations from chance, and tested the actual proportion against that null model.

For *explanatory power*, we considered only traits that showed significant differences and not those that were statistically similar between leaf types. Thus, the chance probability for a single hypothesis finding the right leaf type to have the higher value was 0.5. For the explanatory power of all three hypotheses combined a weighted chance probability was calculated accounting for the number of hypotheses that applied for each trait; while the chance probability of a single hypothesis being supported was 0.5, that for two hypotheses was 1-(1-0.5)2 = 0.75, and that for three hypotheses was 1-(1-0.75)3 = 0.875. We multiplied these probabilities by the proportion of traits with expectations from one, two or three hypotheses respectively to determine the overall probability of the trait expectations being supported from chance, and tested the actual proportion against that null model.**References for Supplementary Methods**

Bernacchi C.J., Pimentel C. & Long S.P. (2003) *In vivo* temperature response functions of parameters required to model RuBP-limited photosynthesis. *Plant Cell and Environment*, **26**, 1419-1430.

Bernacchi C.J., Singsaas E.L., Pimentel C., Portis A.R. & Long S.P. (2001) Improved temperature response functions for models of Rubisco-limited photosynthesis. *Plant Cell and Environment*, **24**, 253-259.

Cochard H., Nardini A. & Coll L. (2004) Hydraulic architecture of leaf blades: where is the main resistance? *Plant, Cell & Environment*, **27**, 1257-1267.

Cochard H., Venisse J.S., Barigah T.S., Brunel N., Herbette S., Guilliot A., Tyree M.T. & Sakr S. (2007) Putative role of aquaporins in variable hydraulic conductance of leaves in response to light. *Plant Physiology*, **143**, 122-133.

Farquhar G.D., Caemmerer S.V. & Berry J.A. (1980) A biochemical model of photosynthetic CO2 assimilation in leaves of C3 species. *Planta*, **149**, 78-90.

Franks P.J. & Farquhar G.D. (2007) The mechanical diversity of stomata and its significance in gas exchange control. *Plant Physiology*, **143**, 78-87.

Fry B., R. G., Thoulke K., Neill C., Michner R.H., Mersch F.J. & Brand W. (1996) Cryoflow: cryofocusing nanomole amounts of CO2, N2 and SO2, from an elemental analyzer for stable isotopic analysis. *Rapid Communications in Mass Spectrometry* **10**, 953-958.

Givnish T.J., Montgomery R.A. & Goldstein G. (2004) Adaptive radiation of photosynthetic physiology in the Hawaiian lobeliads: Light regimes, static light responses, and whole-plant compensation points. *American Journal of Botany*, **91**, 228-246.

Kerstiens G. (1996) Cuticular water permeability and its physiological significance. *Journal of Experimental Botany*, **47**, 1813-1832.

Koide R.T., Robichaux R.H., Morse S.R. & Smith C.M. (2000) Plant water status, hydraulic resistance and capacitance. In: *Plant Physiological Ecology: Field Methods and Instrumentation* (eds R.W. Pearcy, J.R. Ehleringer, H.A. Mooney, & P.W. Rundel), pp. 161-183. Kluwer, Dordrecht, the Netherlands.

Kubiske M.E. & Abrams M.D. (1991) Rehydration effects on pressure-volume relationships in four temperate woody species: variability with site, time of season and drought conditions. *Oecologia*, **85**, 537-542.

Lewis A.M. & Boose E.R. (1995) Estimating volume flow rates through xylem conduits. *American Journal of Botany*, **82**, 1112-1116.

Long S.P. & Bernacchi C.J. (2003) Gas exchange measurements, what can they tell us about the underlying limitations to photosynthesis? Procedures and sources of error. *Journal of Experimental Botany*, **54**, 2393-2401.

Miller R.O. (1998) High Temperature Oxidation: Dry Ashing. In: *Handbook of Reference Methods for Plant Analysis* (ed Y.P. Kalra), pp. 53-56. CRC Press, Boca Raton, FL.

Pearcy R.W., Schulze E.-D. & Zimmermann R. (2000) Measurement of transpiration and leaf conductance. In: *Plant Physiological Ecology: Field Methods and Instrumentation* (eds R.W. Pearcy, J.R. Ehleringer, H.A. Mooney, & P.W. Rundel), pp. 137-160. Kluwer Academic, Dordrecht.

Porder S., Paytan A. & Vitousek P.M. (2005) Erosion and landscape development affect plant nutrient status in the Hawaiian Islands. *Oecologia*, **142**, 440-449.

Rasband W.S. (1997-2008) *ImageJ,* [*http://rsb.info.nih.gov/ij/*](http://rsb.info.nih.gov/ij/)*,* , Bethesda, Maryland, USA.

Sack L., Cowan P.D., Jaikumar N. & Holbrook N.M. (2003) The 'hydrology' of leaves: co-ordination of structure and function in temperate woody species. *Plant, Cell and Environment*, **26**, 1343-1356.

Sack L. & Frole K. (2006) Leaf structural diversity is related to hydraulic capacity in tropical rain forest trees. *Ecology*, **87**, 483-491.

Sack L., Melcher P.J., Zwieniecki M.A. & Holbrook N.M. (2002) The hydraulic conductance of the angiosperm leaf lamina: a comparison of three measurement methods. *Journal of Experimental Botany*, **53**, 2177-2184.

Scoffoni C., Pou A., Aasamaa K. & Sack L. (2008) The rapid light response of leaf hydraulic conductance: new evidence from two experimental methods. *Plant Cell and Environment*, **31**, 1803-1812.

Sokal R.R. & Rohlf F.J. (1995) *Biometry, 3rd ed.* W.H. Freeman, New York.

Tijms H. (2007) *Understanding Probability*. Cambridge University Press, Cambridge, UK.

Tyree M.T., Nardini A., Salleo S., Sack L. & El Omari B. (2005) The dependence of leaf hydraulic conductance on irradiance during HPFM measurements: any role for stomatal response? *Journal of Experimental Botany*, **56**, 737-744.

Weast R.C., ed. (1974) *Handbook of Chemistry and Physics, 54th ed.* (54th ed.). CRC Press, Cleveland, Ohio.

**Table S1.** Leaf morphology traits, symbols, and units, and mean values ± 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. \*0.05 > *P* ≥ 0.01; \*\*0.01 > *P* ≥ 0.001; \*\*\**P* < 0.001. Rows in gray highlight the traits with statistically significant differences at *P* ≤ 0.05.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Leaf morphology traits** | **Symbol** | **Units** | **Leaf rachis** | **Leaflet** | **Leaf** | **Phyllode** | ***t*-test** |
| Thickness |  | mm |  |  | 0.198 ± 0.0155 (7) | 0.425 ± 0.0126 (8) | \*\*\* |
| Density |  | g · cm-3 |  | 0.244 ± 0.011 (7) |  | 0.189 ± 0.0118 (7) | \*\*\* |
| Area |  | cm2 | 21.7 ± 1.97 (7) | Single leaflet:  0.417 ± 0.0358 (7)  All leaflets:  115 ± 10.9  for 276 ± 11.1 leaflets per leaf (7) | 137 ± 11.7 (7) | 26.8 ± 3.56 (7) | \*\*\* |
| Dry mass |  | g |  |  | 0.891 ± 0.0954 (7) | 0.203 ± 0.0157 (8) | \*\*\* |
| Fractional area of leaf |  |  | 0.162 ± 0.0160 (7) | 0.838 ± 0.0160 (7) |  |  |  |
| Fractional mass of leaf |  |  | 0.379 ± 0.0140 (7) | 0.621 ± 0.0140 (7) |  |  |  |
| Leaflet mass per leaflet area |  | g · m-2 |  | 47.4 ± 2.28 (7) |  |  |  |
| Mass per leaf area | *LMA* | g · m-2 | 24.3± 1.49 (7) | 39.7 ± 1.87 (7) | 64.0 ± 2.77 (7) | 78.4 ± 3.17 (7) | \*\* |

**Table S2.** Leaf composition traits, symbols, and units, and mean values ± 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. \*0.05 > *P* ≥ 0.01; \*\*0.01> *P* ≥ 0.001; \*\*\**P* < 0.001. Rows in gray highlight the traits with statistically significant differences at *P* ≤ 0.05.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Leaf composition traits** | **Symbol** | **Units** | **Leaf rachis** | **Leaflet** | **Leaf** | **Phyllode** | ***t*-test** |
| Saturated water content | *SWC* | g · g-1 |  |  | 2.89 ± 1.14 (7) | 2.90 ± 0.239 (5) | NS† |
| Water mass per area |  | g · m-2 |  |  | 166 ± 11.1 (7) | 222 ± 19.8 (5) | \*† |
| Nitrogen per area | *N*area | g · m-2 |  | 1.92 ± 0.112 (7) | 2.50 ± 0.151 (7) | 3.71 ± 0.174 (8) | \*\*\* |
| Nitrogen per mass | *N*mass | % | 2.44 ± 0.239 (7) | 4.86 ± 0.241 (7) | 3.94 ± 0.256 (7) | 4.72 ± 0.270 (8) | \* |
| Phosphorus per area | *P*area | g · m-2 |  | 0.137 ± 0.0114 (7) | 0.256 ± 0.0225 (7) | 0.228 ± 0.00793 (8) | NS |
| Phosphorus per mass | *P*mass | % | 0.493 ± 0.0492 (7) | 0.345 ± 0.0216 (7) | 0.399 ± 0.0262 (7) | 0.286 ± 0.00725 (8) | \*\* |
| Carbon per area | *C*area | g · m-2 |  | 19.0 ± 0.846 (7) | 29.7 ± 1.25 (7) | 36.7 ± 1.62 (8) | \*\* |
| Carbon per mass | *C*mass | % | 43.8 ± 0.193 (7) | 48.0 ± 0.260 (7) | 46.4 ± 0.162 (7) | 46.8 ± 0.289 (8) | \* |
| Nitrogen: phosphorus ratio | *N:P* |  | 5.20 ± 0.688 (7) | 14.7 ± 1.68 (7) | 10.228 ± 1.16 (7) | 16.5 ± 0.900 (8) | \*\* |
| Nitrogen: carbon ratio | *N:C* |  | 0.0558 ± 0.00546 (7) | 0.101 ± 0.00534 (7) | 0.0850 ± 0.00566 (7) | 0.101 ± 0.00622 (8) | \* |
| Carbon isotope ratio | *δ13C* | ‰ | -31.1 ± 0.283 (7) | -32.3 ± 0.331 (7) | -31.8 ± 0.298 (7) | -30.7 ± 0.0354 (8) | \*\* |

**Table S3.** Leaf stomatal traits, symbols, and units, and mean values ± 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. \* 0.05 > *P* ≥ 0.01; \*\*0.01> *P* ≥ 0.001; \*\*\**P* < 0.001. Rows in gray highlight the traits with statistically significant differences at *P* ≤ 0.05.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Stomatal traits** | **Symbol** | **Units** | **Leaf** | **Phyllode** | ***t*-test** |
| Stomatal density adaxial | *SD*ad | mm-2 | 20.6 ± 4.47 (6) | 124 ± 5.58 (6) | \*\*\* |
| Stomatal density abaxial | *SD*ab | mm-2 | 200 ± 3.32 (6) | 118 ± 10.8 (6) | \*\*\* |
| Stomatal density, total | *SD*tot | mm-2 | 221 ± 6.80 (6) | 242 ± 16.2 (6) | NS |
| % stomatal density adaxial | %*SD*ad | mm-2 | 9.12 ± 1.70 (6) | 51.8 ±1.60 (6) | \*\*\* |
| Guard cell length, adaxial | *GCL*ad | µm | 23.9 ± 1.98 (6) | 21.3 ± 1.05 (6) | NS |
| Guard cell length, abaxial | *GCL*ab | µm | 23.4 ± 1.36 (6) | 20.9 ± 0.936 (6) | NS |
| Guard cell width, adaxial | *GCW*ad | µm | 12.2 ± 1.45 (6) | 11.4 ± 1.82 (6) | NS |
| Guard cells width, abaxial | *GCW*ab | µm | 9.46 ± 1.08 (6) | 11.0 ± 2.46 (6) | NS |
| Pore length, adaxial | *PL*ad | µm | 15.1 ± 1.21 (6) | 10.1 ± 1.14 (6) | \* |
| Pore length, abaxial | *PL*ab | µm | 13.0 ± 0.922 (6) | 11.0 ± 0.618 (6) | NS |
| Stomatal pore index, adaxial | *SPI*ad |  | 0.500 ± 0.156 (6) | 1.38 ± 0.340 (6) | \* |
| Stomatal pore index, abaxial | *SPI*ab |  | 3.48 ± 0.531 (6) | 1.51 ± 0.275 (6) | \*\* |
| Stomatal pore index, total | *SPI*tot |  | 3.98 ± 0.569 (6) | 2.89 ± 0.539 (6) | NS |
| % adaxial stomatal pore index | %*SPI*ad |  | 12.8 ± 3.27 (6) | 46.5 ± 5.84 (6) | \*\*\* |
| Theoretical stomatal conductance | *g*th | mol · m-2 · s-1 | 2.065 ± 0.155 (6) | 1.64 ± 0.201 (6) | NS |

**Table S4.** Leaf mesophyll anatomy traits, symbols, and units, and mean values ± 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. \*0.05 > *P* ≥ 0.01; \*\*0.01 > *P* ≥ 0.001; \*\*\**P* < 0.001. Rows in gray highlight the traits with statistically significant differences at *P* ≤ 0.05.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Mesophyll anatomy traits** | **Units** | **Leaf, adaxial half** | **Leaf, abaxial half** | **Leaf** | **Phyllode** | ***t*-test** |
| *Tissue thicknesses* |  |  |  |  |  |  |
| Cuticle thickness | µm | 2.06 ± 0.958 (5) | 1.39 ± 0.661 (5) |  | 4.08 ± 0.660 (5) | \*, NS |
| Epidermal thickness (= cell height) | µm | 13.6 ± 0.963 (5) | 14.5 ± 1.52 (5) |  | 12.7 ± 1.16 (5) | NS, NS |
| Palisade mesophyll thickness | µm | 52.4 ± 3.70 (5) |  |  | 68.6 ± 6.47 (5) | NS |
| Spongy mesophyll thickness | µm |  | 72.1 ± 4.81 (5) |  |  |  |
| Palisade: spongy thickness ratio | µm |  |  | 0.732 ± 0.0451 (5) |  |  |
| Water storage tissue thickness | µm |  |  |  | 155 ± 3.82 (5) |  |
| *Numbers of cells in tissue layer* |  |  |  |  |  |  |
| Palisade mesophyll tissue |  | 1.93 ± 0.221 (5) |  |  | 2.70 ± 0.207 (5) | \* |
| Spongy mesophyll tissue |  |  | 4.73 ± 0.356 (5) |  |  |  |
| Water storage tissue |  |  |  |  | 8.00 ± 0.624 (5) |  |
|  |  |  |  |  |  |  |
| *Cell dimensions* |  |  |  |  |  |  |
| Epidermal cell width |  | 22.7 ± 1.57 (5) | 21.5 ± 2.46 (5) |  | 13.2 ± 1.408 (5) | \*\*,\*\* |
| Palisade cell length | µm | 43.9 ± 3.99 (5) |  |  | 26.2 ± 2.93 (5) | \*\* |
| Palisade cell width | µm | 11.6 ± 0.697 (5) |  |  | 9.43 ± 0.574 (5) | \* |
| Spongy cell diameter | µm |  | 20.1 ± 1.25 (5) |  |  |  |
| Water storage cell diameter | µm |  |  |  | 31.3 ± 2.81 (5) |  |

**Table S5.** Leaf xylem anatomy traits, symbols, and units, and mean values ± standard error for leaves and phyllodes of *Acacia koa* grown under controlled conditions, and significance of paired *t*-tests for comparisons of leaf types. \* 0.05 > *P* ≥ 0.01; \*\*0.01 > *P* ≥ 0.001; \*\*\**P* < 0.001. Rows in gray highlight the traits with statistically significant differences at *P* ≤ 0.05

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Xylem traits** | **Symbol** | **Units** | **Leaf** | **Phyllode** | ***t*-test** |
| *Primary vein* |  |  |  |  |  |
| Number of conduits |  |  | 8.00 ± 1.049 (5) | 43.2 ± 6.47 (5) | \*\* |
| Mean maximum conduit diameter |  | µm | 8.07 ± 0.727 (5) | 13.2 ± 0.672 (5) | \*\*\* |
| Theoretical hydraulic conductivity | *K*t | mmol · m · s-1 · MPa-1, × 10-5 | 6.78 ± 1.62 (5) | 264 ± 56.6 (5) | \*\* |
| Theoretical hydraulic conductivity,  normalized by leaf area and length,  for all primary veins | *K*t' | mmol · m-2 · s-1 · MPa-1 | 181 ± 44.6 (5) | 13.7 ± 3.55 (5) | \* |
| *Minor veins* |  |  |  |  |  |
| Number of conduits |  |  | 2.00 (5) | 6.60 ± 2.088 (5) | NS |
| Mean maximum conduit diameter |  | mmol · m-2 · s-1 · MPa-1, × 10-5 | 7.00 ± 0.814 (5) | 6.94 ± 0.747 (5) | NS |
| Theoretical hydraulic conductivity | *K*t | mmol · m-2 · s-1 · MPa- | 0.799 ± 0.43 (5) | 2.02 ± 0.728 (5) | NS |

**Table S6.** Leaf cuticular conductance and pressure volume curve parameters, symbols, and units, and mean values ± 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 (except paired for cuticular conductance). \* 0.05 > *P* ≥ 0.01; \*\*0.01 > *P* ≥ 0.001; \*\*\**P* < 0.001. Rows in gray highlight the traits with statistically significant differences at *P* ≤ 0.05.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Leaf drought tolerance traits** | **Symbol** | **Units** | **Leaf** | **Phyllode** | ***t*-test** |
| Cuticular conductance | *g*min | mmol · m-2 · s-1 | 3.62 ± 0.349 (13) | 3.98 ± 0.361 (13) | NS |
|  |  |  |  |  |  |
| *Pressure volume curve parameters* |  |  |  |  |  |
| Turgor loss point | πtlp | MPa | -1.52 ± 0.0557 (7) | -1.42 ± 0.620 (5) | NS |
| Osmotic potential full turgor | πo | MPa | -1.32 ± 0.0618 (7) | -1.18 ± 0.0385 (5) | NS |
| Elastic modulus | ε | MPa | 15.0 ± 1.21 (7) | 7.77 ± 1.52 (5) | \*\* |
| Relative capacitance at full turgor | *C*ft | MPa-1 | 0.0600 ± 0.00498 (7) | 0.121 ± 0.213 (5) | \*\* |
| Relative capacitance at turgor loss | *C*tlp | MPa-1 | 0.218 ± 0.0253 (7) | 0.303 ± 0.410 (5) | NS |
| Absolute capacitance per leaf area, at full turgor | *C*ft*\** | g · m-2 · MPa-1 | 15.8 ± 1.81 (7) | 23.1 ± 1.40 (5) | \* |
| Absolute capacitance per leaf area, at turgor loss | *C*tlp\* | g · m-2 · MPa-1 | 32.8 ± 4.30 (7) | 56.2 ± 5.64 (5) | \*\* |
| Relative water content at turgor loss point | *RWC*tlp | % | 91.4 ± 0.590 (7) | 83.9 ± 2.94 (5) | \* |

**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 ± standard error for leaves and phyllodes of *Acacia koa* grown under controlled conditions, and significance of *t*-tests for comparisons of leaf types. \*0.05 > *P* ≥ 0.01; \*\*0.01 > *P* ≥ 0.001; \*\*\**P* < 0.001. †Mean values were determined from fitted functions in Figs 4 and 6, and standard errors determined by propagation of error, except when the parameters of sigmoidal responses had too wide confidence intervals. Except for ‡, *t*-tests were conducted using leaf and phyllode means and standard errors; when standard errors were absent for one leaf type we tested whether its mean value fell within the 95% confidence intervals for the other. Rows in bold highlight the traits with statistically significant differences at *P* ≤ 0.05. Recovery after rehydration for leaf hydraulic conductance, photosynthetic rate and stomatal conductance are expressed as a % of the value expected from the dehydration trajectory. a Not significantly different from 100% recovery for its leaf water potential as expected from the dehydration trajectory.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Parameters of responses to experimental drought** | **Symbol** | **Units** | **Leaf** | **Phyllode** | ***t*-test** |
|  |  |  |  |  |  |
| Leaf hydraulic conductance at full turgor† | *K*leaf, max | mmol · m-2 · s-1 · MPa | 3.73 ± 0.77 | 15.5 | \*\*\* |
| Leaf water potential for transpiring leaf, moist soil | Ψleaf, moist soil | MPa | -0.550 ± 0.0693 | -0.432 ± 0.069 | NS |
| Leaf hydraulic conductance at Ψleaf moist soil† | *K*leaf, moist soil | mmol · m-2 · s-1 · MPa | 3.39 ± 0.81 | 5.99 | \* |
| Ψ at 20% loss of *K*leaf, moist soil† |  | MPa | -1.14 ± 0.32 | -0.533 | NS |
| Ψ at 50% loss of *K*leaf, moist soil† |  | MPa | -1.78 ± 0.37 | -0.746 | \* |
| Ψ at 80% loss of *K*leaf, moist soil† |  | MPa | -2.57 ± 0.58 | -1.16 | \* |
|  |  |  |  |  |  |
| *Stomatal conductance* |  |  |  |  |  |
| Ψ at 20% loss of *g*max† |  | MPa | -1.72 | -1.51 ± 0.067 | \* |
| Ψ at 50% loss of *g*max† |  | MPa | -1.77 | -1.63 ± 0.14 | NS |
| Ψ at 80% loss of *g*max† |  | MPa | -1.82 | -1.75 ± 0.29 | NS |
|  |  |  |  |  |  |
| *Photosynthetic carbon assimilation rate* |  |  |  |  |  |
| Ψ at 20% loss of maximum light-saturated rate† |  | MPa | -1.75 ± 0.062 | -1.17 ± 0.25 | \* |
| Ψ at 50% loss of maximum light-saturated rate† |  | MPa | -1.80 ± 0.14 | -1.61 ± 0.39 | NS |
| Ψ at 80% loss of maximum light-saturated rate† |  | MPa | -1.84 ± 0.32 | -2.07 ± 0.77 | NS |
|  |  |  |  |  |  |
| *Recovery after rehydration* |  |  |  |  |  |
| Recovery in water potential for rehydrated shoots |  | MPa | 0.50 ± 0.12 (6) | 0.66 ± 0.05 (8) | NS‡ |
| Recovery of *K*leaf for rehydrated shoots |  | % | No recovery | 123a |  |
| Recovery of *K*leaf for rehydrated plants |  | % | No recovery | 166a |  |
| Recovery of stomatal conductance for rehydrated plants |  | % | 87a | 79 a | NS |
| Recovery of photosynthetic rate for rehydrated plants |  | % | >100a | 87 a | NS |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Photosynthetic Light**  **Response Traits** | **Symbol** | **Units** | **Leaflet** | **Leaf** | **Phyllode** | ***t*-test** |
| Quantum efficiency  per area | *QE*area | mol CO2 (mol photons)-1 | 0.0573 ± 000514 (13) | 0.0480 ± 0.00440 (7)† | 0.0494 ± 0.00490 (13) | NS, NS‡ |
| Quantum efficiency  per mass | *QE*mass | mmol CO2 · g-1 (mol photons m-2)-1 | 1.87 ± 0.179 (13) | 1.16 ± 0.114 (7)† | 0.636 ± 0.0683 (13) | \*\*\*,\*\*‡ |
| Respiration rate per area | *R*area | µmol CO2 · m-2 · s-1 | 0.951 ± 0.185 (13) | 0.797 ± 0.156 (7)† | 1.00 ± 0.164 (13) | NS, NS‡ |
| Respiration rate per mass | *R*mass | nmol CO2 · g-1 · s-1 | 0.0309 ± 0.00595 (13) | 0.0192 ± 0.00372 (7)† | 0.0128 ± 0.00225 (13) | \*\*, NS‡ |
| Light saturated assimilation  per area | *A*area | µmol CO2 · m-2 · s-1 | 12.0 ± 0.958 (13) | 10.0 ± 0.825 (7)† | 11.8 ± 0.590 (13) | NS, NS‡ |
| Light saturated assimilation  per mass | *A*mass | µmol CO2 · g-1 · s-1 | 0.397 ± 0.0428 (13) | 0.246 ± 0.0272 (7)† | 0.150 ± 0.01 (13) | \*\*\*,\*‡ |
| Light saturated assimilation  per nitrogen | *A/N* | mmol CO2 · (mol N)-1 · s-1 | 0.229 ± 0.0272 (7) | 0.175 ± 0.0224 (7)† | 0.0890 ± 0.00781 (8) | \*\*‡,\*\*‡ |
| Saturation irradiance | *I*s | µmol photons · m-2 · s-1 | 621 ± 59.8 (13) |  | 837 ± 81.7 (13) | NS |
| Light compensation point | *I*c | µmol photons· m-2 · s-1 | 16.0 ± 2.21 (13) |  | 19.4 ± 2.06 (13) | NS |
| Maximum stomatal  conductance | *g*max | mol · m-2 · s-1 | 0.102 ± 0.0121 (13) | 0.0857 ± 0.0102 (7)† | 0.0988 ± 0.00781 (11) | NS, NS‡ |
| Instantaneous water use  efficiency | *WUE* | mmol H20 · (mol CO2)-1 | 9.90 ± 0.736 (13) | 9.90 ± 0.736 (13)† | 8.69 ± 0.541 (11) | NS, NS |

**Table S8.** Leaf photosynthetic light response traits, symbols, and units, and mean values ± standard error for leaflets, leaves and phyllodes of *Acacia koa* grown under controlled conditions, and significance of *t*-tests for comparisons of leaf types (leaflet versus phyllode and whole leaf versus phyllode respectively). †Values estimated from gas exchange values made on leaflets, and mass and area allocation to leaflets and rachis, assuming that the rachis had negligible stomatal conductance, photosynthesis and respiration (see Supplemetary Methods, “*Gas exchange: estimated values for whole leaves”*); standard errors determined by propagation of error. \*0.05 > *P* ≥ 0.01; \*\*0.01 > *P* ≥ 0.001; \*\*\**P* < 0.001. ‡The *t*-tests were conducted on means and standard errors. Rows in gray highlight the traits with statistically significant differences at *P* ≤ 0.05.

**Table S9.** Parameters of the leaf photosynthetic response to intercellular CO2 concentration (ci), symbols, and units, and mean values ± standard error for leaflets, leaves and phyllodes of *Acacia koa* grown under controlled conditions, and significance of *t*-tests for comparisons of leaf types (leaflet versus phyllode and whole leaf versus phyllode respectively). †Values estimated from gas exchange values made on leaflets, and mass and area allocation to leaflets and rachis, assuming that the rachis had negligible photosynthesis (see Supplementary Methods, “*Gas exchange: estimated values for whole leaves”*); standard errors determined by propagation of error. x*P* = 0.08; \*0.05 > *P* ≥ 0.01; \*\*0.01> *P* ≥ 0.001; \*\*\**P* < 0.001. ‡The *t*-tests were conducted on means and standard errors. Rows in gray highlight the traits with statistically significant differences at *P* ≤ 0.05.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **A-ci parameters** | **Symbol** | **Units** | **Leaflet** | **Leaf** | **Phyllode** | ***t*-test** |
| Maximum carboxylation velocity  per area, 25°C | *V*c,max | µmol CO2 · m-2 · s-1 | 139 ± 17.3 (11) | 117 ± 14.6† (7) | 85.1 ± 6.25 (10) | \*, x‡ |
| Electron transport capacity, 25°C | [*J*max](mailto:Jmax@25/area) | µmol e- · m-2 · s-1 | 235 ± 13.0 (11) | 197 ± 11.5† (7) | 162 ± 9.54 (10) | \*\*, \*‡ |
| Quantum efficiency of photosystem II | ФPSII |  | 0.280 ± 0.0212 (11) |  | 0.268 ± 0.0114 (10) | NS |
| Maximum carboxylation velocity per area,  25°C per nitrogen | *V*c,max/*N* | mmol CO2 · (mol N)–1 · s-1 | 2.23 ± 0.240 (7) | 1.71 ± 0.201 (7)† | 0.634 ± 0.0540 (8) | \*\*, \*\*‡ |
| Electron transport capacity, 25°C,  per nitrogen | *J*max/*N* | mmol e- · (mol N)–1 · s–1 | 3.82 ± 0.311 (7) | 2.93 ± 0.276 (7)† | 1.21 ± 0.099 (8) | \*\*\*, \*\*‡ |

**Table S10.** Leaf traits measured relating to vapor pressure deficit, symbols, and units, and mean values ± standard error for leaflets, leaves and phyllodes of *Acacia koa* grown under controlled conditions, and significance of one-tailed *t*-tests; *n* = 5 for all parameters. \*0.05 ≥ *P* ≥ 0.01; \*\*0.01 > *P* ≥ 0.001. Rows in gray highlight the traits with statistically significant differences at *P* ≤ 0.05.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **VPD parameters** | **Symbol** | **Units** | **Leaflet** | **Phyllode** | ***t*-test** |
| Stomatal conductance ratio | *g*2/*g*1 |  | 0.723 ± 0.0685 | 0.497 ± 0.0783 | \* |
| Photosynthetic rate ratio | *A*2/*A*1 |  | 0.796 ± 0.0164 | 0.611 ± 0.0749 | *NS* |
| Transpiration rate ratio | *E*2/*E*1 |  | 1.45 ± 0.136 | 1.01 ± 0.149 | *NS* |
| Time for 50% stomatal closure | *t*50% | min | 13.8 ± 2.57 | 12.1 ± 2.63 | *NS* |
| Time for 95% stomatal closure | *t*95% | min | 18.4 ± 4.37 | 22.6 ± 7.78 | *NS* |
| Time of ‘wrong-way response’ | *t*WWR | min | 9.32 ± 3.23 | 7.46 ± 0.683 | *NS* |