

Adjustment of structure and function of Hawaiian *Metrosideros polymorpha* at high vs. low precipitation

W. K. CORNWELL*†‡, R. BHASKAR§, L. SACK¶, S. CORDELL|| and
C. K. LUNCH*

*Department of Biological Sciences, Stanford University, Stanford, CA 94305, USA, §Department of Integrative Biology, University of California, Berkeley, CA 94720, USA, ¶Department of Botany, University of Hawai'i at Manoa, Honolulu, HI 96822, USA, ||USDA Forest Service, Hilo, HI 96720, USA

Summary

1. Populations of *Metrosideros polymorpha* establish across a broad range of precipitation in Hawai'i – from <400 to >10000 mm per year. To determine whether adjustment of hydraulic and photosynthetic traits could contribute to this success in both high and low rainfall, we sampled populations on the wet and the dry sides of Hawai'i Island, replicated on two different-aged lava flows at similar elevation and mean annual temperature.
2. We quantified 24 stem and leaf traits in an integrated study of hydraulics, gas exchange, leaf chemistry and anatomy.
3. Values for traits associated with capacity for water transport through the plant and gas exchange per leaf area were higher at dry sites, including photosynthetic capacity, nitrogen per leaf area and hydraulic conductivity on both sapwood area and leaf area basis. These adjustments, due to plastic and/or heritable differentiation, would partially compensate for generally lower water availability.
4. Specific leaf area shifted towards lower values at the dry sites. However, several other traits associated directly with drought tolerance were inflexible across sites, including stem vulnerability to embolism and leaf cuticular conductance, indicating that the ability to persist through dry periods is sustained across the species' range.
5. The ability of *M. polymorpha* to establish across a wide range of habitats is associated with, on one hand, adjustments in traits that would enable sustained growth across a dramatic range of moisture supply while simultaneously maintaining as fixed other traits that would contribute to survival through extended drought.

Key-words: conductance, percent loss conductance, photosynthesis, drought tolerance, precipitation gradient

Functional Ecology (2007) **21**, 1063–1071
doi: 10.1111/j.1365-2435.2007.01323.x

Introduction

The ability of some species to succeed in widely varying environmental conditions – to have a wide 'ecological amplitude' – has long been a fundamental empirical observation. Understanding the physiological basis that allows this type of commonness is an important goal of current research (Sultan *et al.* 1998; Maherali *et al.* 2002; Cavender-Bares, Kitajima & Bazzaz 2004). An understanding of ecological amplitude would be

particularly relevant for the Hawaiian Islands, where one plant species, *Metrosideros polymorpha* Gaud., has a distribution that spans one of the largest environmental ranges of any tree species. *Metrosideros polymorpha* dominates vegetation from sea level to greater than 2500 m elevation, on very recent lava flows as well as on soils greater than 4 million years old, and at sites ranging in rainfall from less than 400 mm per year to greater than 10 000 mm per year (Cordell *et al.* 1998).

Shifts in functional traits – via heritable differentiation or plasticity – can contribute to the ability of species to succeed in a wide range of conditions (Sultan *et al.* 1998; Pigliucci & Schmitt 1999; Maseda & Fernandez 2006; Valladares, Sanchez-Gomez & Zavala 2006), enabling the occupation of a wide environmental range. Variation in leaf traits of *M. polymorpha*, including

†Author to whom correspondence should be addressed. E-mail: will.cornwell@falw.vu.nl

‡Present address: Department of Systems Ecology, Faculty of Earth and Life Sciences, Vrije Universiteit, De Boelelaan 1087, 1081 HV Amsterdam, the Netherlands.

specific leaf area (SLA; leaf area per mass), anatomy, nutrient concentrations, photosynthetic rates and carbon isotope composition (Vitousek, Field & Matson 1990; Geeske, Aplet & Vitousek 1994; Kitayama *et al.* 1997; Cordell *et al.* 1998) has been found across both a range of elevations and the 4 million year substrate age gradient. However, despite the striking ability of *M. polymorpha* to establish at very wet and dry ends of enormous precipitation gradients, little is known about how variation in specific traits may allow for the species' success in such varied conditions.

Two inter-related sets of traits are important in mitigating the negative consequences of both soil and atmospheric drought: first, 'flux-related traits' promote a capacity for high rates of both water transport through the plant and photosynthesis on a per leaf area basis. Second, among evergreen taxa, drought tolerance traits allow for survival and the maintenance of leaf area and functional xylem through extended dry periods.

Leaf-specific hydraulic transport capacity facilitates the transport of water from the soil to the leaves, and thus permits a high stomatal conductance at a given water potential gradient (Carey, Callaway & Delucia 1998; Katul, Leuning & Oren 2003; Sack *et al.* 2003a; Sack & Holbrook 2006). An increase in leaf specific conductance could result from greater investment in sapwood area per leaf area and/or from building sapwood with greater conductivity (see Maherali & DeLucia 2001; Brodribb, Holbrook & Gutierrez 2002). If the soil water potential is close to a species' physiological limit, or if atmospheric demand is large (i.e. high vapor pressure deficit (VPD)), then high hydraulic capacity can be crucial in maintaining a long-term positive carbon balance (Mencuccini 2003; Maseda & Fernandez 2006).

In addition to maximum capacity, the physiological characteristics of the plant can have a profound effect on the ratio of carbon to water fluxes – the leaf-level water-use efficiency (WUE) (see Field, Merino & Mooney 1983). Building leaves with greater concentrations of nitrogen and other components of photosynthetic machinery per unit leaf area would have two benefits in dry environments. First, it would allow for higher maximum rates of photosynthesis when conditions are favourable. Second, when water is less available, higher photosynthetic capacity could allow maintenance of the same rate of photosynthesis while reducing stomatal conductance and thus water loss (Wright, Reich & Westoby 2003).

An additional set of traits would confer direct tolerance of drought, allowing persistence through prolonged dry periods. The plant may build xylem tissue that is less vulnerable to embolism at highly negative water potentials (Sperry *et al.* 2002). The ability to survive and maintain leaf area through extreme drought may also be increased by producing leaves with low surface-area-to-volume ratio (Hadley & Smith 1990; Sack, Grubb & Marañón 2003b) and low cuticular conductance (Kerstiens 1996).

We assessed these morphological, physiological, structural and chemical traits among *M. polymorpha* populations at high and low precipitation, replicated on two substrate ages. We hypothesized that *M. polymorpha* at dry sites would show strong shifts towards: (i) higher capacity for water transport per leaf area and gas exchange per leaf area; (ii) higher intrinsic WUE; and (iii) increased drought tolerance, including greater resistance to embolism and ability to restrict unregulated water loss.

Materials and methods

STUDY SITES

We sampled individuals of *M. polymorpha* Gaud. at four sites on the island of Hawai'i between June 2004 and June 2005, matched by elevation and mean annual temperature (Table 1). Two study sites were on the eastern wet side of the island while two were on the dry western side. The wet sites had eightfold higher mean annual rainfall, 7% higher mean relative humidity, and thus, given the similar temperatures, on average 54% lower mean vapour pressure deficit (Pearcy *et al.* 2000).

Plants were sampled on soils of two different ages at each moisture regime (Table 1). The sites on the younger substrates were on almost identical basalts derived from Mauna Loa. On the wet side, the older site was a *kipuka* – an island of substrate surrounded by younger flows with mapped age of 3000–5000 years; on the dry side, the older soil site was derived from a flow from Hualalai volcano, with a mapped age of 1000–3000 years (Wolfe & Morris 1996). All four soils in this study were at a stage of development when nutrients, especially nitrogen, are accumulating (Vitousek *et al.* 1992).

Individual trees were haphazardly sampled from the population of reproductively mature, canopy trees and were representative of the stands at the respective sites. Across the precipitation contrast, trees were of similar stature on the young lava flows; stature could not be controlled across the other sites (Table 1). Individuals at the wet site on old soils were especially tall and leaves were not easily accessible. As a result, for traits that required attached leaves (e.g. $A-c_i$), we could only make measurements to compare wet vs. dry sites on the younger soils.

Trait selection and measurement

SHOOT WATER POTENTIAL

In June 2004, pre-dawn and mid-day water potentials (ψ_{PD} and ψ_{MD} , respectively) were measured on terminal shoots using a pressure chamber (Plant Moisture Stress, Albany, OR, USA) for seven individuals at each site. ψ_{PD} was expected to reflect water potential in the root zone; plastic bags were placed over the shoots

Table 1. Site characteristics for the study of *Metrosideros polymorpha* populations on wet and dry soil and on older and younger soil, on the island of Hawai'i

	Sites			
	Dry young 19°49'N, 155°49'W	Dry old 19°46'N, 155°56'W	Wet young 19°41'N, 155°12'W	Wet old 19°41'N, 155°12'W
Coordinates				
Mean annual rainfall (mm per year)*	c. 600–700	c. 600–700	c. 5406	c. 5406
Mean relative humidity (%)†	74–80	74–80	82–88	82–88
Mapped date of lava flow‡	1859 flow	1000–3000 years	1855 flow	Older than 3000–5000 years
<i>Metrosideros polymorpha</i> mean height (m)	2–4	6–8	2–4	6–10
Vegetation	Colonizing on bare lava, widely spaced	Widely spaced among exotic fountain grass <i>Pennisetum setaceum</i> (Forsk.)	Widely spaced among mat- forming fern <i>Dicranopteris</i> <i>linearis</i> (Burm. f.) Underw.; Gleicheniaceae	In diverse, closed-canopy forest

All sites were at 600–800 m elevation, with mean annual temperature c. 20 °C (Giambelluca *et al.* 1986).

*From > 15-year data sets for closest weather stations; National Climate Data Center: <<http://cdo.ncdc.noaa.gov/CDO/cdo>>. At the dry sites, the seasonality of precipitation is largely unpredictable, but typically, most rain falls during large winter storms; lengthy droughts can occur during any time of the year.

†Hawai'i Weather/Climate Modeling Ohana: <<http://ecpc.ucsd.edu/projects/pdc/pdc.html>>.

‡Wolfe & Morris (1996).

the previous evening to reduce soil–leaf disequilibria due to potential nocturnal transpiration (Donovan, Linton & Richards 2001).

STEM HYDRAULIC CONDUCTIVITY

Branches from each individual (seven per site) were collected in the early morning, placed in moist plastic bags and brought back to the laboratory. Maximum xylem hydraulic conductivity was measured on stem segments >30 cm using a portable low pressure flow meter (LPFM; Feild, Brodribb & Holbrook 2002). In our system, flow solution passed from an elevated reservoir (4–5 kPa) through polyetheretherketone (PEEK) tubing in series with the stem segment; the flow rate through the system was calculated by multiplying the known conductance of the PEEK tubing by the drop in pressure across the tubing, as measured with a pressure transducer (PX136, Omega Engineering, Stamford, CT, USA) with output to a voltmeter. Stem segments were injected with pressurized air underwater to ensure that the segment length was longer than that of the longest vessel (Zimmermann & Jeje 1981). Flow solution of 10 mM KCl in filtered water was used to maximize flow through pit membranes (Zwieniecki, Melcher & Holbrook 2001). Prior to conductance measurements, stems were flushed at 150 kPa for 30 min to remove emboli and both ends shaved with a fresh razor blade. Stem cross-sections were photographed and sapwood area analyzed using IMAGEJ <<http://rsb.info.nih.gov/ij/>>. We multiplied maximum xylem conductance by stem length and divided by sapwood area to calculate sapwood area specific conductivity, K_S ($\text{kg m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$), and divided by total distal leaf area (see Methods below) to calculate leaf area specific conductivity, K_L ($\text{kg m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$).

RESISTANCE TO EMBOLISM

We constructed stem xylem vulnerability curves to determine sensitivity to water-stress-induced embolism (Sperry, Donnelly & Tyree 1988). From each site, three to four sun-exposed branches were collected from each of seven individuals, and dried on a bench to a range of water potentials. Due to difficulty of sampling large amounts of material from the tall forest at the wet old site, we excluded that site for this trait. Each branch was bagged 45 min prior to measurement to allow water potential equilibration. We measured water potential for a side shoot, and hydraulic conductance for a paired branch. A short segment of c. 5 cm was cut underwater, and hydraulic conductance was measured using the LPFM with <2 kPa delivery pressure (Brodribb *et al.* 2003). We measured conductance again after a high-pressure flush (175 kPa) lasting more than 5 min; the percent loss in stem conductance (PLC) was calculated as the conductance of the flushed stem minus that of the pre-flushed stem divided by that of the flushed stem. A vulnerability curve was generated for each site by plotting PLC against increasing xylem tension. Data were fitted with an exponential sigmoidal curve (Pammenter & Vander Willigen 1998), allowing determination of the xylem tension that corresponded to 50% PLC (PLC_{50}).

LEAF TO SAPWOOD AREA RATIO AND SPECIFIC LEAF AREA

We harvested shoots c. 15 cm in length from all individuals at the four sites. We obtained digital images of leaf areas using a scanner, and sapwood areas from digital micrographs of stem cross-sections. All areas were calculated using IMAGEJ. We calculated leaf to

sapwood area ratio (LA : SA) as the total leaf area distal to the cut section / sapwood area, and SLA as fresh area/dry mass.

LEAF GAS EXCHANGE AND RESPONSE TO CARBON DIOXIDE

In June 2004, CO₂ assimilation rate per area (A_{area}) and stomatal conductance (g) were measured on the same individual trees twice, mid-morning (10.30–11.30 h) and mid-day (11.45–12.30 h), with the cuvette set to a photosynthetic photon flux density (PPFD) of 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$, which the preliminary measurements showed as saturating in *M. polymorpha*, and a constant CO₂ partial pressure of 38.5 Pa. We held flow rate constant and used ambient temperature and humidity. Intrinsic instantaneous WUE was calculated as A/g , rather than the traditional A divided by transpiration, which allowed us to minimize the effect of cuvette VPD, which varied between 1.0 and 2.3 kPa. We weighed leaves after oven drying (70 °C for at least 72 h), to determine CO₂ assimilation per mass (A_{mass}).

In May and June of 2005, we measured the response of photosynthesis to the internal concentration of CO₂ (c_i ; LI-6400, LI-COR Inc., Lincoln, NE, USA) for 9–11 individuals at each site. We used a PPFD of 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$, a constant flow rate and ambient temperature and relative humidity. We stepped down cuvette CO₂ concentrations from 40 Pa partial pressure to 0 Pa and then up to 90 Pa. At each of nine cuvette CO₂ partial pressures, we used pre-set stability thresholds to record leaf gas exchange rates. We fitted response curves using non-linear regression (Farquhar, Von Caemmerer & Berry 1980; Lambers, Chapin & Pons 1998) and calculated the maximum rate of electron transport, J_{max} , and the maximum rate of carboxylation, V_{cmax} .

CUTICULAR CONDUCTANCE

We determined cuticular conductance (minimum conductance, g_{min} *sensu* Kerstiens (1996)) for leaves of the most recent fully expanded flush (one leaf per tree for five trees per site). We hydrated leaves overnight in water, wrapped in plastic and then dried them at PPFD < 5 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, for >2 h, to drive stomatal closure, after which we weighed the leaves at five intervals of 10–45 min, during which time they showed a linear slope for water loss vs. time ($R^2 > 0.99$). The g_{min} was calculated as the slope of water loss vs. time divided by the mole fraction water vapour gradient from leaf to air, assuming the leaf airspaces to be saturated (Pearcy *et al.* 2000). Mean and ranges of ambient temperature and relative humidity were 26.9 ± 0.3 °C and $64\% \pm 2\%$, respectively.

LEAF ANATOMY

We measured anatomical traits for leaves of the most recent expanded flush (one leaf per tree for five trees

per site), fixed in formalin acetic acid. Cross-sections of central lamina to the right of the midrib were made by cryostat (Leica CM 1850; Leica Microsystems Inc., Bannockburn, IL, USA), and photographed at 100 \times . We measured the thicknesses of the whole lamina, adaxial and abaxial cuticle and epidermises, hypodermis and mesophyll.

CHEMICAL COMPOSITION AND CARBON ISOTOPE RATIO

Dried leaf samples were finely ground and analysed for %N and $\delta^{13}\text{C}$ (PDZ Europa Scientific 20/20 Mass Spectrometer; Center for Stable Isotope Biogeochemistry at UC Berkeley).

STATISTICAL ANALYSIS

We analysed each trait for the effect of site – soil age and moisture level – using two-way ANOVAS; data were log-transformed when necessary to achieve normality. In cases when data were only available for the young soil contrast and when data were not normally distributed, we used a Wilcoxon rank sum test.

Results

PLANT WATER STATUS

The trees at the dry young sites had more negative Ψ_{PD} compared to the wet young site (Table 2). For trees transpiring at mid-day, shoots had fallen to a similar Ψ_{MD} at all sites, with no significant difference among sites; these ranged from -1.25 MPa for trees at the dry young site to -1.07 MPa at the wet young site.

TRAITS RELATING TO THE CAPACITY FOR WATER TRANSPORT AND GAS EXCHANGE PER LEAF AREA

The trees at the dry sites showed upward adjustment in traits relating to the capacity for water transport per leaf area and gas exchange per leaf area. Values for K_L were fivefold to sevenfold higher at dry sites (Fig. 1a). This variation arose due to 90%–130% higher values for K_S at dry sites (Fig. 1b) and 40%–48% lower values for LA : SA (Fig. 1c). Leaves from trees on different soil ages did not differ in K_L , but K_S was greater and LA : SA larger on older vs. younger soil (Fig. 1). Light-saturated V_{cmax} and maximum values of g , A_{area} and A_{mass} were higher at the dry young site relative to the wet young site (Table 2).

FLUX-RELATED TRAITS AND WATER-USE EFFICIENCY

Trees at dry sites showed adjustment in traits that would increase WUE. On young soils, trees at dry sites had a 29% higher V_{cmax} than at wet sites (Table 2). Trees

Table 2. Mean values (SE) for physiological functional traits for *Metrosideros polymorpha* from young lava flows on Hawaii with highly contrasting precipitation levels. Bold values indicate $P < 0.05$

	Dry	Wet	<i>N</i>	Wilcoxon <i>P</i>
Instantaneous shoot water potentials (Summer 2004)				
Pre-dawn Ψ (MPa)	-0.22 (0.01)	-0.075 (0.01)	7	0.002
Mid-day Ψ (MPa)	-1.25 (0.06)	-1.07 (0.07)	7	0.10
Instantaneous leaf gas exchange				
Summer 2004*				
A_{area} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)†	16.5 (0.6)	8.0 (0.7)	7	<0.001
A_{mass} ($\text{nmol g}^{-1} \text{s}^{-1}$)†	72.8 (3.1)	37.0 (3.4)	7	<0.001
g ($\text{mol m}^{-2} \text{s}^{-1}$)†	0.24 (0.02)	0.13 (0.01)	7	0.004
Morning A/g ($\mu\text{mol}/\text{mmol}$)	69.2 (4.2)	57.7 (3.5)	7	0.038
Mid-day A/g ($\mu\text{mol}/\text{mmol}$)	67.6 (2.3)	67.8 (2.0)	7	NS
Summer 2005‡				
A_{area} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	12.0 (0.5)	8.3 (0.7)	9–11	<0.001
g ($\text{mol m}^{-2} \text{s}^{-1}$)	0.14 (0.01)	0.10 (0.01)	9–11	0.13
A/g ($\mu\text{mol}/\text{mmol}$)	87 (6.1)	84 (6.1)	9–11	NS
Photosynthetic capacity (Summer 2005)				
J_{max} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	102.5 (5.2)	79.6 (9.1)	9–11	0.08
V_{cmax} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	86.1 (9.7)	55.6 (6.0)	9–11	0.002

*Measurement with cuvette at a CO_2 partial pressure of 38.5 Pa.

†Values for 2004 are the mean of the maximum rate for each individual including the morning and mid-day measurements.

‡Measurement taken mid-morning with cuvette CO_2 partial pressure of 40 Pa; this data point was used as part of $A-c_i$ curve fit.

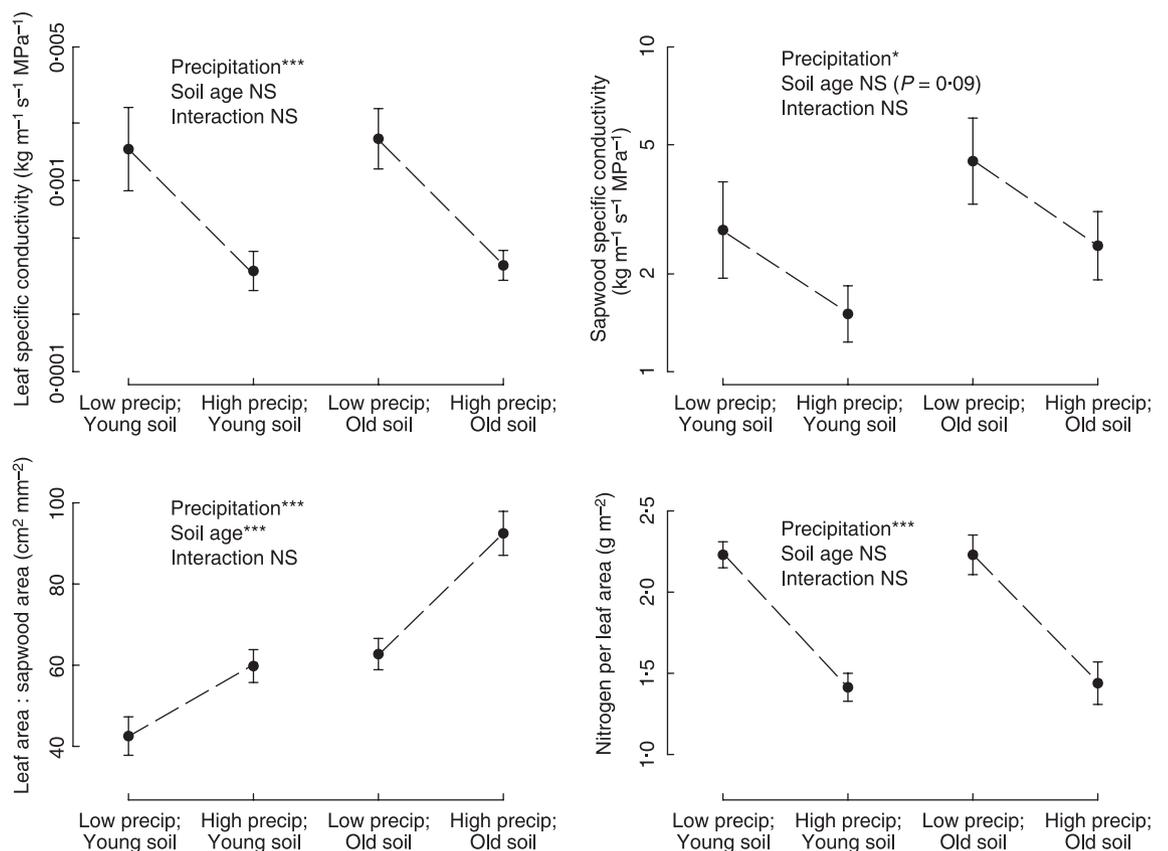


Fig. 1. Leaf-specific conductivity, sapwood specific conductivity, leaf area to sapwood area ratio and nitrogen per leaf area for *Metrosideros polymorpha* at four contrasting sites. Grey dotted lines connect precipitation contrasts on similar age soils. * represents $0.01 < P < 0.05$, ** $0.001 < P < 0.01$, *** $P < 0.001$ for each factor within a two-way ANOVA.

at the dry sites had 54%–59% higher N_{area} than at wet sites (Fig. 1d). This difference between wet and dry sites arose from a different mechanism for young and old soils. On the young soil, the dry site trees had

higher N_{area} due to an almost 60% higher N_{mass} , but similar SLA values to the wet site trees ($N_{\text{area}} = N_{\text{mass}}/\text{SLA}$), whereas on the older soil, the dry site trees had a 65% lower SLA, but similar N_{mass} to the wet site trees.

Table 3. Mean values (\pm SE) for leaf and stem functional traits for *Metrosideros polymorpha* from four sites on Hawaii Island. Bold values indicate $P < 0.05$

	Sites					ANOVA results (P values)		
	Dry young	Dry old	Wet young	Wet old	N	Precipitation	Age	Precipitation : age
Resistance to embolism								
PLC ₅₀ (MPa)	-2.15 (0.26)	-2.02 (0.23)	-2.35 (0.20)	nm	22–26			
Leaf composition								
SLA (cm ² g ⁻¹)	44.2 (1.4)	46.1 (1.6)	46.3 (1.8)	76.3 (5.4)	7, lt	<0.001	<0.001	<0.001
$\delta^{13}\text{C}$ (‰)	-27.5 (0.39)	-27.9 (0.36)	-28.8 (0.42)	-31.1 (0.25)	7	<0.001	<0.001	0.01
Cuticular conductance (g_{min}) (mmol m ⁻² s ⁻¹)	4.00 (0.58)	3.10 (0.94)	3.35 (0.62)	2.79 (0.63)	5	0.15	0.03	0.59
N_{mass} (%)	1.04 (0.09)	0.99 (0.05)	0.65 (0.03)	1.08 (0.09)	7, lt	0.004	<0.001	0.008
Leaf tissue thicknesses (μm)								
Whole lamina	428 (21)	403 (9.2)	472 (32.3)	322 (19)	5	0.38	<0.001	0.005
Adaxial cuticle	12.2 (0.6)	9.0 (0.9)	8.5 (1.3)	3.6 (0.4)	5	<0.001	<0.001	0.40
Adaxial epidermis	12.9 (1.0)	10.7 (0.6)	11.3 (1.3)	9.6 (0.7)	5	0.15	0.04	0.77
Hypodermal thickness	95.9 (9.0)	85.7 (3.5)	98.1 (12.0)	62.4 (4.2)	5	0.15	0.04	0.09
Mesophyll thickness	277 (9.5)	280 (16)	324 (18)	221 (13)	5	0.67	0.001	0.002
Abaxial epidermis	10.5 (0.7)	9.9 (0.3)	11.7 (0.6)	11.5 (1.3)	5	0.06	0.54	0.81
Abaxial cuticle	7.6 (0.4)	4.8 (0.4)	7.0 (0.6)	3.4 (0.5)	5	0.06	<0.001	0.46

'nm' indicates that the trait was not measured at a site and 'lt' indicates that the values were log-transformed for statistical analysis. Note that ANOVA is not appropriate for the curve fit parameters for resistance to embolism measurements, but SEs for the parameter estimates do not suggest statistically different values.

We observed 20% higher A/g at the dry young site for the mid-morning sampling in June 2004 (Table 2), but there was no significant difference in A/g for the mid-day point in 2004 or for the morning in 2005. Leaves were significantly enriched in ¹³C, on dry vs. wet sites, and on young vs. old soils (Table 3), with an interaction indicating a greater effect of soil age at the wet sites.

TRAITS RELATING TO DROUGHT TOLERANCE

Several traits providing a measure of drought tolerance did not vary between wet and dry sites. The trees' PLC₅₀ did not differ significantly between wet and dry sites, ranging from -2.02 to -2.35 MPa (Table 3). Cuticular conductance did not vary significantly between wet and dry sites (Table 3). Leaves were of statistically similar thickness across the moisture gradient, as were the mesophyll, hypodermis and adaxial epidermis (Table 3). However, the adaxial cuticle was 43%–150% thicker at dry vs. wet sites. Notably, leaves had lower SLA at dry sites relative to wet sites, from 5% lower on young soil to 40% lower on old soil. This difference corresponded to greater leaf pubescence at dry sites (cf. Geeske *et al.* 1994; Hoof *et al.* 2007). Trees on young soil seemed to show adjustment in leaf features relative to trees on older soil. Individuals on young soil had thicker lamina and component tissues and lower SLA than trees on old soil, but on average 25% higher g_{min} than trees on old soils (Table 3).

Discussion

Our findings for *M. polymorpha* across an eightfold precipitation gradient support the importance of trait

adjustments for a species with very wide ecological amplitude. We found large shifts in functional traits, reflecting both long-term developmental processes (e.g. leaf structure and composition) and traits that can be modified quickly (e.g. LA : SA). We found a shift towards higher capacity for water transport and gas exchange at dry sites. These adjustments represent a combination of plastic and heritable differentiation; previous studies have shown that both factors contribute to leaf variation in *M. polymorpha* across gradients of elevation and soil age (Cordell *et al.* 1998). Because our study focused on field measurements, we cannot partition the observed variance in flux traits to plastic and heritable components.

The adjustments quantified here would contribute to the ability of *M. polymorpha* to maintain a favourable carbon balance and fitness across the range of habitats (Westoby *et al.* 2002). Shifts in functional traits would contribute to the trees' ability to increase water transport and gas exchange rates at dry sites while maintaining as fixed several important traits associated with survival through dry conditions.

COMPENSATORY ADJUSTMENT IN FLUX-RELATED TRAITS

Metrosideros polymorpha showed large variation in flux-related traits, with higher water transport capacity and gas exchange rates on dry soil. K_L was fivefold to sevenfold greater at dry vs. wet sites, due to both a substantially higher K_S and lower LA : SA ($K_L = K_S / \text{LA} : \text{SA}$). These adjustments would allow a higher water transport capacity at a given water potential gradient (Tyree & Zimmermann 2002). Notably, a field-based

study of *Pinus ponderosa* also found increased K_L under high VPD (Maherali & DeLucia 2001). In contrast, experimental seedlings of temperate trees (Shumway, Steiner & Kolb 1993) and forestry plantations of *Eucalyptus* clones (Vander Willigen & Pammenter 1998) showed decreased K_L with lower water availability. The similar results for *M. polymorpha* and *P. ponderosa* may reflect long-term acclimation and/or adaptation; whereas short-term effects of dry conditions might more typically lead to reduced K_L and gas exchange (Mencuccini 2003).

Metrosideros polymorpha showed a greater maximum carboxylation rate and photosynthetic rate at the young dry site (Table 2). The shift to higher photosynthetic capacity could partially offset the increased carbon costs associated with lower SLA and greater sapwood area per leaf area in dry conditions (see Carey *et al.* 1998). The higher A_{area} and A_{mass} also could allow a more sustained performance as soil dries, and/or a stronger performance during wet periods (Grubb 1998).

At mid-day, trees at all sites had equivalent Ψ_{MD} , suggesting 'isohydr' for *M. polymorpha* (Fisher *et al.* 2006). This behaviour would allow *M. polymorpha* to open stomata sufficiently for transpiration rates to bring leaf water potential to an approximate set point, across the range of water availabilities experienced at the different sites. As discussed above, the higher K_L for trees at the dry sites would allow a higher rate of gas exchange for this leaf water potential while avoiding dangerously negative water potentials. Consistent with this pattern, we found that measurements of g from 2004 were all higher at the young dry site relative to the young wet site (Table 2). This difference was not explained by differences in water availability at the time of measurement, as the dry site trees had more negative Ψ_{PD} ; the performance at drier sites might thus have been yet higher if access to water had been equal across sites.

THE CONTRIBUTION OF GREATER PHOTOSYNTHETIC CAPACITY TO INCREASED INTRINSIC WATER-USE EFFICIENCY

We predicted that one compensatory response to low precipitation would be to increase intrinsic WUE – the ratio of carbon fixed to stomatal conductance (A/g). This can be achieved by increasing photosynthetic capacity per leaf area in situations where improved WUE is advantageous (Wright *et al.* 2003). Our measurement of 29% greater V_{cmax} at the dry young site relative to the wet young site is consistent with this prediction. Calculations using our estimates of J_{max} and V_{cmax} and the Farquhar *et al.* (1980) model suggest that at a given value of g , dry site leaves will consistently have a higher intrinsic WUE. For example, using measured photosynthetic capacity values (Table 2) at a stomatal conductance of $0.1 \text{ mol m}^{-2} \text{ s}^{-1}$, we calculate that A and therefore A/g should be 18% higher for the dry site leaves. However, the Farquhar *et al.* (1980)

model also implies that A/g is strongly dependent on the value of g , with A/g declining as g increases from 0.1 to $0.2 \text{ mol m}^{-2} \text{ s}^{-1}$. Given the different diel and seasonal regulation of stomatal conductance in contrasting environments (Maherali *et al.* 2002), this adds considerable complexity to the interpretation of instantaneous measurements of A/g . We observed 20% greater A/g at the dry site only at the mid-morning sampling point in 2004 (Table 2) and no significant difference for the mid-day 2004 or 2005 measurements, perhaps due to larger g at the dry site.

Leaf carbon isotope composition provides a longer term index of the CO_2 concentration at the site of carboxylation (c_c) (Ehleringer & Osmond 2000). The carbon isotopic composition of *M. polymorpha* has been explored across gradients in soil age and elevation (Vitousek *et al.* 1990; Meinzer *et al.* 1992; Cordell *et al.* 1999), demonstrating that $\delta^{13}\text{C}$ integrates photosynthetic capacity, stomatal behaviour, the timing of photosynthesis and internal resistance to CO_2 diffusion. In previous work (e.g. Stewart *et al.* 1995), leaves enriched in ^{13}C have been interpreted as indicating higher A/g on average at dry sites. Consistent with these results, we found that for *M. polymorpha*, leaves at the dry sites were enriched in ^{13}C (Table 3), indicating a lower CO_2 concentration at the site of carboxylation (c_c) during the time when the carbon comprising the leaf was fixed. We note that a lower c_c at dry sites could arise from either a higher A/g or greater mesophyll resistance or both (Vitousek *et al.* 1990).

'FIXED' DROUGHT TOLERANCE TRAITS IN *METROSIDEROS POLYMORPHA* CONTRIBUTE TO A CONSTITUTIVE DROUGHT TOLERANCE ACROSS SITES

Metrosideros polymorpha possessed traits contributing to a fixed drought tolerance across the moisture gradient. The PLC_{50} for *M. polymorpha* values were moderately low, at -2.0 to -2.4 MPa (cf. median values of -0.9 MPa and -2.4 MPa , respectively, for trees of tropical rain forest and of tropical dry forest (Maherali, Pockman & Jackson 2004)). The Ψ_{MD} across the range was from -1.25 to -1.07 MPa , considerably above the measured values for PLC_{50} . The mean cuticular conductance (g_{min}) for *M. polymorpha* was low, $3.31 \text{ mmol m}^{-2} \text{ s}^{-1}$, similar to that of drought-tolerant temperate and tropical oak species (Kerstiens 1996). Given the lengthy dry periods in this system, a low g_{min} and low SLA would contribute to the long-term survival of shoots (Pratt *et al.* 2005).

Notably, the inflexibility of traits associated with drought tolerance for *M. polymorpha* growing even at wet sites may contribute to an ability to withstand occasional dry periods even at very high precipitation sites. *Metrosideros polymorpha* establishes on porous and shallow soils with low water-holding capacity, often surrounded by black lava rock with low albedo; drought tolerance traits may be advantageous even in

these very high precipitation environments. Notably, the lack of variation within *M. polymorpha* is similar to the observation of invariant drought tolerance traits for *Acer grandidentatum* along a local soil moisture gradient (Alder, Sperry & Pockman 1996).

ADJUSTMENTS ACROSS SOIL AGES AND COMPARISON TO ADJUSTMENTS ACROSS ELEVATION GRADIENTS

Metrosideros polymorpha showed strong patterning of traits across soil ages. The adjustments in the photosynthetic traits across soil ages confirmed and extended those shown in previous studies: g , A_{area} , A_{mass} and N_{area} were higher on older soils (see Cordell *et al.* 2001). Additionally, trees on older soils had higher LA : SA, which may be the optimal strategy, given the taller stature of the canopy at those sites (Buckley & Roberts 2006). We also note that the higher LA : SA was balanced by a slightly higher K_s , leading to a similar K_L across soil ages (Fig. 1). Traits associated with drought tolerance show a complex relationship with soil age. We found that leaf tissue thicknesses were greater on younger soils, as previously reported (Geeske *et al.* 1994; Cordell *et al.* 1998); these differences may contribute to the longer life span for leaves from younger sites (Reich *et al.* 1999; Cordell *et al.* 2001). However, the leaves from older sites had a lower g_{min} than leaves from younger sites.

Previous work has demonstrated that across elevation gradients, *M. polymorpha* shows adjustments in many traits. At higher elevations, *M. polymorpha* shows higher g , A_{area} , A_{mass} and N_{area} , which was termed an ecophysiological 'homeostasis' (Cordell *et al.* 1999). Notably, as found in this study across a moisture gradient, PLC₅₀ for *M. polymorpha* was similar across an elevation gradient, with PLC₅₀ declining substantially only in alpine habitats (Santiago, Jones & Goldstein, in press).

The contrast for *M. polymorpha* between the large intraspecific variation in hydraulic transport and photosynthetic capacity, and lack of intraspecific variation in resistance to embolism, is striking. Further studies of detailed physiology are needed for other species with success in a wide range of environmental conditions; such work would establish whether this set of adjustments and fixed traits is a common mechanism for achieving a wide ecological amplitude, and whether *M. polymorpha* is a model species in this respect.

Acknowledgements

We thank Peter Vitousek and Heraldo Farrington for logistical assistance; Kristen Frole for assistance with the anatomical measurements; the State of Hawaii DOFAW, Kamehameha Schools, and the Dry Forest Working Group for providing access to sites; and NSF for a graduate fellowship and a dissertation improvement grant to W.K.C. and for grant No. IOB-#0546784

to L.S. We also thank David Ackerly, Hafiz Maherali and Todd Dawson for helpful comments on the manuscript.

References

- Alder, N.N., Sperry, J.S. & Pockman, W.T. (1996) Root and stem xylem embolism, stomatal conductance, and leaf turgor in *Acer grandidentatum* populations along a soil moisture gradient. *Oecologia*, **105**, 293–301.
- Brodribb, T.J., Holbrook, N.M., Edwards, E.J. & Gutierrez, M.V. (2003) Relations between stomatal closure, leaf turgor and xylem vulnerability in eight tropical dry forest trees. *Plant, Cell and Environment*, **26**, 443–450.
- Brodribb, T.J., Holbrook, N.M. & Gutierrez, M.V. (2002) Hydraulic and photosynthetic co-ordination in seasonally dry tropical forest trees. *Plant, Cell and Environment*, **25**, 1435–1444.
- Buckley, T. & Roberts, D. (2006) How should leaf area, sapwood area, and stomatal conductance vary with tree height to maximize growth? *Tree Physiology*, **26**, 145–157.
- Carey, E.V., Callaway, R.M. & Delucia, E.H. (1998) Increased photosynthesis offsets costs of allocation to sapwood in an arid environment. *Ecology*, **79**, 2281–2291.
- Cavender-Bares, J., Kitajima, K. & Bazzaz, F.A. (2004) Multiple trait associations in relation to habitat differentiation among 17 Floridian oak species. *Ecological Monographs*, **74**, 635–662.
- Cordell, S., Goldstein, G., Meinzer, F.C. & Handley, L.L. (1999) Allocation of nitrogen and carbon in leaves of *Metrosideros polymorpha* regulates carboxylation capacity and $\delta^{13}\text{C}$ along an altitudinal gradient. *Functional Ecology*, **13**, 811–818.
- Cordell, S., Goldstein, G., Meinzer, F.C. & Vitousek, P.M. (2001) Regulation of leaf life-span and nutrient-use efficiency of *Metrosideros polymorpha* trees at two extremes of a long chronosequence in Hawaii. *Oecologia*, **127**, 198–206.
- Cordell, S., Goldstein, G., Mueller-Dombois, D., Webb, D. & Vitousek, P.M. (1998) Physiological and morphological variation in *Metrosideros polymorpha*, a dominant Hawaiian tree species, along an altitudinal gradient: the role of phenotypic plasticity. *Oecologia*, **113**, 188–196.
- Donovan, L.A., Linton, M.J. & Richards, J.H. (2001) Predawn plant water potential does not necessarily equilibrate with soil water potential under well-watered conditions. *Oecologia*, **129**, 328–335.
- Ehleringer, J.R. & Osmond, C.B. (2000) Stable isotopes. *Plant Physiological Ecology: Field Methods and Instrumentation* (eds R.W. Pearcy, J. Ehleringer, H.A. Mooney & P.W. Rundel), pp. 281–300. Kluwer Academic Publishers, Dordrecht.
- Farquhar, G.D., Von Caemmerer, S. & Berry, J.A. (1980) A biochemical model of photosynthetic CO₂ assimilation in leaves of C-3 species. *Planta*, **149**, 78–90.
- Feild, T.S., Brodribb, T. & Holbrook, M. (2002) Hardly a relict: freezing and the evolution of vesselless wood in Winteraceae. *Evolution*, **56**, 464–478.
- Field, C., Merino, J. & Mooney, H.A. (1983) Compromises between water-use efficiency and nitrogen-use efficiency in 5 species of California evergreens. *Oecologia*, **60**, 384–389.
- Fisher, R.A., Williams, M., Do Vale, R.L., Da Costa, A.L. & Meir, P. (2006) Evidence from Amazonian forests is consistent with isohydric control of leaf water potential. *Plant, Cell and Environment*, **29**, 151–165.
- Geeske, J., Aplet, G. & Vitousek, P.M. (1994) Leaf morphology along environmental gradients in Hawaiian *Metrosideros polymorpha*. *Biotropica*, **26**, 17–22.
- Giambelluca, T.W., Nullet, M.A. & Schroeder, T.A. (1986) Rainfall Atlas of Hawaii. Department of Land and Natural Resources, Honolulu, HI.

- Grubb, P.J. (1998) A reassessment of strategies of plants which cope with shortages of resources. *Perspectives in Plant Ecology and Evolution*, **1**, 3–31.
- Hadley, J.L. & Smith, W.K. (1990) Influence of leaf surface wax and leaf-area to water-content ratio on cuticular transpiration in western Conifers, USA. *Canadian Journal of Forest Research*, **20**, 1306–1311.
- Hoof, J., Sack, L., Webb, D.T. & Nilsen, E.T. (2007) Contrasting structure and function of pubescent and glabrous varieties of Hawaiian *Metrosideros polymorpha* at high elevation. *Biotropica*, in press. DOI: 10.1111/j.1744-7429.2007.00325.x.
- Katul, G., Leuning, R. & Oren, R. (2003) Relationship between plant hydraulic and biochemical properties derived from a steady-state coupled water and carbon transport model. *Plant, Cell and Environment*, **26**, 339–350.
- Kerstiens, G. (1996) Cuticular water permeability and its physiological significance. *Journal of Experimental Botany*, **47**, 1813–1832.
- Kitayama, K., Pattison, R., Cordell, S., Webb, D. & Mueller-Dombois, D. (1997) Ecological and genetic implications of foliar polymorphism in *Metrosideros polymorpha* Gaud. (Myrtaceae) in a habitat matrix on Mauna Loa, Hawaii. *Annals of Botany*, **80**, 491–497.
- Labbers, H., Chapin, F.S. & Pons, T.L. (1998) *Plant Physiological Ecology*. Springer, New York.
- Maherali, H. & Delucia, E.H. (2001) Influence of climate-driven shifts in biomass allocation on water transport and storage in ponderosa pine. *Oecologia*, **129**, 481–491.
- Maherali, H., Pockman, W.T. & Jackson, R.B. (2004) Adaptive variation in the vulnerability of woody plants to xylem cavitation. *Ecology*, **85**, 2184–2199.
- Maherali, H., Williams, B.L., Paige, K.N. & Delucia, E.H. (2002) Hydraulic differentiation of Ponderosa pine populations along a climate gradient is not associated with ecotypic divergence. *Functional Ecology*, **16**, 510–521.
- Maseda, P.H. & Fernandez, R.J. (2006) Stay wet or else: three ways in which plants can adjust hydraulically to their environment. *Journal of Experimental Botany*, **57**, 3963–3977.
- Meinzer, F.C., Rundel, P.W., Goldstein, G. & Sharifi, M.R. (1992) Carbon isotope composition in relation to leaf gas exchange and environmental conditions in Hawaiian *Metrosideros polymorpha* populations. *Oecologia*, **91**, 305–311.
- Mencuccini, M. (2003) The ecological significance of long-distance water transport: short-term regulation, long-term acclimation and the hydraulic costs of stature across plant life forms. *Plant, Cell and Environment*, **26**, 163–182.
- Pammenter, N.W. & Vander Willigen, C. (1998) A mathematical and statistical analysis of the curves illustrating vulnerability of xylem to cavitation. *Tree Physiology*, **18**, 589–593.
- Pearcy, R.W., Ehleringer, J., Mooney, H.A. & Rundel, P.W. (2000) *Plant Physiological Ecology: Field Methods and Instrumentation*. Kluwer Academic Publishers, Dordrecht.
- Pigliucci, M. & Schmitt, J. (1999) Genes affecting phenotypic plasticity in Arabidopsis: pleiotropic effects and reproductive fitness of photomorphogenic mutants. *Journal of Evolutionary Biology*, **12**, 551–562.
- Pratt, R.B., Ewers, F.W., Lawson, M.C., Jacobsen, A.L., Brediger, M.M. & Davis, S.D. (2005) Mechanisms for tolerating freeze-thaw stress of two evergreen chaparral species: *Rhus ovata* and *Malosma laurina* (Anacardiaceae). *American Journal of Botany*, **92**, 1102–1113.
- Reich, P.B., Ellsworth, D.S., Walters, M.B., Vose, J.M., Gresham, C., Volin, J.C. & Bowman, W.D. (1999) Generality of leaf trait relationships: a test across six biomes. *Ecology*, **80**, 1955–1969.
- Sack, L. & Holbrook, N.M. (2006) Leaf hydraulics. *Annual Review of Plant Biology*, **57**, 361–381.
- Sack, L., Cowan, P.D., Jaikumar, N. & Holbrook, N.M. (2003a) The ‘hydrology’ of leaves: co-ordination of structure and function in temperate woody species. *Plant, Cell and Environment*, **26**, 1343–1356.
- Sack, L., Grubb, P.J. & Marañón, T. (2003b) The functional morphology of juvenile plants tolerant of strong summer drought in shaded forest understories in southern Spain. *Plant Ecology*, **168**, 139–163.
- Santiago, L.S., Jones, T. & Goldstein, G. (in press) Physiological variation in Hawaiian *Metrosideros polymorpha* across a range of habitats: from dry forests to cloud forests. *Mountains in the Mist: Science for Conserving And Managing Tropical Montane Cloud Forests* (eds L.A. Bruijnzeel, F.N. Scatena, J.G. Hamilton, J.O. Juvik & P. Bubb). University of Hawaii Press, Honolulu.
- Shumway, D.L., Steiner, K.C. & Kolb, T.E. (1993) Variation in seedling hydraulic architecture as a function of species and environment. *Tree Physiology*, **12**, 41–54.
- Sperry, J.S., Donnelly, J.R. & Tyree, M.T. (1988) A method for measuring hydraulic conductivity and embolism in xylem. *Plant, Cell and Environment*, **11**, 35–40.
- Sperry, J.S., Hacke, U.G., Oren, R. & Comstock, J.P. (2002) Water deficits and hydraulic limits to leaf water supply. *Plant, Cell and Environment*, **25**, 251–263.
- Stewart, G.R., Turnbull, M.H., Schmidt, S. & Erskine, P.D. (1995) C-13 natural abundance in plant communities along a rainfall gradient—a biological integrator of water availability. *Australian Journal of Plant Physiology*, **22**, 51–55.
- Sultan, S.E., Wilczek, A.M., Bell, D.L. & Hand, G. (1998) Physiological response to complex environments in annual Polygonum species of contrasting ecological breadth. *Oecologia*, **115**, 564–578.
- Tyree, M.T. & Zimmermann, M.H. (2002) *Xylem Structure and the Ascent of Sap*. Springer, Berlin.
- Valladares, F., Sanchez-Gomez, D. & Zavala, M.A. (2006) Quantitative estimation of phenotypic plasticity: bridging the gap between the evolutionary concept and its ecological applications. *Journal of Ecology*, **94**, 1103–1116.
- Vander Willigen, C. & Pammenter, N.W. (1998) Relationship between growth and xylem hydraulic characteristics of clones of *Eucalyptus* spp. at contrasting sites. *Tree Physiology*, **18**, 595–600.
- Vitousek, P.M., Aplet, G., Turner, D. & Lockwood, J.J. (1992) The Mauna Loa environmental matrix: foliar and soil nutrients. *Oecologia*, **89**, 372–382.
- Vitousek, P.M., Field, C.B. & Matson, P.A. (1990) Variation in foliar $\delta^{13}\text{C}$ in Hawaiian *Metrosideros polymorpha* – a case of internal resistance. *Oecologia*, **84**, 362–370.
- Westoby, M., Falster, D.S., Moles, A.T., Vesk, P.A. & Wright, I.J. (2002) Plant ecological strategies: some leading dimensions of variation between species. *Annual Review of Ecology and Systematics*, **33**, 125–159.
- Wolfe, E.W. & Morris, J. (1996) *Geologic Map of the Island of Hawai'i*. U.S. Geological Survey, Washington DC.
- Wright, I.J., Reich, P.B. & Westoby, M. (2003) Least-cost input mixtures of water and nitrogen for photosynthesis. *American Naturalist*, **161**, 98–111.
- Zimmermann, M.H. & Jeje, A.A. (1981) Vessel length distribution in stems of some American woody plants. *Canadian Journal of Botany – Revue Canadienne De Botanique*, **59**, 1882–1892.
- Zwieniecki, M.A., Melcher, P.J. & Holbrook, N.M. (2001) Hydrogel control of xylem hydraulic resistance in plants. *Science*, **291**, 1059–1062.

Received 20 March 2007; accepted 28 June 2007
 Handling Editor: Ken Thompson