

Does rainfall explain variation in leaf morphology and physiology among populations of red ironbark (*Eucalyptus sideroxylon* subsp. *tricarpa*) grown in a common garden?

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Summary We investigated adaptation of leaf morphology and physiology of red ironbark (*Eucalyptus sideroxylon* Cunn. Ex. Wools subsp. *tricarpa* L.A.S. Johnson) in a common garden experiment. Fifteen populations, representing a rainfall range of 500 to 1055 mm per annum at the sites of seed collection, were grown at the same site. Because environmental variables other than rainfall did not vary significantly among populations, we were able to test if leaf morphology and physiology were related to seed-source rainfall. There were large differences among and within populations in all measured variables. Most univariate relationships with seed-source rainfall were not significant. Notable exceptions were the weak positive correlation of specific leaf area with seed-source rainfall—consistent with expectations—and the weak negative correlation of photosynthesis and stomatal conductance with seed-source rainfall—the opposite of what we predicted. In many cases, populations collected from sites of similar rainfall differed greatly in leaf morphology and physiology. Principal component analysis (PCA) reduced the 13 input variables to five principal components (PC) explaining 73.0% of the total variance in the original data. Some of the PC axes could be interpreted in terms of adaptation to drought (i.e., to seed-source rainfall), but relationships of accumulated variables (the PC axes) with seed-source rainfall were significant for only one PC axis. Hence, among red ironbark populations grown in a common garden, there was significant genetic variation in leaf morphology and physiology, but for most traits, this variation was unrelated to rainfall at the site of seed collection. This study adds to a growing body of common garden literature showing weak within-species relationships of leaf morphology and physiology with seed-source rainfall, in contrast to the consistently stronger relationships among species growing at different points along broad environmental gradients.

Keywords: carbon isotope, drought, leaf thickness, leaf width, nitrogen, oxygen isotope, photosynthesis, stomatal conductance, water-use efficiency.

Introduction

There are generally consistent relationships between several leaf attributes and environmental factors, especially water or nutrient availability (e.g., Reich et al. 2004). One of the most commonly observed relationships is the reduction in leaf size with decreasing rainfall (Givnish 1984, Fonseca et al. 2000), which is typically accompanied by an increase in leaf thickness and a decrease in specific leaf area (SLA, leaf area per unit dry mass) (Cunningham et al. 1999, Fonseca et al. 2000). There are several theories that may explain this relationship. One theory draws on the relationship between leaf size, especially leaf width, and boundary layer conductance. Small leaves track air temperature closely, whereas large leaves may be heated well above ambient (Gates et al. 1968) and suffer from over-heating, particularly when water is scarce (Smith 1978). Thus, a narrow leaf form is viewed as an adaptation to hot, sunny and dry environments (Werger and Ellenbroek 1978). Another theory suggests that thick or dense leaves provide greater structural reinforcement and consequently an increased ability to withstand wilting (Maximov 1929, Oertli 1989).

An alternative explanation is that leaf morphology is selected indirectly owing to its relationship with physiological and growth traits. Species with smaller and thicker leaves (low SLA) generally have lower mass-based rates of photosynthesis (A_{mass}) (Reich et al. 1999), lower relative growth rates as seedlings (Wright and Westoby 1999) and lower aboveground productivity per unit canopy mass (Warren and Adams 2000). Low SLA may be selected for in water-limited habitats because SLA is negatively correlated with instantaneous water-use efficiency (WUE) (Lamont et al. 2002, Sefton et al. 2002). However, there are putative effects of nutrient supply on SLA, A_{mass} and leaf life spans (Wright et al. 2002). Low SLA, A_{mass} and long leaf life spans have long been generalized in the literature as adaptations to resource-poor habitats (e.g., Grime 1977).

The majority of studies on trends in leaf morphology and

physiology in response to variations in environmental factors have included sequences of species that occur at different locations in a replacement series along an environmental gradient. Results of studies with numerous tree species reveal marked phenotypic plasticity in leaf morphology or physiology, or both (Cordell et al. 1998, Hovenden and Vander Schoor 2003). Confounding environmental factors with genetic factors is thus a common problem in studies of adaptive responses to environment. It is often useful, therefore, to determine whether adaptive responses of leaf morphology and physiology to water availability are observed in different populations of plants grown under identical environmental conditions (i.e., in a common garden experiment).

Red ironbark (*Eucalyptus sideroxylon* cunn. Ex. Woolls subsp. *tricarpa* L.A.S. Johnson) grows in central, southern and eastern Victoria, and extends north along the south coast of New South Wales (Boland et al. 1984, Bramwells and Whiffin 1984). It occurs naturally across relatively small ranges of latitude and altitude within Australia. Hence the many populations of red ironbark experience negligible environmental variation except in rainfall, which varies from about 500 mm per annum to more than 1000 mm (see Table 1). Leaf (and fruit) morphology varies widely among populations (Bramwells and Whiffin 1984), but the underlying causes of this variation have not been examined. For example, when examined at natural sites, leaf thickness and leaf width of red ironbark were found to be inconsistently related to rainfall (Bramwells and Whiffin 1984), and both genetic and environmental influences may have been responsible for the observed variation in measured characters.

To identify genetic variation among populations in leaf morphology and physiology, we designed a common garden experiment in which populations of red ironbark, represented by seeds collected from different habitats, were grown at the same site under identical environmental conditions. Measurements were made in spring 2003 when the soil was at, or close to, field capacity and physiological measurements could be made under favorable conditions for growth without significant limitations due to temperature or soil or atmospheric water deficits. Based on differences in rainfall at the seed source, we tested several hypotheses: (1) leaf width and leaf size are negatively related to seed-source rainfall; (2) SLA is positively related to seed-source rainfall, as a result not only of changes in leaf thickness but also because of changes in the saturated water content and density of leaf tissue; (3) A_{mass} and photosynthesis per unit N (PNUE) are positively related to seed-source rainfall; and (4) WUE is negatively related to seed-source rainfall, and high WUE in dry-habitat populations is a result of low stomatal conductance (as indicated by a positive relationship between $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$) (Scheidegger et al. 2000).

Morphological and physiological adaptation typically comprise a coordinated pattern of change in several characteristics. The application of exploratory multivariate statistical tools, such as principal component analysis (PCA), enables identification of multivariate patterns of change that cannot be identified by analysis of single parameters (Tausz et al. 2002). If

consistent patterns of morphological and physiological traits are identified by PCA, but are unrelated to seed-source rainfall, they may reflect random genetic drift rather than a result of selection pressure. Hence we tested the hypothesis that leaf morphological and physiological traits exhibit consistent multivariate patterns related to seed-source rainfall.

Materials and methods

Seed collection and the common garden site

The common garden experiment included 15 populations grown under uniform conditions. Seed had been collected from sites spanning the natural range of red ironbark (Table 1) and separated from each other by more than 20 km. At each site, seed was collected by shotgun from one healthy dominant tree, as described elsewhere (Stackpole and Tibbits 2001 and CSIRO Australian Tree Seed Centre, www.ffp.csiro.au/tigr/atcmain/). We obtained long-term climate data from the Australian Bureau of Meteorology (www.bom.gov.au/climate/averages/) for each seed collection site.

The common garden experiment was established in the goldfields area of central Victoria, Australia (36.62° S, 144.30° E, 175 m a.s.l.). The site is located on ex-pasture land with a north to north easterly aspect of less than 4°. The soil has a gradational profile, from reddish-brown loam with ironstone (the top 20 cm), to orange-brown medium clay (20 to 80 cm), to greyer mottled clays (up to a depth of 80 to 150 cm or more).

The closest meteorological station with long-term climatic records is Bendigo Prison (Australian Bureau of Meteorology, Site No. 081003, 36.75° S, 144.28° E). In July (midwinter), mean daily minimum and maximum temperatures are 3.5 and 12.1 °C, respectively. In January (midsummer), mean daily minimum and maximum temperatures are 14.3 and 28.9 °C, respectively. Mean annual point potential evapotranspiration (Morton 1983) for this area is between 1400 and 1600 mm. Mean annual rainfall is 550 mm year⁻¹. However, measurements were made in the spring of 2003 when rainfall for the preceding 12 months had been only 80% of the long-term mean.

In mid-June of 2000, the site was ripped to a depth of 60 cm with a winged ripper. Rip lines were subsequently bedded with a mound-plough, giving rise to 20-cm mounds. Seedlings were planted on September 25 and 26, 2000, with five trees per row, at a spacing of 4.0 (between rows) × 1.8 m.

Measurements were made in spring 2003 when the soil was at field capacity and trees were 3 years old, had adult foliage and were between 1.5 and 3 m tall.

Photosynthetic measurements

The maximum rate of light-saturated photosynthesis (A_{max}) was measured on five trees per population over two warm (about 15 to 20 °C maximum) cloudless days in spring 2003. Two measurements were made per tree and averaged. Populations were measured in random order throughout the 2 days to avoid confounding population with diurnal effects. All mea-

Measurements were made on the youngest fully expanded foliage from the north (sunlit) side of the tree. Measurements were made with an open, infra-red gas analyzer (LI-6400, Li-Cor, Lincoln, NE) at 360 to 370 $\mu\text{mol mol}^{-1} \text{CO}_2$, 20 °C leaf temperature, 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetic photon flux (PPF), and a leaf-to-air vapor pressure deficit of 1.0–1.3 kPa. Initial experiments established that this PPF was above the light-saturation point of red ironbark. Photosynthesis, transpiration and intercellular carbon dioxide concentration (C_i) were allowed to stabilize before measurements were taken. Additional measurements were made on five nearby (~250 m distant) trees of the Bodalla population because initial measurements indicated that the Bodalla population included in the common garden had a much lower A_{max} than the other populations. However, the A_{max} of the adjacent population was also low (t test, $P > 0.1$), confirming that a low A_{max} is characteristic of this population. Immediately following measurement of photosynthesis, five leaves per tree were collected for subsequent morphological measurements and chemical analyses.

Chlorophyll fluorescence analysis

After the gas exchange measurements, chlorophyll fluorescence of the leaves used for the gas exchange measurements was measured with the Li-Cor 6400-40 leaf chamber fluorometer. Photochemical quantum yield of photosystem II (PSII) (Φ_{PSII}) was calculated according to Maxwell and Johnson (2000).

Morphological measurements

For each of the five leaves per tree, we made a series of morphological measurements. Leaf thickness was measured in two places between major veins with a Vernier calliper (Mitutoyo, Japan). Total length and maximum width were measured to the nearest 1 mm with a steel ruler. The projected leaf area was measured with a leaf area meter (LI-3000A + LI-3050A, Li-Cor). The fully saturated (turgid) mass of leaves was measured after rehydration in a saturated atmosphere in a sealed plastic bag at 4 °C for 24–48 h. Dry mass (DM) was determined after 72 h in an oven at 60 °C. Specific leaf area was calculated as projected area divided by dry mass. Density was calculated as 1/SLA divided by thickness. Saturated water content (in units of $\text{g H}_2\text{O g}_{\text{DM}}^{-1}$) was determined as (turgid mass – dry mass)/dry mass

Stable carbon and oxygen isotope compositions and N determination

For isotopic analysis, pooled samples, comprising five dried leaves per tree, were ground to a fine powder in a matrix mill (MM 301, Retsch, Haan, Germany). We determined $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ by isotope ratio mass spectrometry (DELTA plus XL, ThermoFinnigan MAT, Bremen, Germany) at the West Australian Biogeochemistry Centre. Subsamples were analyzed for total N by Dumas combustion (CHN-2000, LECO, St Joseph, MI).

Statistical analysis

Statistical analysis was performed with Statistica (StatSoft, Tulsa, OK). Because of the relatively small sample size ($n = 5$) for each population, differences in morphological and physiological traits among populations were assessed by the Kruskal-Wallis test, a non-parametric test analogous to a one-way analysis of variance. For the same reason, we report medians and median deviations of traits for each population (Sachs 1992). Relationships between traits and rainfall at the site of seed origin were assessed by non-parametric correlation (Spearman's ρ), because the rainfall values were unevenly distributed along the x -axes. Relationships among traits across all populations were determined by correlation analysis (Pearson's r).

Multivariate statistical analysis was performed as described by Tausz et al. (1998). Principal component analysis was undertaken to identify patterns in the original data set and to facilitate the extraction of accumulated variables not directly accessible for measurement. The analysis was based on 13 key variables (see Table 5) measured in $n = 75$ individual trees. We chose variables on the basis that they were not necessarily auto-correlated (as, for example, is the case for density, thickness, SLA, N_{mass} and N_{area}). We included some variables that showed significant correlation in the present data set, but only where this correlation was not trivial (e.g., A_{mass} , Φ_{PSII} and g_s). According to the Shapiro-Wilk W test, none of the input variables deviated significantly from normal distribution. An acceptable principal component (PC) solution was determined based on visual examination of the Scree plot and the Kaiser criterion (all eigenvalues greater than 1). Component scores and PC loadings were determined after Varimax axis rotation to maximize the variance of the squared loadings (Johnson and Wichern 1992).

Results

Variation in climate at the site of seed origin

Rainfall was the main environmental variable that differed among the seed sources (Table 1). Among the 15 seed sources, mean annual rainfall varied from a low of 500 mm year^{-1} at Heathcote and Tarnagulla to a high of 1055 mm year^{-1} at Bodalla. Trends among sites in the 1st decile of annual rainfall (i.e., the lowest 10% of rainfall records) were generally similar. However, the 1st decile of annual rainfall varied from 255 to 803 mm year^{-1} , which would correspond to severe drought at the driest site and moderate drought at the wettest site. The amount of rainfall from the beginning of November until the end of March is perhaps most relevant physiologically because it is during this period that temperatures and evaporative demand are greatest. Summer rainfall varied from a mean of 135 mm year^{-1} at Tarnagulla to 476 mm year^{-1} at Bodalla. Mean modeled evaporation varied little among the seed sources. Altitude varied among sites from 110 to 500 m and may have indirect effects (Austin and Smith 1989) on leaf morphology and physiology; however, direct effects of altitude were

Table 1. Origins of sampled populations of red ironbark. Sites are ranked from lowest to highest mean annual rainfall, as determined from data obtained at the nearest meteorological station. Dec. 1 is the first decile and Dec. 9 is the 9th decile; these are the median of the lowest 10% of annual rainfall records and the median of the highest 10% of records. Evaporation is annual point potential evapotranspiration (Morton 1983). Seeds were collected by the Centre for Forest Tree Technology, except where noted otherwise.

Location	Latitude	Longitude	Altitude (m)	Annual evaporation (mm)	Annual rainfall (mm)			November–March mean rainfall (mm)
					Dec. 1	Dec. 9	Mean	
Heathcote	36.98	144.75	280	1400–1600	225	661	500	153
Tarnagulla ¹	36.75	143.75	500	1400–1600	280	572	500	135
Craigie	37.07	143.75	250	1400–1600	370	690	530	168
Bealiba	36.80	143.63	300	1400–1600	357	719	550	171
Whroo	36.70	144.97	160	1400–1600	357	719	550	171
Douglas dam	36.82	144.82	240	1400–1600	377	739	570	180
Clunes	37.27	143.73	340	1200–1400	399	746	587	187
Heyfield	37.93	146.72	120	1200–1400	512	850	700	290
Martins Creek	37.47	148.55	300	1200–1400	615	1136	858	343
Lorne	38.47	144.05	110	1200–1400	663	1060	860	256
Nowa Nowa	37.70	148.10	110	1200–1400	570	1207	865	352
Tucker box	37.63	148.25	340	1200–1400	570	1207	865	352
Narooma ²	36.28	149.28	160	1200–1400	613	1417	914	431
Christmas Hills	37.68	145.38	200	1000–1200	803	1237	1039	386
Bodalla ³	36.35	149.35	200	1200–1400	605	1873	1055	476

¹ CSIRO seedlot 19816.

² CSIRO seedlot 15307.

³ CSIRO seedlot 19103.

Table 2. Summary of leaf morphology and chemistry of red ironbark populations collected from different locations (Table 1) and grown in a common garden experiment. Measured traits were: the total length of leaves, maximum width of the leaf lamina, lamina width as a fraction of lamina length, specific leaf area (SLA), lamina thickness, leaf density, the saturated water content of leaves, mass-based nitrogen concentration (N_{mass}), and nitrogen content per unit leaf area (N_{area}). Data are medians (median deviations) of five trees per population. The significance P (Kruskal–Wallis test) of differences among populations is indicated in the last row.

Population	Total length (mm)	Max width (mm)	Lamina width/lamina length	SLA ($\text{m}^2 \text{kg}^{-1}$)	Lamina thickness (mm)	Density (kg m^{-3})	H ₂ O content (g g^{-1})	N_{mass} (mg g^{-1})	N_{area} (g m^{-2})
Heathcote	93 (9)	25 (2)	0.30 (0.01)	3.8 (0.2)	0.51 (0.01)	513 (16)	1.03 (0.05)	15.8 (0.2)	4.1 (0.1)
Tarnagulla	82 (6)	21 (2)	0.33 (0.05)	3.7 (0.3)	0.54 (0.02)	502 (11)	1.10 (0.08)	13.6 (1.5)	3.7 (0.4)
Craigie	96 (8)	21 (3)	0.27 (0.04)	3.5 (0.3)	0.54 (0.04)	545 (14)	0.93 (0.03)	11.6 (0.5)	3.5 (0.3)
Bealiba	106 (6)	29 (3)	0.30 (0.06)	3.9 (0.2)	0.51 (0.02)	550 (31)	1.10 (0.11)	12.5 (1.0)	3.2 (0.4)
Whroo	74 (7)	27 (1)	0.42 (0.04)	3.6 (0.2)	0.53 (0.04)	530 (5)	0.94 (0.02)	14.4 (0.4)	4.0 (0.4)
Douglas	81 (3)	31 (2)	0.44 (0.05)	4.2 (0.1)	0.45 (0.03)	537 (37)	1.04 (0.04)	12.1 (0.9)	2.9 (0.2)
Clunes	106 (16)	31 (2)	0.39 (0.06)	3.5 (0.3)	0.52 (0.03)	521 (9)	1.01 (0.01)	14.0 (1.2)	4.0 (0.4)
Heyfield	87 (9)	32 (3)	0.46 (0.05)	4.3 (0.1)	0.45 (0.04)	553 (23)	1.10 (0.03)	13.7 (0.7)	3.1 (0.2)
Martin	101 (13)	27 (4)	0.32 (0.02)	3.8 (0.1)	0.50 (0.02)	488 (11)	1.17 (0.08)	12.2 (0.5)	3.2 (0.2)
Lorne	95 (11)	33 (2)	0.42 (0.01)	3.8 (0.1)	0.50 (0.01)	518 (14)	1.06 (0.03)	16.0 (0.1)	4.2 (0.1)
Nowa	97 (10)	30 (2)	0.36 (0.05)	3.7 (0.2)	0.51 (0.02)	538 (12)	1.02 (0.02)	12.2 (0.4)	3.3 (0.2)
Tuckerbox	87 (2)	26 (3)	0.35 (0.05)	3.8 (0.3)	0.49 (0.01)	539 (25)	1.01 (0.04)	14.2 (1.0)	3.9 (0.3)
Narooma	112 (4)	27 (2)	0.25 (0.01)	4.3 (0.1)	0.43 (0.03)	545 (16)	1.09 (0.02)	13.8 (1.7)	3.1(0.3)
Christmas Hills	102 (10)	26 (1)	0.34 (0.03)	3.9 (0.1)	0.47 (0.04)	551 (36)	0.94 (0.02)	10.9 (0.6)	2.9 (0.2)
Bodalla	91 (4)	19 (1)	0.25 (0.02)	4.0 (0.2)	0.48 (0.01)	515 (8)	0.98 (0.02)	11.0 (0.3)	2.6 (0.3)
P	0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.251	< 0.001	0.001	0.002

small because the range in atmospheric pressure and partial pressure was less than 5%.

Variations in leaf morphology and physiology

Morphological traits, with the exception of leaf density, varied

significantly between populations (Table 2). Mass-based N concentration (N_{mass}) and N content per unit leaf area (N_{area}) were the most variable traits within populations (as indicated by the large median deviations) and among populations (> 60% difference between median maxima and minima). The

Table 3. Physiological traits of red ironbark populations (Table 1) grown in a common garden. Measured traits were: the rate of photosynthesis per unit area (A_{area}), the rate of photosynthesis per unit mass (A_{mass}), the rate of photosynthesis per unit leaf water ($A_{\text{H}_2\text{O}}$), photosynthetic nitrogen-use efficiency (PNUE), stomatal conductance (g_s), instantaneous water-use efficiency (WUE), photosystem II effective quantum yield (Φ_{PSII}), stable carbon isotope discrimination ($\delta^{13}\text{C}$), and stable oxygen isotope discrimination ($\delta^{18}\text{O}$). Data are medians (median deviations) of five trees per population. The significance P (Kruskal-Wallis test) of differences among populations is indicated in the last row.

Population	A_{area} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	A_{mass} ($\text{nmol g}^{-1} \text{s}^{-1}$)	$A_{\text{H}_2\text{O}}$ ($\text{nmol g}^{-1} \text{H}_2\text{O s}^{-1}$)	PNUE ($\mu\text{mol g}^{-1} \text{N s}^{-1}$)	g_s ($\text{mol m}^{-2} \text{s}^{-1}$)	WUE (mmol mol^{-1})	Φ_{PSII}	$\delta^{13}\text{C}$ (‰)	$\delta^{18}\text{O}$ (‰)
Heathcote	18 (4)	66 (22)	65 (13)	2 (1)	0.21 (0.09)	6.8 (0.5)	0.186 (0.027)	-26.0 (0.2)	22 (1)
Tarnagulla	18 (2)	64 (13)	60 (3)	5 (1)	0.19 (0.02)	7.9 (1.0)	0.172 (0.009)	-26.0 (0.4)	17 (1)
Craigie	17 (3)	64 (18)	69 (2)	4 (1)	0.15 (0.01)	9.4 (0.6)	0.172 (0.010)	-26.4 (0.6)	23 (1)
Bealiba	17 (3)	68 (13)	62 (5)	5 (1)	0.21 (0.05)	8.7 (0.9)	0.159 (0.007)	-26.3 (0.3)	22 (2)
Whroo	17 (5)	67 (21)	71 (24)	5 (2)	0.20 (0.02)	8.1 (0.7)	0.157 (0.024)	-25.4 (0.2)	21 (1)
Douglas	16 (1)	70 (3)	64 (7)	5 (2)	0.19 (0.03)	7.1 (0.6)	0.145 (0.009)	-26.9 (0.2)	22 (1)
Clunes	17 (3)	60 (6)	59 (6)	4 (2)	0.23 (0.03)	7.2 (1.3)	0.173 (0.018)	-25.9 (0.8)	21 (2)
Heyfield	11 (2)	45 (7)	41 (12)	3 (1)	0.09 (0.03)	8.1 (0.4)	0.130 (0.003)	-26.3 (0.)	20 (2)
Martin	12 (3)	50 (8)	43 (2)	4 (1)	0.09 (0.01)	9.2 (0.2)	0.123 (0.006)	-26.2 (0.4)	25 (1)
Lorne	15 (3)	58 (10)	56 (2)	4 (1)	0.12 (0.02)	9.3 (2.2)	0.185 (0.010)	-25.5 (0.7)	24 (2)
Nowa	14 (2)	45 (4)	50 (4)	4 (1)	0.10 (0.03)	9.7 (1.1)	0.130 (0.018)	-26.3 (0.3)	22 (2)
Tuckerbox	10 (1)	39 (2)	37 (1)	2 (1)	0.14 (0.01)	4.9 (0.7)	0.135 (0.011)	-25.2 (0.5)	24 (1)
Narooma	12 (6)	49 (19)	44 (5)	3 (2)	0.10 (0.03)	9.2 (2.3)	0.103 (0.041)	-26.1 (0.4)	20 (2)
Christmas Hills	18 (1)	73 (7)	79 (5)	9 (2)	0.20 (0.03)	8.4 (0.5)	0.160 (0.006)	-26.3 (0.5)	23 (2)
Bodalla	6 (3)	25 (9)	25 (9)	3 (1)	0.08 (0.02)	4.5 (0.4)	0.086 (0.021)	-27.1 (0.8)	24 (2)
P	0.002	0.012	0.003	0.018	0.007	0.024	0.001	0.073	0.210

two indices of leaf size (lamina length and lamina width) varied among populations by 36–50%, whereas leaf shape (as indicated by lamina width/lamina length) varied by 58%. The variations in leaf size and shape were considerably greater than the variations in SLA, leaf thickness and leaf water content, all of which varied by around 20%.

There was significant variation in the instantaneous physiological traits of photosynthesis (per unit area, mass, H_2O and N), g_s and WUE among populations (Table 3). Each of the different measures of photosynthesis varied among populations by about 90%, g_s by 75% and WUE by 60%. The longer-term, assimilation-weighted traits of $\delta^{13}C$ and $\delta^{18}O$ did not vary significantly among populations, largely because of high within-population variability.

Relationships between leaf traits and rainfall

Relationships between leaf traits and seed-source (mean annual) rainfall were either not significant ($P > 0.05$), or signifi-

cant but weak (Spearman's $\rho < 0.50$). Lamina length was positively related to seed-source rainfall, increasing by an average of 15% from the driest to the wettest sites (Table 4). Petiole length varied little among populations and was unrelated to seed-source rainfall (data not shown). Thus, petiole length/lamina length decreased with increasing seed-source rainfall. Specific leaf area was positively related to seed-source rainfall, whereas leaf thickness was negatively related to seed-source rainfall. The size of these changes was rather small, however, with the 555 mm increase in seed-source rainfall from the driest to the wettest sites being associated with an 8% increase in SLA and a 9% decrease in thickness. Both N_{mass} and N_{area} decreased with increasing seed-source rainfall, with this change being smaller for N_{mass} (–10%) than for N_{area} (–18%) owing to the compensating effect of changes in SLA.

Photosynthetic rates (per unit area, mass and water), Φ_{PSII} and g_s were negatively related to seed-source rainfall, with each parameter decreasing by around 30% as seed-source rainfall increased from 500 to 1055 mm. Neither PNUE, WUE, $\delta^{13}C$ nor $\delta^{18}O$ was related to seed-source rainfall. In general, the relationships between leaf traits and the 1st and 9th deciles of total rainfall or summer rainfall at the seed source were the same as the relationships between leaf traits and mean annual rainfall at the seed source (data not shown).

Relationships among morphological and physiological traits—multivariate patterns within the data set

Principal component analysis reduced the 13 input variables to five PCs explaining 73.0% of the total variance in the original data (Table 5). Communality values (a measure of how well the input variables are explained by the five resulting PCs) were greater than 0.6 for all variables except for petiole/lamina length, which had a communality of 0.51, and $\delta^{18}O$ which had a communality of only 0.28. The relationship of the original variables with PCs is indicated by PC loadings, which are akin to correlation coefficients between original characters and the PC (Table 5). Scores in PC1 were positively related to A_{mass} , Φ_{PSII} and g_s . Scores in PC2 were positively related to leaf width, SLA and saturated water content. Scores in PC3 were negatively related to leaf length but positively related to leaf width (indicated by the negative loading of leaf lamina length and the positive loading of the leaf wide/length ratio on PC3). Scores in PC4 were positively related to leaf width and WUE (Table 5). Scores in PC5 were positively related to $\delta^{13}C$ and N_{mass} .

The PC scores of PC1 (accumulated variable containing information on photosynthetic capacity, Φ_{PSII} and high g_s) were weakly and negatively correlated with mean annual rainfall at the seed source (Figure 1). Scores of the other PCs were not significantly correlated with mean annual seed-source rainfall. In general, correlations of PC scores with the 1st and 9th deciles of total rainfall and summer rainfall were nearly identical to correlations with mean annual rainfall.

Table 4. Summary of non-parametric correlations (Spearman's ρ) between leaf traits and seed-source rainfall (mm year⁻¹) for red ironbark. The final column shows the significance (P) of correlations. Abbreviations: SLA = specific leaf area; A_{area} = rate of photosynthesis per unit area; A_{mass} = rate of photosynthesis per unit mass; A_{H_2O} = rate of photosynthesis per unit leaf water; PNUE = photosynthetic nitrogen-use efficiency; g_s = stomatal conductance; WUE = instantaneous water-use efficiency; Φ_{PSII} = photosystem II effective quantum yield; $\delta^{13}C$ = stable carbon isotope discrimination; $\delta^{18}O$ = stable oxygen isotope discrimination; N_{mass} = mass-based nitrogen concentration; N_{area} = nitrogen content per unit leaf area; and ns = not significant ($P > 0.05$).

Parameter	Spearman's ρ	P
<i>Leaf morphology</i>		
Petiole length/lamina length	0.33	0.005
Lamina width/lamina length	–	ns
Lamina length (mm)	0.29	0.012
Lamina width (mm)	–	ns
Lamina thickness (mm)	–0.44	< 0.001
SLA (m ² kg ⁻¹)	0.30	0.009
Density (kg m ⁻³)	–	ns
Water content (g g ⁻¹)	–	ns
<i>Instantaneous physiological traits</i>		
A_{area} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	–0.35	0.002
A_{mass} ($\text{nmol g}^{-1} \text{s}^{-1}$)	–0.28	0.016
A_{H_2O} ($\text{nmol g}^{-1} \text{H}_2\text{O s}^{-1}$)	–0.29	0.014
PNUE ($\mu\text{mol g}^{-1} \text{N s}^{-1}$)	–	ns
g_s ($\text{mol m}^{-2} \text{s}^{-1}$)	–0.31	0.007
WUE (mmol mol^{-1})	–	ns
Φ_{PSII}	–0.47	< 0.001
<i>Long-term physiological traits</i>		
$\delta^{13}C$ (‰)	–	ns
$\delta^{18}O$ (‰)	–	ns
<i>Leaf chemistry</i>		
N_{mass} (mg g ⁻¹)	–0.26	0.036
N_{area} (g m ⁻²)	–0.37	0.003

Table 5. Principal components (PC) solution of 13 variables measured in leaves from different populations of red ironbark. Data are PC loadings and communalities determined after Varimax axis rotation. The PC loadings < 0.5 are not shown. Abbreviations: A_{mass} = rate of photosynthesis per unit mass; g_s = stomatal conductance; Φ_{PSII} = photosystem II effective quantum yield; WUE = instantaneous water-use efficiency; SLA = specific leaf area; $\delta^{13}\text{C}$ = stable carbon isotope discrimination; $\delta^{18}\text{O}$ = stable oxygen isotope discrimination; N_{mass} = mass-based nitrogen concentration.

Variable	PC1	PC2	PC3	PC4	PC5	Communality
A_{mass}	0.92	–	–	–	–	0.92
g_s	0.90	–	–	–	–	0.89
Φ_{PSII}	0.85	–	–	–	–	0.82
WUE	–	–	–	0.82	–	0.77
Lamina length	–	–	–0.87	–	–	0.89
Petiole/lamina length	–	–	–	–	–	0.51
Lamina width	–	0.53	–	0.66	–	0.79
Width/length	–	–	0.83	–	–	0.92
SLA	–	0.73	–	–	–	0.72
Leaf water content	–	0.79	–	–	–	0.64
$\delta^{13}\text{C}$	–	–	–	–	0.74	0.64
$\delta^{18}\text{O}$	–	–	–	–	–	0.28
N_{mass}	–	–	–	–	0.80	0.70
Explained variance (%)	21	15	14	12	11	

Discussion

There were large differences in all measured variables among and within populations of red ironbark (Tables 2 and 3). Mean annual rainfall (500–1055 mm per annum) was the major difference among seed sources, and we expected that variation in leaf morphology and physiology would be related to this. However, in many cases, populations collected from sites with similar rainfall differed greatly in leaf morphology and physiology (e.g., Bodalla versus Christmas Hills). There were few consistent relationships between single variables and mean annual rainfall at the seed source, and where these relationships were significant, they were either weak (SLA, thickness) or the opposite of that predicted (A_{mass} , g_s). Principal component analysis indicated that, among and within red ironbark populations, there were several directions of variation in morphology and physiology, represented by single PCs, and these were independent of each other. Some of these axes could be interpreted in terms of drought adaptation, but the independence of the different axes argues against co-occurrence of these drought-adaptation traits within the present data set (e.g., variation in SLA, leaf width and water content was independent of variation in $\delta^{13}\text{C}$ or WUE). Hence, despite ample evidence of conserved genetic variation in leaf morphology and physiology (as seen in differences among populations), this variation could not be interpreted in terms of adaptation to drought. This is consistent with a previous, albeit less quantitative, analysis of natural populations of red ironbark (Bramwells and Whiffin 1984).

When examining broad gradients composed of many species, there are consistent relationships between leaf morphology and physiology and rainfall (e.g., Fonseca et al. 2000, Wright et al. 2001), whereas within a single species such relationships are often inconsistent (e.g., red ironbark, this study; among *E. camaldulensis* Dehnh. clones, James and Bell

(1995); among *E. leucoxydon* F. J. Muell. White I. populations, Boland (1978); among *Pinus taeda* L. populations, Bongarten and Teskey (1986); among *P. ponderosa* Dougl. ex P. Laws. & C. Laws. populations, Zhang et al. (1997)). This inconsistency is especially perplexing when one considers that the same processes that determine the distribution of species also control the distribution of populations within species. Reich (1993) suggested that the apparent paradox of weak or minimal within-species relationships could be reconciled with overall broad relationships if there was little variation in either the environmental gradient or the measured trait. Both seem unlikely explanations in the present case because mean annual rainfall at the seed source varied from 500 mm year⁻¹ to a little over 1000 mm year⁻¹ and there were similar twofold differences in the 1st decile of annual rainfall and mean rainfall over the five warmest months (Table 1). Similar rainfall ranges in multi-species comparisons have resulted in consistent relationships (e.g., Wright et al. 2001). Given that we found high variability in most traits among and within populations, we can only conclude that the measured traits are demonstrably unrelated to rainfall.

Part of the reason that leaf traits may be unrelated to rainfall is that the climatic distribution of genotypes is not solely determined by physiological traits. It is well known that the action of environmental selection on physiological traits produces a broad “fundamental niche,” but genotypes occupy a much narrower “realized niche” as a result of density-dependent selection (Ellenberg 1953, Rehfeldt et al. 1999). Moreover, density-dependent selection tends to relegate genotypes to physiologically suboptimal environments, as has been observed in *Pinus contorta* Dougl. ex Loud. (Rehfeldt et al. 1999). Following similar logic, the lack of correlation between rainfall and leaf traits among populations of red ironbark might be associated with density-dependent selection forcing genotypes to occupy

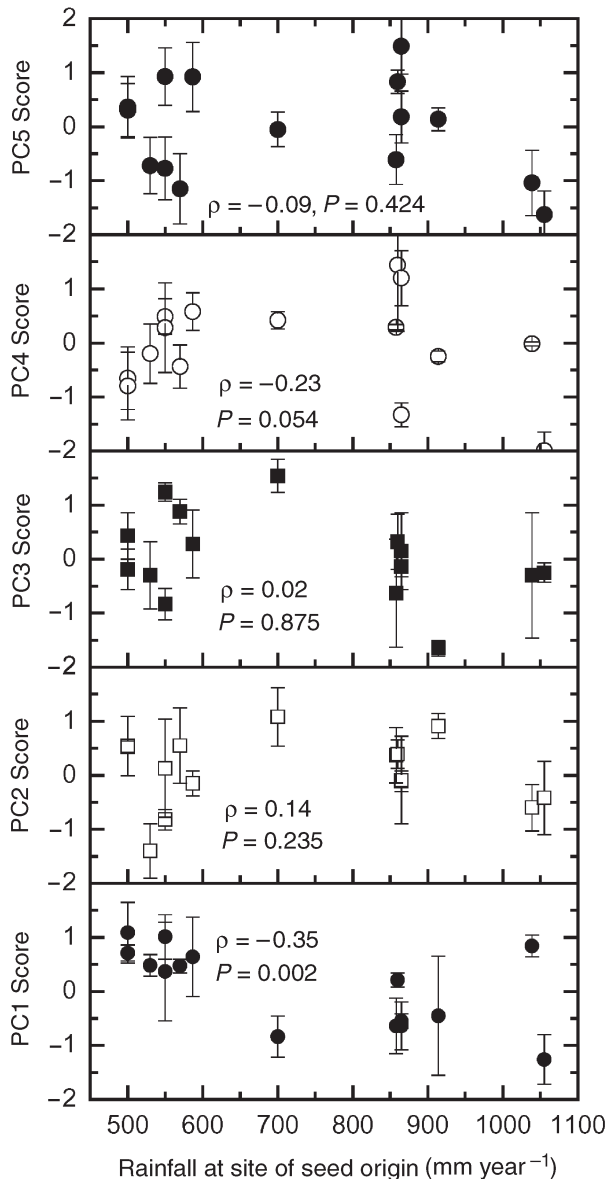


Figure 1. Principal component scores (PC1–5, for explanation see Table 5) versus population mean annual rainfall. Data are medians and median deviations of $n = 5$ trees per population. Spearman's ρ was calculated on all data points ($n = 75$).

climatically suboptimal environments.

Use of a common garden experiment and making measurements in spring under identical favorable growing conditions overcame the problem of confounding environmental effects with genotypic differences, but raised other questions. Differences in physiological traits (e.g., A_{\max} , WUE, $\delta^{13}\text{C}$) among genotypes may well be more or better related to population rainfall when plants are exposed to water stress. For example, in *P. ponderosa* seedlings, differences in $\delta^{13}\text{C}$ among genotypes were greater under water-stress conditions than under well-watered conditions (Olivas-Garcia et al. 2000). In natural habitats, the incidence and severity of water stress varies

among genotypes (e.g., Table 1), and thus the traits we measured would be expressed to varying degrees among genotypes. This is an intractable problem that can only be resolved by measuring populations in their natural habitat.

Mean annual rainfall and other long-term rainfall measures (e.g., November–March summer rainfall, 1st decile annual rainfall) can be poor indicators of water availability. Evaporative demand, the amount of competing (transpiring) vegetation, the water-release characteristics of the soil, and soil depth are important components of water availability. In our study, evaporative demand increased from wet to dry sites (Table 1) and thus serves to accentuate the rainfall gradient. We acknowledge that some of the factors determining water availability (e.g., soil depth) vary over small scales (m), and that water availability depends as much on position within the habitat or landscape (e.g., Adams 1996) as on mean values for rainfall or soil properties (e.g., Farley and McNeilly 2000).

Principal component analysis indicated that there were several multivariate directions of variation in the morphological and physiological traits across and within the red ironbark populations investigated, which are represented by single PCs. Variation in all input variables, except $\delta^{18}\text{O}$ and leaf lamina/petiole length ratio, was adequately explained by five principal components (accumulated variables, PCs). Because of the nature of PCA, these axes of variation were independent of each other. Some of these axes could be interpreted in terms of drought adaptation, but their independence from each other argues against co-occurrence of these drought adaptation traits within the present data set (e.g., variation in SLA, width or water content was independent of variation in $\delta^{13}\text{C}$ or WUE). Hence, there are consistent relationships among these variables, and these almost certainly represent fundamental constraints on leaf form and function within the gene pool of this taxon. Only one of these components, PC1, which combined A_{mass} , Φ_{PSII} and g_s —instantaneous physiological traits related to photosynthesis and gas exchange—was significantly correlated with population rainfall. High scores in PC1 suggest high photosynthetic capacity, high photochemical efficiency and high stomatal conductance, traits that we would also expect in fast-growing species (Reich et al. 1999) or in populations from wetter areas. The relationships of PC1 scores with rainfall, however, were negative, a result supporting the negative univariate correlations of A_{mass} , Φ_{PSII} and g_s with population rainfall (Table 4). One possible explanation is that the common garden site was located in a low rainfall area, and thus populations originating from drier habitats were experiencing close-to-normal environmental conditions compared with populations from wetter habitats that were growing outside their normal range and may consequently have experienced more stress and thus lower A_{mass} , Φ_{PSII} and g_s . The result would therefore reflect phenotypical and physiological acclimation rather than genetic constitution.

A combination of wide leaves, high SLA and high leaf water content has commonly been reported in fast-growing species (Poorter and Bergkotte 1992, Wright and Westoby 1999), but in red ironbark, this multivariate trait was unrelated to seed-

source rainfall (see PC2 score). Other within-species comparisons have also shown inconsistent relationships between these traits and rainfall (in contrast to among-species comparisons) (Cunningham et al. 1999, Fonseca et al. 2000). For example, in *Cistus ladanifer* L., populations from dry areas had smaller leaves (Nunéz-Oliviera et al. 1996), whereas in the congeneric *C. salvifolius* L., the opposite trend was found (Farley and McNeilly 2000). For *E. camaldulensis* clones grown in a common garden, there was also no correlation between leaf characteristics and seed-source climate, and it was argued that groundwater availability, root structure and internal transport were more important to drought tolerance than leaf characteristics (James and Bell 1995).

High WUE is frequently proposed as a key characteristic of drought-adapted species with low SLA (Sun et al. 1996, Cregg and Zhang 2001, Lamont et al. 2002, Sefton et al. 2002, Wright et al. 2003); however, there was no evidence that this was the case in red ironbark. Among red ironbark populations, instantaneous WUE and $\delta^{13}\text{C}$ (an indirect assimilation-weighted, long-term estimate of WUE) were unrelated to seed-source rainfall. Furthermore, $\delta^{13}\text{C}$ was combined with N_{mass} in PC5, but we found no relationship between PC5 scores and rainfall. The absence of a correlation between $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ (data not shown) (Scheidegger et al. 2000) suggests that photosynthesis and stomatal conductance are coordinated so as to maintain a constant C_i or WUE. Results of this study contribute to the significant body of evidence and logic that cast doubt on the paradigm that drought-adapted genotypes have high WUE (see also Lauteri et al. 1997, Zhang et al. 1997, Warren et al. 2001). Indeed, the ability to use water readily when available (low WUE) but hardly at all when water is scarce (high WUE) is, logically, a more effective competitive strategy in many circumstances.

One reason that leaf characteristics may be unrelated to rainfall (within species) is that they are selectively neutral, an assertion that is supported by their high plasticity. In terms of whole-plant performance, SLA and thickness are effectively neutral owing to the compensating effects of differences in foliage mass, and the trade-off of light capture with rates of photosynthesis (Gutschick and Wiegel 1988, Warren and Adams 2000, Gutschick and BassiriRad 2003). Relationships between rainfall and leaf morphology and physiology are affected by leaf absorptance and leaf orientation (e.g., Smith et al. 1998) and it may be that absorptance or orientation, or both, varied among genotypes and thus confounded the predicted relationships between rainfall and leaf morphology and physiology. Although rainfall ranks among the more important selection pressures, it probably first acts on traits other than leaf morphology and physiology. These other traits (e.g., root and hydraulic architecture, leaf orientation and absorptance) may have more impact on drought adaptation than leaf morphology and physiology.

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