

Original Article

The influence of leaf size and shape on leaf thermal dynamics: does theory hold up under natural conditions?

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ABSTRACT

Laboratory studies on artificial leaves suggest that leaf thermal dynamics are strongly influenced by the two-dimensional size and shape of leaves and associated boundary layer thickness. Hot environments are therefore said to favour selection for small, narrow or dissected leaves. Empirical evidence from real leaves under field conditions is scant and traditionally based on point measurements that do not capture spatial variation in heat load. We used thermal imagery under field conditions to measure the leaf thermal time constant (τ) in summer and the leaf-to-air temperature difference (ΔT) and temperature range across laminae (T_{range}) during winter, autumn and summer for 68 Proteaceae species. We investigated the influence of leaf area and margin complexity relative to effective leaf width (w_e), the latter being a more direct indicator of boundary layer thickness. Normalized difference of margin complexity had no or weak effects on thermal dynamics, but w_e strongly predicted τ and ΔT , whereas leaf area influenced T_{range} . Unlike artificial leaves, however, spatial temperature distribution in large leaves appeared to be governed largely by structural variation. Therefore, we agree that small size, specifically w_e , has adaptive value in hot environments but not with the idea that thermal regulation is the primary evolutionary driver of leaf dissection.

Key-words: boundary layer; cooling time constant; effective leaf width; infrared imagery; leaf dissection; leaf shape; leaf size; leaf temperature; thermal dynamics.

INTRODUCTION

Among the explanations for the adaptive significance of leaf morphological variation, perhaps the most prominent is the role of a leaf's size and shape in its thermal regulation. In particular, the two-dimensional proportions of a leaf are said to govern its temperature via the thickness of its air boundary layer, in which heat transfer is slow relative to the more turbulent air beyond it (Drake *et al.*, 1970; Gates, 1968; Givnish, 1979; Gottschlich & Smith, 1982; Grace *et al.*, 1980; Monteith

& Unsworth, 1990; Parkhurst & Loucks, 1972; Raschke, 1960; Schuepp, 1993; Vogel, 1970). All other things being equal, the thickness of a leaf boundary layer increases with distance from the windward edge and therefore with leaf size, such that the rate of heat convection per unit area is greater between leaf and air for small leaves than large leaves. This leads to equilibrium temperatures closer to the air for small than large leaves and is the most widely accepted explanation for the presence of smaller leaves in regions such as deserts (e.g., Gibson, 1998). In a similar way to size, the shape of leaves potentially can affect heat transfer: a leaf lamina with dissected margin or lobes functioning like many small leaves, making it a more suitable shape for hot, exposed environments than a less-dissected or entire leaf of equivalent area (Givnish, 1978; Gurevitch & Schuepp, 1990; Lewis, 1972; Winn, 1999). Related to, but distinct from leaf size (more specifically, area) and shape (margin complexity or dissection) is effective leaf width (w_e): the diameter of the largest circle that can be inscribed within the margin (e.g., McDonald *et al.*, 2003). Rather than relating thermal regulation simply to a total leaf size, w_e accounts for the fact that a leaf of given area may have a larger or smaller distance across the lamina, depending on the extent of margin dissection (also known as the 'characteristic dimension', Taylor, 1975). With respect to thermal regulation, therefore, w_e might be expected to have a greater influence than either leaf area or shape *per se*.

Empirical work investigating the influence of the two-dimensional shape of leaves on their thermal dynamics has been carried out on leaf replicas, allowing specific measurements of boundary layer resistance. For shaped metal plates in wind tunnels, heat dissipates more rapidly from deeply dissected or lobed plates than those with shallow or no lobes (Gottschlich & Smith, 1982; Grace *et al.*, 1980; Parkhurst *et al.*, 1968; Vogel, 1970). An inherent problem with using metal plates is that their thermal properties differ from those of real leaves. A leaf's lamina varies spatially, with undulations, veins and hairs contributing to surface irregularities that alter boundary layer conductance (Grace *et al.*, 1980; Grace & Wilson, 1976; Schuepp, 1993). Three-dimensional structure has been accounted for in studies of heat convection in fluid tunnels using real leaves coated in metal (Gurevitch & Schuepp, 1990; Schuepp, 1972). These studies more accurately reflect natural thermodynamic properties of leaves, yet they still do

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not incorporate the microclimatic variability experienced by leaves in their natural environment, such as local irradiance, irregular wind speed, movement of leaves, time of day, and so on. Such features might override effects of two-dimensional morphology on heat dissipation, limiting our ability to translate laboratory findings to what occurs in nature. A few field-based studies have used thermocouples under natural conditions to assess the influence of leaf shape on leaf temperature (Hegazy & El Amry, 1998; Winn, 1999). Thermocouples, however, provide only a point reading on a leaf, as well as measuring a weighted average of temperature within the boundary layer, rather than the actual leaf surface. Thermocouples therefore may not accurately represent the average or critical leaf temperatures key to metabolic function, a particular issue for larger leaves, which may have a temperature gradient across the lamina. Field-based measurements of whole leaves are required to confirm predictions on how the two-dimensional shape of leaves influences their temperature in nature.

Not only are field data on leaf temperature lacking but also distributional patterns of different leaf shapes (as distinct from sizes) in the environment are inconclusive. In spite of the seemingly obvious benefit of dissected leaves in preventing excessively high leaf temperatures, there is scant evidence that dissected leaf shapes occur more frequently in hot environments than elsewhere (Moles *et al.*, 2014; Nicotra *et al.*, 2008). Whereas increased leaf dissection with warmer environmental temperatures across a geographic range (Lewis, 1969), season (Winn, 1999), or canopy of an individual plant (Zwieniecki *et al.*, 2004) can be found within a single species, the same pattern rarely is observed across multiple species. If anything, transcontinental studies across thousands of species suggest that leaf dissection, or specifically 'toothiness', decreases with mean annual temperature (Bailey & Sinnott, 1915; Royer *et al.*, 2005). These strong, global-scale patterns call into question any generalizations about the adaptive function of leaf dissection with respect to thermal regulation in high temperature regions. For w_e , we know of one cross-species South African study showing narrow leaves associated with hot environments; however this relationship is not clear cut because narrow leaves also co-occur with low soil nutrients and wet winters (Yates *et al.*, 2010).

The inconclusive links between the two-dimensional proportions of leaves and environmental temperature, coupled with the lack of field-based research on leaf temperature variation with leaf morphology, motivated the current study. Here, we examined the relationship between leaf dimensions – area, margin complexity and w_e – and leaf thermal regulation within the Proteaceae, a Gondwanan plant family having its greatest diversity in Australia (Weston, 2007). Species in this family typically have sclerophyllous leaves that are long-lived, sometimes over 13 or even up to 20 years old (Witkowski *et al.*, 1992; G Jordan, Pers. Comm. 2007). For high cost, long-lived leaves, avoiding temperature stress and potentially leaf death is especially important and reducing leaf area or width or increasing leaf dissection could minimize excessively high leaf heat loads. In Australia, Proteaceae species are native to almost every habitat type and display an extraordinary variety of leaf sizes and shapes. Anecdotally, leaf area in this family tends to be

smaller in dry heath lands and arid zones, and larger in rainforests in Australia (Weston, 2007). Leaf shape, however, varies less predictably with climate in the Proteaceae: dissected or lobed leaves seem to occur in rainforests as often as they do in heath lands, and entire leaves dominate in the arid zones (Weston, 2007). Such distributional relationships suggest that either a) predictions of thermal regulation based on model leaves do not apply to leaves in nature, b) dissected leaf shape is a poor proxy for the ability to thermally regulate and/or c) thermal regulation may be a stronger evolutionary driver of leaf size (width and potentially area) than shape. We here investigate the extent to which the area, shape and/or w_e of real leaves have a biologically significant effect on their thermal dynamics under natural conditions.

MATERIALS AND METHODS

Sampling regime

Experimental work was carried out at the Australian Botanic Garden, Mt Annan, New South Wales, Australia, during three sampling periods: June/July (winter), March (autumn) and January (summer). We measured leaves of 68 woody shrub and tree species from 17 genera and 10 tribes in the Proteaceae (Table S1). As well as encompassing a broad phylogenetic breadth within the family, species were selected to incorporate a wide range of shapes and sizes. When species possessed adult leaves of both an entire and dissected leaf type, both leaf types were measured (*Alloxylon flammeum*, *Buckinghamia celcissima*, *Grevillea hilliana* and *Grevillea venusta*, Table S1). In each sampling period, a large subset of the complete sampling set was measured, with phylogenetic and morphological diversity being maximized within each sub-sample: 43 leaves in winter (41 species), 72 in autumn (65 species) and 29 in summer (29 species). After taking thermal images of leaves *in situ*, morphological measurements were made in the laboratory on each leaf.

During the winter sampling period, conditions were overcast (\sim PAR $500 \mu\text{mol}^{-1} \text{sec}^{-1}$), with ambient temperature averaging 14°C and relative humidity averaging 56%. In autumn, conditions also were overcast (\sim PAR $700 \mu\text{mol}^{-1} \text{sec}^{-1}$), with ambient temperature averaging 23°C and relative humidity averaging 48%. In summer, images were taken under hot, sunny conditions (\sim PAR $2000 \mu\text{mol}^{-1} \text{sec}^{-1}$), with ambient temperatures averaging 35°C and relative humidity averaging 40%. Measurements were made when leaves were stationary, with wind speeds not exceeding 0.5 m sec^{-1} (measured with a Vaisala, WAAI5A Anemometer, Helsinki, Finland; connected to a Datalogger, DT500 Data logger, Rowville, Australia).

Thermography

We obtained infrared images of leaves using a ThermoCAM SC2000 infrared camera (Flir Systems AB, USA). In the camera controls, leaf emissivity was set at 0.95, within the range of known values for leaves (Jones, 1999; Jones *et al.*, 2002; Monteith & Unsworth, 1990). Ambient temperature and relative humidity, recorded with a hygrometer/thermometer (Oregon

Scientific, Oregon, USA), and the distance between the leaf and the lens were entered prior to each measurement. The camera lens was set perpendicular to the main plane of the leaf surface at a distance of 0.5–1 m from the leaf when using the standard 24° built-in lens and 0.1–0.2 m when using a close-up lens for smaller leaves. Measurements were made over 3–4 consecutive days for each sampling season, and the camera was turned off and on several times during each day and recalibrated prior to each measurement to minimize the possibility of systematic error of the camera calibration affecting measurements on a sample day.

To analyse infrared images, we used the ThermoCAM Researcher 2000 software on a personal computer. Leaf images were scrutinized for pixels with potentially aberrant temperature readings. We then used the software to draw an outline tracing the entire perimeter of each leaf, at least three pixels inside the margin. This border was selected to ensure that in estimating leaf temperature we did not include pixels representing surrounding objects or air adjacent to the leaf margin. Within this area, we obtained measurements of the average temperature, minimum temperature (coldest pixel) and maximum temperature (hottest pixel). To investigate the influence of the size and shape of leaves on the rate at which heat was lost from their surface, in the summer sampling period, we measured the time constant for cooling τ . For each leaf, an image was recorded every second as it cooled after being shaded, generating a decay curve. The cooling time constant τ was calculated from the negative inverse of the slope of the straight line fitted to a plot of the logarithm of the measured leaf-temperature *versus* time (Leigh *et al.*, 2006). For leaves in all sampling seasons, we calculated the leaf-to-air temperature difference, ΔT , by subtracting leaf temperature from air temperature. We made these calculations based on three different measures of leaf temperature: the difference between ambient temperature and mean, minimum and maximum leaf temperatures (ΔT , ΔT_{\min} and ΔT_{\max} , respectively). We also calculated the within-leaf temperature range (T_{range}) for leaves measured in each sampling period by subtracting the temperature of the hottest pixel from that of the coldest pixel on each imaged leaf surface.

Leaf morphology

After imaging, leaves were collected and placed in sealed plastic bags, wrapped in moist paper towel for transport back to the laboratory at the Australian National University in Canberra. Each leaf was scanned on a flatbed scanner, and leaf area and perimeter (both without petioles) were measured using the Image-J public domain image processing program (Rasband, 1997–2006). Leaves were oven-dried for a minimum of 2 days and weighed. For leaves measured in winter and autumn, fresh weight also was measured prior to scanning. Individual leaf dry weights were subtracted from fresh weights to obtain total water content, which was normalized by area to obtain the water content per unit leaf area, used to calculate a predicted time constant for each leaf in the study.

Three measures of two-dimensional proportions were made for each leaf: leaf area, effective leaf width (w_e) and an index of leaf shape, the normalized difference of margin complexity

(NDMC). NDMC was calculated using the perimeters of the leaf margin and its convex hull: $(\text{margin} - \text{convex hull})/(\text{margin} + \text{convex hull})$, with the convex hull being the smallest convex envelope containing the leaf margin on a two-dimensional plane (Fig. 1). The NDMC of compound leaves was calculated for whole leaves, rather than on a leaflet basis, for two reasons. Firstly, compound leaves of the species in this study are shed whole (A. Leigh, Pers. Obs.) and therefore function as one large leaf from an ecological standpoint. Secondly, given the indistinct transition between compound and deeply dissected simple leaves in our data set, any decision we should make regarding what constitutes a thermal 'unit' with respect to leaflets would be arbitrary and subjective.

Predicted leaf thermal dynamics

We estimated the thickness of the boundary layer of each leaf following the standard formula for a flat leaf (Nobel, 1999):

$$\delta = 4.0\sqrt{(w_e/\mu)}, \quad (1)$$

where δ is the average boundary layer thickness in mm; the factor 4.0 is a constant, with units of $\text{mm s}^{-0.5}$ (Nobel, 1975); μ is the wind speed in m s^{-1} ; and w_e is the effective leaf width. For the purposes of Eqn 1, the units of w_e are metres, whereas our measures of w_e as expressed hereafter are in millimetres. We based our calculations on a wind speed of 0.5 m s^{-1} . Note that this calculation of boundary layer thickness, based on wind speed and effective leaf width, does not account for other potentially influential factors, such as the surface roughness and thickness of the leaf, and its angle, relative to wind direction (Nobel, 1999).

Using the calculated boundary layer values, we predicted the time constant for cooling (τ) for each leaf:

$$\tau = C\delta/2\kappa, \quad (2)$$

where C is the heat capacity of the leaf per unit area, obtained by multiplying the water content (g) per unit area for that species by the heat capacity of water ($4.18 \text{ Joules g}^{-1} \text{ } ^\circ\text{C}^{-1}$); κ is the thermal conductivity coefficient of air ($2.6 \times 10^{-2} \text{ Joules } ^\circ\text{C}^{-1} \text{ m}^{-1} \text{ s}^{-1}$); the multiplier 2 accounts for the two sides of the leaves. In Eqn 2, δ is in units of m. This equation expresses a scaling for the thermal time constant that, on consideration of the heat equation, we might expect to be followed by a surface with C at a given temperature embedded in a medium (in our case air) with thermal conductivity κ (Hahn & Ozisik, 2012). The time constant should scale linearly with C , inversely with the conductivity of the medium and be proportional to a length scale that characterizes the problem. In this case, we expect the length scale to be on the order of the boundary layer thickness. Equation 2 assumes that other factors involved in cooling, such as transpiration, are insignificant, an assumption we return to later.

Calculations of ΔT resulted in some negative values, that is some leaves were cooler than ambient (see Results section). To determine the extent to which negative ΔT values could be explained by the effects of latent heat loss, leaf transpiration rates were modelled for all leaves in all sampling periods.

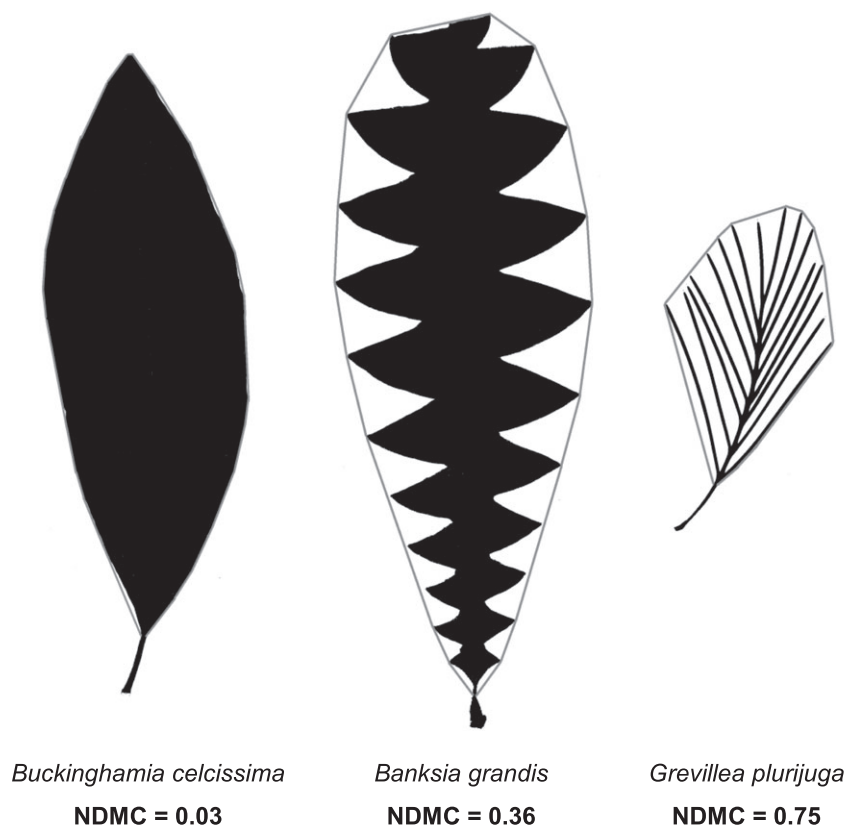


Figure 1. Example convex hulls of three Proteaceae leaves with contrasting shapes, where the convex hull is the smallest convex envelope that can fit around the leaf margin on a two-dimensional plane. To quantify leaf shape – or the extent of dissection to the leaf margin – the normalized difference margin complexity (NDMC) was calculated as the difference between the perimeters of the margin and convex hull, normalized by the sum of these perimeters. Images are not to scale.

Transpiration rates were calculated using the leaf temperature model of Leigh *et al.* (2012). This model calculates leaf temperature from known environmental inputs (radiative load, air temperature and wind speed, measured in this study) and leaf properties (leaf width, thickness, spectral absorptance and thermal capacitance, measured or estimated in this study). To estimate transpiration rates, we first used the model to calculate average leaf temperatures for all leaves based on measured leaf size and environmental conditions assuming no transpiration. Using these modelled leaf temperatures, we determined ΔT for non-transpiring leaves ($\Delta T_{\text{NOTRANS}}^{\text{P}}$). We then calculated the latent heat loss rate that would account for the difference between this modelled $\Delta T_{\text{NOTRANS}}^{\text{P}}$ and the observed ΔT we measured in the field. Assuming the modelled transpiration rates were within a realistic range, any mismatch between the $\Delta T_{\text{NOTRANS}}^{\text{P}}$ and the observed ΔT , with the former being higher than the latter, would indicate that the corresponding observed leaves were transpiring.

Data analyses

All analyses were carried out using SPSS (IBM SPSS Statistics, Chicago, IL, USA; version 23). Statistical analyses of leaf thermal dynamic traits were carried out using Pearson's correlations to look for relationships among leaf dimension traits

and generalized linear models to investigate the effects of leaf dimensions on leaf temperature. Prior to analyses, to address non-normality, area and NDMC were transformed using the natural log, and w_e was square root transformed. When selecting species to include in this study, our aim was to maximize the range of sizes, shapes and genera. Some genera have very few representative species (the same is true for tribe and subfamily), often growing in distinct habitats, such as rainforest, with leaf morphology markedly different to that found in larger Proteaceae groups (e.g. very large and glabrous *versus* small and sclerophyllous, respectively). We therefore considered these taxa important to include in our data set, and our design was inherently unbalanced with regard to genus. In addition, genus incorporated variation in leaf morphology such as hairy, glossy or glaucous surfaces that could influence leaf temperature. We included genus as a factor in all models except for those involving only summer leaves, where there were insufficient degrees of freedom. As expected, we found a significant effect of genus in nearly all analyses, and our results account for the effect of genus; however, as our primary interest was in the effects of leaf traits on leaf thermal dynamics, we make no inference about differences among genera hereafter.

Along with genus, models incorporated season as a factor, with the leaf traits, area, NDMC and w_e , included as continuous predictors, along with selected interactions. In particular,

we tested for an interaction between area and NDMC because we expected the effect of the latter on leaf thermal dynamics to be greater for large leaves. In other words, our prediction was that when leaves were small, their shape would not contribute greatly to thermal regulation, but for larger leaves, being deeply dissected would reduce their effective width and corresponding heat load. Because we expected that season would influence leaf temperature, interactions with season were included for models containing ΔT and T_{range} , which were measured across season, but not for models with τ , which were measured only in summer. For models examining the effects of leaf area and NDMC, the interactions between area and NDMC, and the three-way interaction between area, NDMC and season were deemed of interest *a priori*; other interaction terms were not fitted. Including w_e with area and NDMC resulted in loss of degrees of freedom and less parsimonious models (higher corrected Akaike Information Criterion scores), so separate models were used to examine the effects of w_e on leaf thermal dynamics. Where significant interactions were found, we reanalysed the corresponding leaf trait-temperature relationship individually within each season. In all models, we used the robust estimator for the covariance matrix as this is best suited to over-dispersed data (Garson, 2013). The ratio of Pearson chi-squared values to degrees of freedom for models including data from the three seasons (ΔT and T_{range}) were all close to one, whereas models for τ , including only summer values had ratios larger than two, probably as a result of smaller sample sizes. Nevertheless, all likelihood ratio chi-squared omnibus test scores were significant, indicating good to very good fits for the models. For the observed τ data set in summer, we conducted outlier tests using Tukey's outlier formula to generate lower and upper bounds (lower bound: $Q1 - [1.5(Q3 - Q1)]$; upper bound: $Q3 + [1.5(Q3 - Q1)]$), against which

the most extreme lower and upper values of the data were compared. Any lower or upper extreme values falling below or above these bounds, respectively, were considered to be outliers.

RESULTS

Leaf dimensions

With respect to leaf dimensions, we were interested in the extent to which leaf thermal dynamics were influenced by size (area) or margin shape complexity *per se*, relative to effective leaf width, the latter being a more direct indicator for boundary layer thickness. Our data set incorporated the remarkable breadth of variation in leaf size and shape in the Proteaceae (Fig. 2). Leaf area ranged from 1.5 to 435 cm², effective leaf width (w_e) ranged from 1.0 to 80 mm and NDMC ranged from 0.01 to 0.85. Leaf area was positively correlated with both w_e and NDMC (Table 1). That leaf margin complexity increased with leaf size reflected the higher incidence of lobed and compound leaves among large-leaved rainforest species in our data set. By contrast, w_e and NDMC were not significantly correlated (Table 1), explained by the fact that a given w_e can be achieved through either a deeply dissected leaf or an entire, narrow leaf.

Leaf time constant

Examining the relationship between the observed time constant for cooling, τ measured in summer and τ predicted by Eqn 2 for summer leaves, we found a significant correlation ($r = 0.62$; $P < 0.001$). By either measure, we expected leaves with a greater area and/or w_e or with a lower NDMC to cool

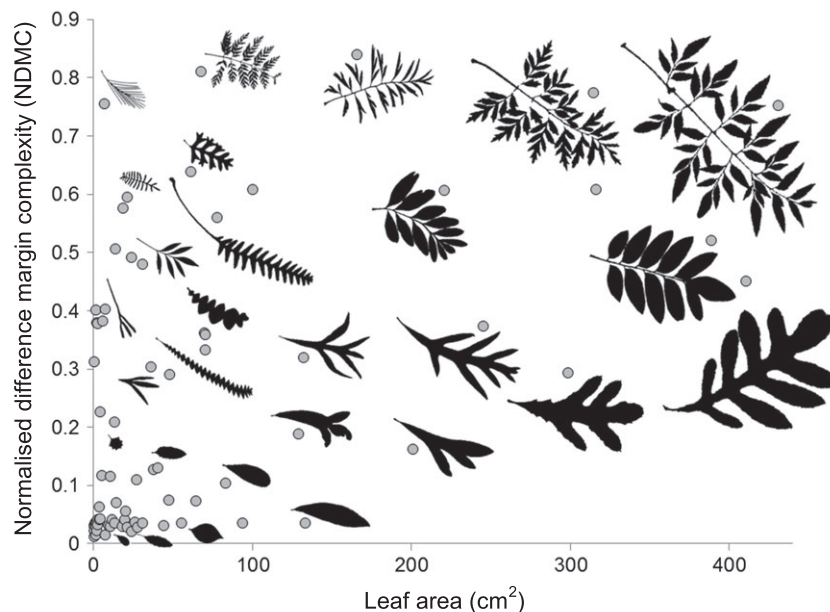


Figure 2. Selected images of leaf silhouettes are overlaid onto observed data to illustrate the range in shape (NDMC) and size (area) of the Proteaceae leaves measured in this study. Each data point represents the size-shape dimensions for one leaf, and data are presented here untransformed to show the actual range of dimensions. Images are not to scale.

Table 1. Pearson correlations between three leaf traits, leaf area, margin complexity (NDMC) and effective width (w_e) of 145 Proteaceae leaves

		Area	NDMC	w_e
Area	Pearson correlation	1.000		
	Significance (2-tailed)	–		
NDMC	Pearson correlation	0.518**	1.000	
	Significance (2-tailed)	0.000	–	
w_e	Pearson correlation	0.748**	0.051	1.000
	Significance (2-tailed)	0.000	0.540	–

Analyses were conducted on transformed data (natural log for Area and NDMC; square root for w_e). Significance indicated in bold (** $P < 0.01$)

NDMC, normalized difference of margin complexity.

more slowly (have a longer time constant) than small, narrow or deeply dissected leaves. No significant main effects of leaf area or NDMC were found for either observed or predicted τ (Fig. 3a, b, d and e; Table 2). There were, however, weakly significant interactions between leaf area and NDMC for both measures of τ : with greater leaf area, the cooling time constant decreased for leaves with more dissected margins (Table 2; interaction not depicted graphically). Both observed and predicted τ were significantly influenced by w_e : the time constant increased as leaf width increased (Fig. 3c and f; Table 2). For observed τ , a particularly high reading for a small leaf, *Grevillea steiglitziana*, most likely due to a transient lull in wind speed to $<0.3 \text{ m s}^{-1}$ was recorded (marked point in Fig. 3a–c). Outlier tests indicated that the data set contained no outliers. In any

case, running the analyses for observed τ without this point did not change significance levels (results not shown). The results we report therefore are based on all of the data.

Leaf-to-air temperature difference

Having established an influence of the two-dimensional proportions of leaves on the rate at which they cool, we then investigated their effect on leaf-to-air temperature difference. This effect varied depending on whether the average, minimum or maximum temperature (coldest or hottest pixel) recorded on the lamina was considered. When the leaf-to-air temperature difference was calculated from average lamina temperature (ΔT), absolute values ranged from 0.0 to 9.7°C . Based on the maximum temperature on the lamina, absolute ΔT_{max} values ranged from 0.2 to 13.9°C , contrasting absolute ΔT_{min} , with a range of 0.0 to 6.3°C . The leaf-to-air temperature difference also varied significantly among sampling seasons, being greatest in summer and lowest in autumn (Table 2). Indeed, for all leaves in summer and most leaves in winter, observed average leaf temperatures remained above ambient air temperature (positive values of ΔT), whereas in autumn, many ΔT values were negative (Fig. 4a–c). By contrast, all modelled $\Delta T_{\text{NOTRANS}}^{\text{P}}$ values, which were calculated for non-transpiring leaves for each sampling period, were positive (Fig. 4d–f). In calculating the modelled $\Delta T_{\text{TRANS}}^{\text{P}}$ data, which assumed leaves were transpiring, our derived leaf temperatures correlated strongly with observed average leaf temperatures in the field ($r = 0.95$, $P < 0.001$), suggesting that our model parameterization was robust. The calculated average transpiration rates

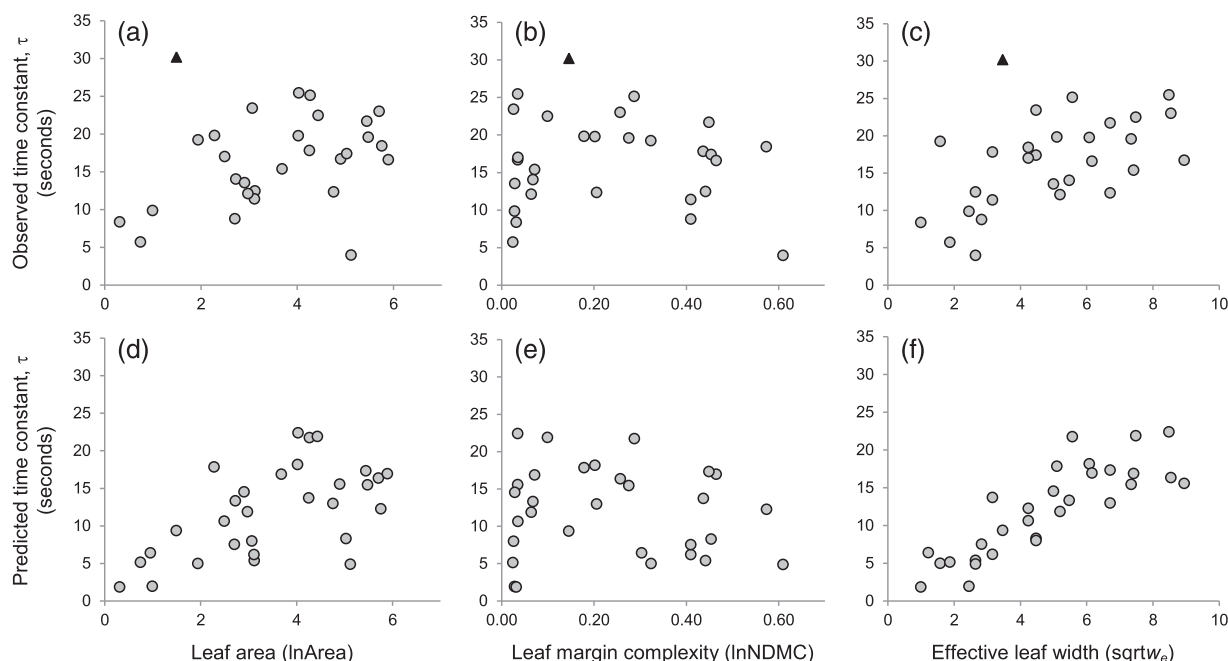


Figure 3. Comparison of the observed (a–c) versus predicted (d–f) time constant for leaf cooling (τ) as a function of leaf area (a and d), margin complexity (NDMC, b and e) and effective leaf width (w_e , c and f) for Proteaceae leaves in summer. Larger values for τ denote slower leaf cooling speeds. The marked point in panels a–c (black triangle) is referred to in the Results section. For analyses, all independent leaf traits were transformed to address non-normality, as indicated on the axes; however, for visual clarity, leaf area and NDMC are presented here as $\ln(\text{Area} + 1)$ and $\ln(\text{NDMC} + 1)$, respectively.

Table 2. Relationships between leaf area and leaf margin complexity (NDMC) and effective leaf width (w_e) on the predicted and observed time constants for leaf cooling (τ), leaf-to-air temperature difference, based on mean (ΔT), minimum (ΔT_{\min}) and maximum (ΔT_{\max}) leaf temperatures, and the temperature range across the surface (T_{range}) of Proteaceae leaves. Because we expected that the effects of NDMC on leaf temperature would vary with leaf area, we tested for an interaction between NDMC and area and analysed the relationship between w_e and thermal dynamics separately. Generalized linear models included genus and sampling season as factors and leaf area, NDMC and w_e as covariates. Values under factors and covariates are p -values; significant effects are indicated in bold.

Variate	Tests for effects of leaf area, margin complexity and their interaction						Tests for effects of effective leaf width			
	<i>d.f.</i>	Genus	Season	Area	NDMC	Area \times NDMC	Season \times area \times NDMC	<i>d.f.</i>	Genus	Season
Predicted τ	4,25	–	–	0.081	0.574	0.010	–	2,28	–	–
Observed τ	4,25	–	–	0.583	0.185	0.030	–	2,27	–	–
ΔT	25,120	0.000	0.000	0.772	0.401	–	0.026	23,122	0.000	0.001
ΔT_{\min}	25,120	0.000	0.000	0.273	0.529	–	0.955	23,122	0.000	0.021
ΔT_{\max}	25,120	0.000	0.000	0.122	0.486	–	0.117	23,122	0.000	0.004
T_{range}	25,120	0.211	0.000	0.005	0.735	–	0.225	23,122	0.000	0.000
									0.000	0.007

Because we expected that the effects of NDMC on leaf temperature would vary with leaf area, we tested for an interaction between NDMC and area and analysed the relationship between w_e and thermal dynamics separately. Generalized linear models included genus and sampling season as factors and leaf area, NDMC and w_e as covariates. Values under factors and covariates are p -values; significant effects are indicated in bold.

NDMC, normalized difference of margin complexity; *df*, degrees of freedom.

ranged between 2.2 (± 1.2) and 2.4 (± 1.2) mmol m⁻²s⁻¹ in autumn and winter, respectively, to 5.1 (± 2.9) mmol m⁻²s⁻¹ in the summer. Note that these averages were based on only those cases where the observed ΔT was smaller than $\Delta T_{\text{NOTRANS}}$ that is those leaves assumed to be transpiring, and this percentage varied markedly with season. In the summer, 70% of the leaves, in the autumn 9% and in the winter 14% showed minimal or no transpiration. Therefore, although average transpiration rate was much higher in summer, this average is based on only a small number of leaves with very high rates, whereas transpiration rates in the winter and autumn were lower but occurred in more leaves.

Relationships between leaf dimensions and the leaf-to-air temperature difference were influenced not only by how leaf temperature was defined (ΔT , ΔT_{\min} or ΔT_{\max}) but also by sampling season (Figs 4 and 5; Table 2). As a main effect in the full season models, neither leaf area nor margin complexity influenced any measure of ΔT ; however, a weak interaction was found with season: in summer, ΔT increased significantly with leaf area (Figs 4a and b, and 5a, b, d and e; Table 2). Although not evident through an interaction in the full season models, when examining effects of leaf area within each season, we were interested to note that ΔT_{\min} significantly decreased with leaf area in autumn ($P = 0.011$; Fig. 5a). The positive influence of increasing w_e on ΔT was stronger than for leaf area, again being clearest in summer, as was its effect on ΔT_{\max} (Figs 4c and 5f; Table 2).

Leaf temperature range

The range of temperatures across the surface of individual Proteaceae leaves, T_{range} , varied from 0.7 to 14.7 °C. Leaf temperature ranges were significantly greater in summer, with average values three times higher than for leaves measured in winter and autumn (Figs 5 and 6; Table 2). There was a strong main effect of leaf area on T_{range} , which held for all sampling seasons (Fig. 5h; Table 2). No significant influence of leaf margin complexity on T_{range} was found (Fig. 5i; Table 2). Contrasting leaf area, the main effect of leaf width on T_{range} was weaker, but there was a significant interaction between w_e and season, such that the effect of increasing leaf width on T_{range} was significant in summer (Fig. 5j; Table 2).

DISCUSSION

By using thermal imagery on real leaves under field conditions, this research provided an empirical test of theoretical and laboratory-based predictions about the influence of leaf size and shape on leaf thermal dynamics, predominantly through altering the leaf boundary layer. To a certain extent, our findings present support for these predictions: leaf cooling time constants were longer (τ increased) with increasing leaf width and, for large leaves, τ decreased with increasing margin complexity; leaf-to-air temperature differences increased with leaf width and in summer also with leaf area; temperature ranges across the leaf surface were unaffected by leaf margin complexity, but increased significantly with leaf area, and in summer also for leaf width. These findings are particularly compelling

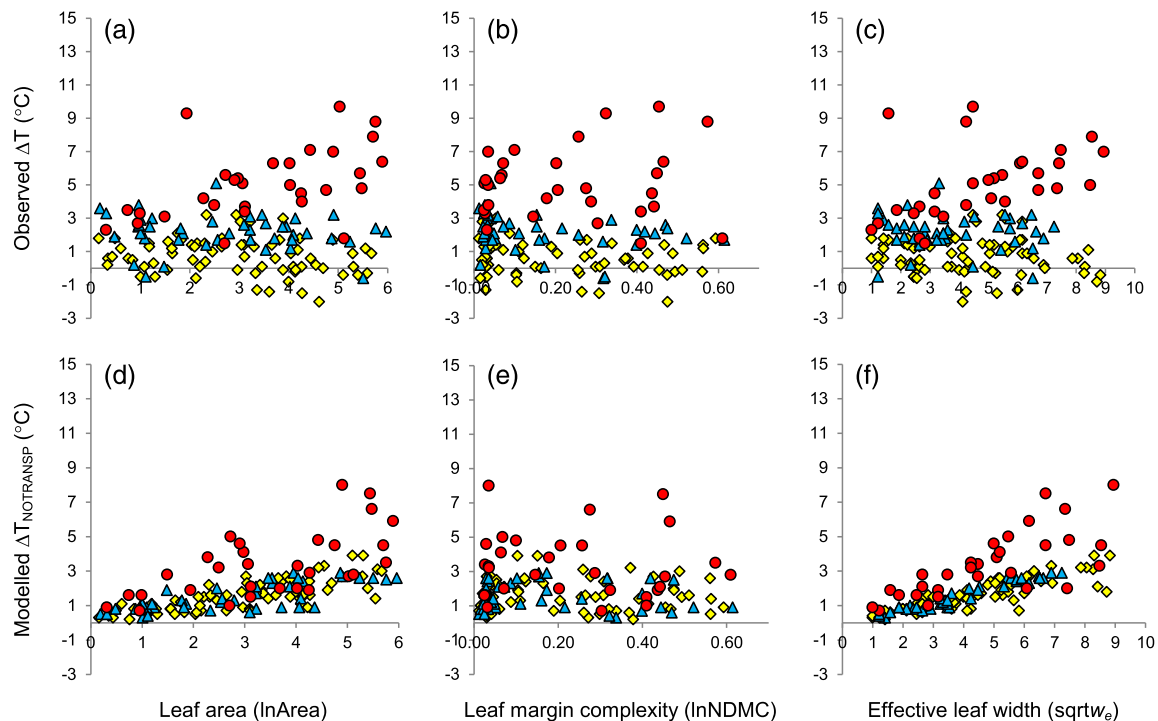


Figure 4. Comparison of the measured *versus* modelled leaf-to-air temperature difference (ΔT and $\Delta T_{\text{NOTRANSPIR}}$ respectively) as a function of leaf area (a and d), margin complexity (NDMC, b and e) and effective leaf width (w_e , c and f) for Proteaceae leaves. Observed ΔT values (a–c) were based on the average leaf temperature across each leaf relative to ambient temperature at the time of measurement. Modelled $\Delta T_{\text{NOTRANSPIR}}$ (d–f) used the same leaf and ambient parameters but assumed that leaves were not transpiring. Data are for leaves from three sampling seasons: winter (blue triangles), autumn (yellow diamonds) and summer (red circles). Data transformation and presentation as for Fig. 3.

given the potential for additional environmental factors to influence the leaf temperatures we measured in the field, such as variation in leaf angles and small fluctuations in air movement. Importantly, however, of the three two-dimensional leaf measures, w_e was by far the strongest predictor of responses relating to dynamic temperature flux, τ and ΔT , contrasting leaf area, which related mainly to T_{range} . Moreover, thermal images suggest that interpretation of the leaf area– T_{range} relationship should be made with caution because the reasons for it are likely to be more complex than physical predictions based on leaf replicas in laboratory conditions.

Leaf temperatures in the field

The diverse leaf types in our study would not normally occur together. Our common garden environment allowed us to compare how leaf thermal dynamics among species adapted to different habitats varied, both within and across seasons. Results measured in the two cooler seasons could give the impression that leaf size and shape had a negligible or even negative influence on leaf temperature relative to ambient temperature. Of particular interest were the autumn ΔT values, which were low and progressively more negative with increasing leaf area. Although counter to what would occur purely through heat convection from artificial leaves, real leaves are subject to the added effect of latent heat loss through transpiration. For larger leaves, an increased boundary layer depth causes a rise in leaf temperature and saturation vapour pressure within the

internal air spaces, which in turn increases the water vapour concentration gradient between the internal air spaces outside air, leading to a higher rate of transpiration and latent heat loss. Provided there is sufficient water to maintain open stomata and radiation is not too high, large leaves can potentially cool more effectively via transpiration and maintain lower temperatures than smaller leaves (Gates & Papian, 1971, Smith & Geller, 1980). That the negative ΔT values we observed in autumn were the result of increasing transpiration with leaf area is supported by the modelled $\Delta T_{\text{NOTRANSPIR}}$ values for the same leaves assuming no transpiration: all were above ambient and increased with leaf area (Fig. 4d). Observed leaf temperatures were seldom below ambient in winter, suggesting slightly lower rates of transpiration, most likely because of a cooler leaf (Matsumoto *et al.*, 2005) and lower soil temperatures (Wan *et al.*, 2004). Considering these combined effects of latent heat loss and low radiative load, a weak or mixed influence of leaf dimension on leaf temperature in these cooler months is unsurprising.

The strongest influence of leaf area and w_e in driving leaf temperature above that of air was evident in summer, when irradiance was very high and air temperatures reached over 41°C . The fact that τ also varied with leaf dimensions, notably w_e , for these summer leaves, suggests an influence of τ on ΔT in summer. These hot sampling conditions followed an extended period of drought, with low relative humidity and soil moisture. Not only do such conditions often lead to stomatal closure (Trifilo *et al.*, 2004, Valladares & Pearcy, 1997, Yao *et al.*,

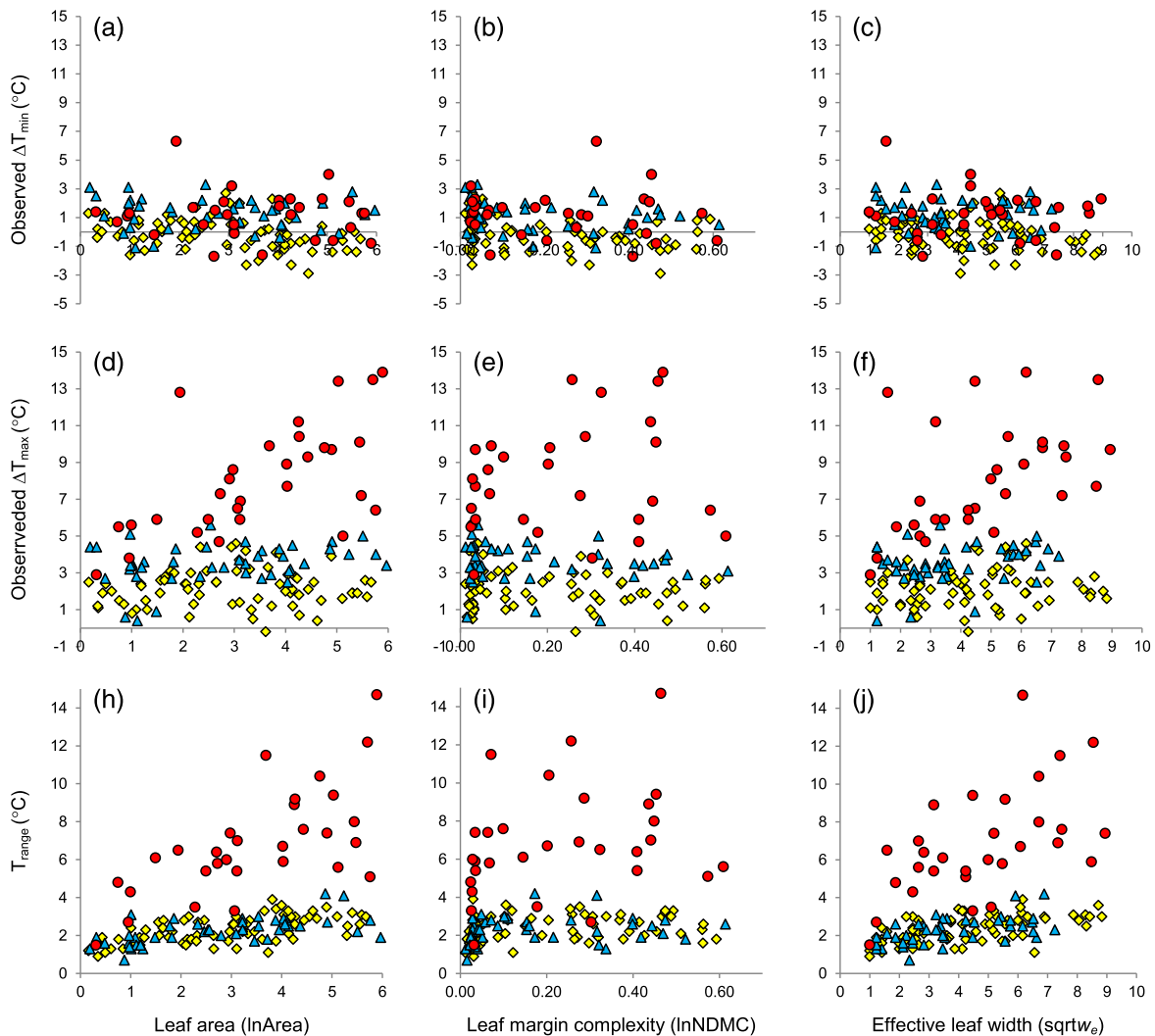


Figure 5. Leaf-to-air temperature difference based on the coolest and warmest point on the leaf (ΔT_{\min} , a–c, and ΔT_{\max} , e–g, respectively) and the range of temperatures across the surface of leaves (T_{range} , h–j) as a function of leaf area (a, d and h), margin complexity (NDMC, b, e and i) and effective leaf width (w_e , c, f and j) in Proteaceae leaves. Data are for leaves from three sampling seasons: winter (blue triangles), autumn (yellow diamonds) and summer (red circles). Data transformation and presentation as for Fig. 3.

2001) but also high absorbed radiant energy can substantially reduce the relative contribution of transpiration to leaf cooling (Smith, 1978, Smith & Geller, 1980). Under such conditions, and as our findings suggest, field measured leaves with closed stomata will most closely represent the theoretical response of leaf replicas, with boundary layer convection dominating heat transfer.

Inherent leaf properties additional to boundary layer depth can affect leaf temperature, a possibility that became visibly discernible when we investigated the range and spatial distribution of temperature across leaf laminae. T_{range} was strongly influenced by leaf area, with temperature ranges across the lamina of large leaves reaching well over 10 °C in summer (Figs 5h and 6). In the absence of an image accompanying numeric measurements, one might assume that the thermal distribution across the leaf surface followed a pattern of heat convection across a boundary layer that was thickest in the centre, following an even, outwardly spreading pattern,

resulting in cooler tissue at the edges of the lamina. Such a neat pattern, however, was by no means the rule for our leaves, particularly those with large area, where the temperature distribution across the lamina was spatially patchy and irregular. This spatial irregularity is likely to have more than one cause. The first is undulations, curvature and irregularities of the leaf surface, resulting in a temperature distribution across a single leaf contingent upon the angle of a given portion of the lamina relative to the direction of the sun (e.g. Fig. 6a and b). A subsequent effect that could arise is patchy stomatal conductance, where localized variation in heat load would alter the corresponding vapour pressure gradient and in turn, stomatal aperture (Mott & Buckley, 2000, Mott & Franks, 2001). This structural influence on T_{range} would be amplified in larger leaves, which are less likely than small leaves to project as a single flat plane but rather to curve, fold or undulate (Niklas, 1999), presenting multiple angles with respect to incident radiation.

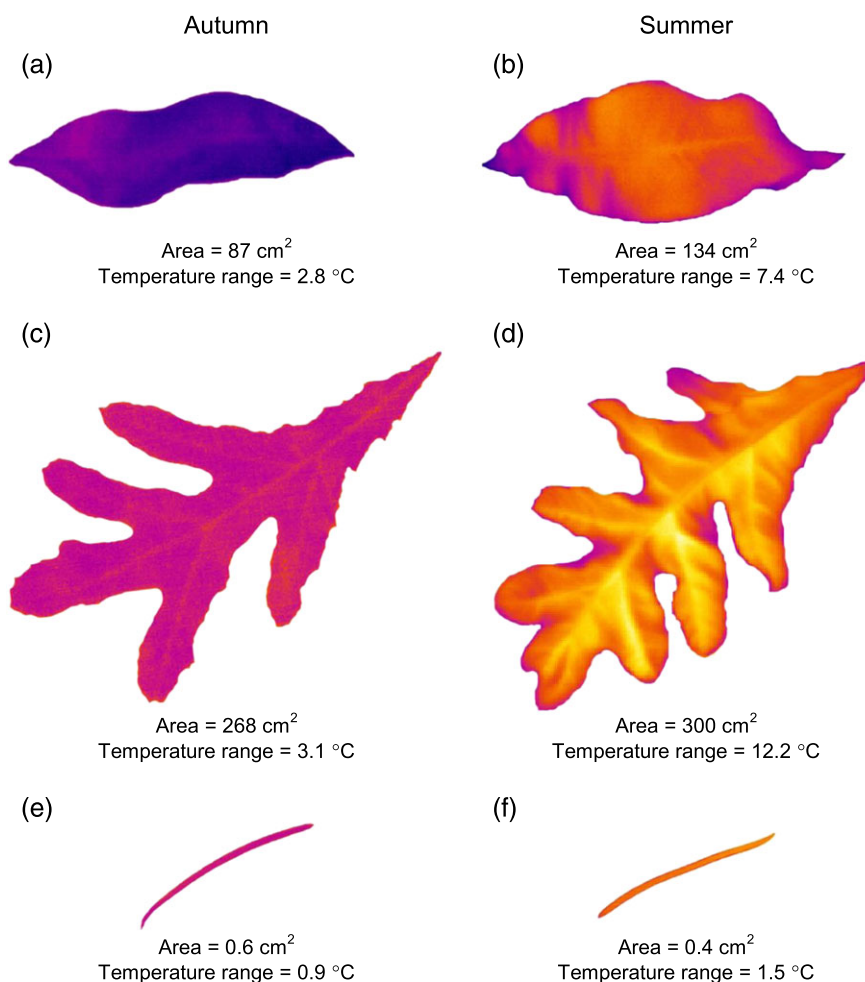


Figure 6. Infrared images of *Athertonia diversifolia* (a and b), *Buckinghamia celcissima* (c and d) and *Grevillea pinaster* (e and f) leaves, illustrating variation in heat load across the leaf surface under mild, overcast conditions in autumn (a, c and e) versus hot, sunny conditions in summer (b, d and f). Images are not to scale.

A second feature likely to affect the spatial thermal profile is the uneven distribution of water within the leaves (Fig. 6c and d). As water is delivered to the lamina via a series of conduits of ever decreasing diameter, the relative volume of water, and therefore thermal mass, varies spatially. Given the strong influence of thermal mass on τ , different regions of the leaf should cool at different rates, something found to occur for leaves during freezing (Ball *et al.*, 2002). Again, spatial variation in thermal mass will increase with leaf area because of the increasing structural requirement for ever larger veins to support a larger lamina (Givnish, 1979, Niinemets *et al.*, 2007, Roth-Nebelsick *et al.*, 2001), creating a greater diversity of vein diameters in large leaves. We therefore suggest that our observed increase in T_{range} with leaf area is associated with, or at least amplified by, a comparatively greater structural heterogeneity in large leaves (e.g. compare Fig. 6b, d and f).

The relative importance of morphological heterogeneity versus boundary layer convection and latent heat loss in governing leaf temperature is difficult to tease apart. Notwithstanding the within-leaf variation already discussed, our results suggest an influence of boundary layer thickness on the

temperature of large leaves, particularly during summer, when latent heat loss was likely to be playing a minor role. For example, although ΔT and τ did not increase with leaf area or shape complexity, w_e – correlated with area yet more tightly linked to boundary layer dynamics – was a strong predictor. Further, although the interaction was weak, for leaves above a certain size, greater margin dissection did afford more rapid cooling in summer. It is important to note, however, that these effects of NDMC on τ were mostly apparent for the uncommonly large leaves in our data set (average area of 150 cm² and up to 300 cm²; results not shown).

Ecological implications

Large and dissected or compound leaves generally are found in the rainforest understorey. In such environments, high humidity can reduce transpiration for some species (Meinzer *et al.*, 1995) and therefore latent heat loss. Under such a scenario, if sun over a canopy gap lead to a short period of high radiative load, a dissected leaf potentially could reduce boundary layer resistance to heat convection. On the other hand, the

importance of a leaf's convective boundary layer in governing heat transfer diminishes at very low wind speeds, 0.1–0.25 m s⁻¹, to the point where free convection ultimately predominates (Gates & Papian, 1971, Grace *et al.*, 1980). The wind speeds within rainforest canopies can regularly be <0.5 m s⁻¹ (Martin *et al.*, 1999, Monteith & Unsworth, 1990, Stokes *et al.*, 2006) and sometimes <0.25 m s⁻¹ (Meinzer *et al.*, 1995). Under such conditions, the influence of boundary layer depth on heat transfer would be reduced, thereby diminishing the benefit of dissected leaves in mitigating overheating. Another scenario would be found for species with relatively low stomatal resistance, as is often the case for rainforest species (Franks & Farquhar, 1999). Under low wind conditions, such species may have increased transpiration rates, reducing ΔT (Schymanski & Or, 2016) but also the need for a dissected leaf margin. Indeed, many of the large rainforest leaves in the current study represent an understorey form for a given species, with very much smaller, un-lobed leaves of the same plant being produced at the exposed outer canopy (e.g., *Athertonia*, Weston, 1995). For rainforest species, it is likely that reduction in size of these outer leaves functions to reduce heat load, whereas large, dissected or compound leaves in the understorey confer other advantages, for example low cost branching that can be readily shed as the plant grows taller (Givnish, 1976, Niinemets, 1998), penetration of light deeper into the canopy (Niklas, 1989) or mitigating mechanical damage (Chazdon, 1986, Cooley *et al.*, 2004).

Similarly, for species in hot, dry environments, we do not believe that leaf dissection represents a primary adaptation for thermal regulation. In our study, a fair proportion of species from such environments had medium-sized leaves with entire margins or relatively low NDMC, for example *Banksia repens*, *Banksia robur*, *Banksia serrata*, *Banksia grandis*, *Grevillea agrifolia*, *Hakea petiolaris* and *Telopea speciosissima*. The leaves of these species have pubescent, reflective surfaces or are oriented vertically. Such traits provide solutions to minimizing excessive heat load that can serve as effective alternatives to reducing leaf dimensions (Curtis *et al.*, 2012, Leigh *et al.*, 2012). Again, whereas within-species variation in leaf dissection – e.g. sun-leaves *versus* shade-leaves – is well known, across species and biomes, evidence for a generalized and universally applicable relationship between leaf shape and environmental temperature is lacking (Li *et al.*, 2016, Nicotra *et al.*, 2008). Where species in hotter environments do possess dissected leaves, improved thermal regulation may be simply a fortunate by-product of other evolutionary drivers of dissection such as improved hydraulic efficiency (Leigh *et al.*, 2011) or reduced solar interception (Mooney *et al.*, 1977, Niklas, 1989). Finally, in focusing within the Proteaceae, this study was taxonomically limited, yet across families, leaf shape is likely to be constrained by genetics, with certain shapes occurring independent of climate (Jordan, 1997). It therefore is likely that variation in leaf shape, both across and within taxa, has multiple evolutionary drivers.

In summary, on the basis of our findings for real leaves under natural conditions, we agree with theory predicting that leaves of small size or, more specifically, small effective leaf width have adaptive value for plants evolved for hot environments.

For leaf margin complexity or dissection, on the other hand, we cannot support this same argument and therefore reject the idea that temperature is the primary selective driver in the evolution of leaf shape complexity.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Proteaceae species used to measure thermal dynamics and morphological properties of leaves. For the four species bearing both dissected and entire leaves, both leaf types were measured. The right-hand column indicates the sampling period in which each species was measured: winter (W), autumn (A), and summer (S).