

Leaf shape linked to photosynthetic rates and temperature optima in South African *Pelargonium* species

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Received: 23 August 2006 / Accepted: 12 September 2007 / Published online: 18 October 2007
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Abstract The thermal response of gas exchange varies among plant species and with growth conditions. Plants from hot dry climates generally reach maximal photosynthetic rates at higher temperatures than species from temperate climates. Likewise, species in these environments are predicted to have small leaves with more-dissected shapes. We compared eight species of *Pelargonium* (Geraniaceae) selected as phylogenetically independent contrasts on leaf shape to determine whether: (1) the species showed plasticity in thermal response of gas exchange when grown under different water and temperature regimes, (2) there were differences among more- and less-dissected leafed species in trait means or plasticity, and (3) whether climatic variables were correlated with the responses. We found that a higher growth temperature led to higher optimal photosynthetic temperatures, at a cost to photosynthetic capacity. Optimal temperatures for photosynthesis were greater than the highest growth temperature regime. Stomatal conductance responded to growth water regime but not growth temperature, whereas transpiration increased and water use efficiency (WUE) decreased at the higher growth temperature. Strikingly, species with more-dissected leaves had

higher rates of carbon gain and water loss for a given growth condition than those with less-dissected leaves. Species from lower latitudes and lower rainfall tended to have higher photosynthetic maxima and conductance, but leaf dissection did not correlate with climatic variables. Our results suggest that the combination of dissected leaves, higher photosynthetic rates, and relatively low WUE may have evolved as a strategy to optimize water delivery and carbon gain during short-lived periods of high soil moisture. Higher thermal optima, in conjunction with leaf dissection, may reflect selection pressure to protect photosynthetic machinery against excessive leaf temperatures when stomata close in response to water stress.

Keywords Dissection index · Evaporative cooling · Temperature response · Photosynthesis · Stomatal conductance

Introduction

The process of photosynthesis is broadly dependent on temperature and water availability. When temperatures for photosynthesis are optimal, however, water availability is often low. Thermal optima for photosynthesis vary among species, with C_3 plants generally having optima (i.e., able to maintain 90% of maximum rates) at temperatures between 20 and 30°C (Larcher 1995), whereas arid and desert plants, and C_4 and CAM plants have higher optima (30–40°C, Larcher 1995) as well as broader optimal ranges (Berry and Björkman 1980; Downton et al. 1984).

The shape of the temperature response curve is determined by different factors at low, moderate and high temperatures (Hikosaka et al. 1999; Xiong et al. 1999; Haldimann and Feller 2005; Sharkey 2005; Yamori et al.

Communicated by Kouki Hikosaka.

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2005; Hikosaka et al. 2006), with critical points varying depending on growth temperatures (Berry and Björkman 1980), plant water status and even time of day (Lange et al. 1977; Valladares and Pearcy 1997; Medlyn et al. 2002). Within species, thermal optima have been shown to be higher in plants grown at higher temperatures, and a growing body of evidence suggests that critical temperatures for thermal tolerance are often plastic within species (Froux et al. 2004). Recent comparative studies show plasticity, but few consistent differences among species from different environments in thermal tolerance of photosynthesis (Knight and Ackerly 2002; 2003; but see Atkin et al. 2006).

Critical temperatures for damage of photosystem II are usually above 45°C (Sharkey 2005). So under most growing conditions, neither air temperatures nor average leaf temperature will exceed critical temperatures. However, under conditions of water stress, stomata may close before photosynthetically critical temperatures are reached; in the absence of evaporative cooling, the leaves of plants in sunny arid environments may then reach critical temperatures (Valladares and Pearcy 1997). Even though stomata may respond to temperature itself (Shabala 1996), and drought stress has been shown to increase thermotolerance in some species (Havaux 1992; Ladjal et al. 2000), very few species actively cool using the transpiration stream, even under ideal water conditions. Even where reduced leaf temperature has been associated with high stomatal conductance (Radin et al. 1994; Mahan and Upchurch 1988; Upchurch and Mahan 1988), there is limited evidence that stomatal opening is a direct response to high temperatures (but see Dwyer et al. 2007).

Leaf shape is considered to be another way that plants solve problems of high leaf temperatures: lobed or dissected leaves will have thinner boundary layers and therefore greater convective cooling (Nobel 1983; and see references in Schuepp 1993). However, evidence for an effect of leaf shape on leaf temperature in the field is inconclusive (Smith 1978; Hegazy and El Amry 1998), and while leaves are generally smaller in hot and dry climates, leaf dissection has not been shown reliably to be more common under these conditions (McDonald et al. 2003).

With rising global temperatures, there is increasing interest in identifying and evaluating plant traits that determine both photosynthetic optima and thermal tolerance. In particular, it is important to establish to what extent these traits are plastic in response to changes in average growth temperatures and water availability. Relevant traits include photosynthetic rates, water use parameters and morphological features such as leaf shape.

In this study we compared eight South African species of *Pelargonium* (Geraniaceae) selected as phylogenetically independent contrasts (PICs) on leaf shape (Burt 1989).

Pelargonium is an ideal genus with which to address these questions because leaf shape is an evolutionarily labile trait, with more-dissected leaf forms evolving independently multiple times within the genus (Jones et al., in preparation). We asked whether: (1) the species showed plasticity in thermal response of gas exchange when grown under different water and temperature regimes, (2) there were differences among more- and less-dissected leafed species in these traits or their plasticities, and (3) attributes such as latitude or rainfall at the sites of seed origin were correlated with the responses.

Materials and methods

Study species

Eight species of *Pelargonium* were selected as four PICs contrasted on leaf shape using images (van der Walt 1977; van der Walt and Vorster 1981, 1988) and a molecular phylogeny (Bakker et al. 2004 and references therein). Within a section of the genus, species were selected to maximize difference in leaf shape, dependent on availability of seed. Species were paired for growth form and geographic region as possible. Seeds from single populations were collected in the field by Rachel Saunders from Silverhill Seeds (Cape Town, South Africa; Table 1), with the exception of seeds of *Pelargonium klinghardtense*, and *Pelargonium oblongatum*, which were obtained ex hort or from specimens in cultivation at the University of Connecticut Greenhouse.

Propagation and experimental design

In late February and early March 2003, seeds were sown in seedling plugs in fine, washed river sand and kept in a glasshouse at 20/10°C (day/night). In early May, seedling plugs were potted into 150-mm standard plastic pots in a custom soil mix of 1:1 decomposed pine bark fines and coarse washed river sand. The mix was pasteurised at 60°C for 3 h, a medium dose of micronutrients was added (Micromax, 0.5 kg m⁻³), and the soil was then processed in a commercial mixer.

Plants were given 2 weeks to adjust to transplanting, and then experimental treatments were introduced. Plants were randomly allocated to one of two greenhouse compartments under either cool (18/8°C day/night) or hot (28/18°C day/night) conditions. Temperatures were chosen to represent average early- and high late-season natural growing conditions, respectively. Each PIC pair was randomly assigned to a bench in the same position in the two glasshouses. To minimise concerns about pseudo-

Table 1 Leaf shape (a typical leaf from the 18/8°C, low-water treatment, at 25% actual size), section, number of replicates used in gas exchange, habitat characteristics and mean dissection index (perimeter/square root of area) of study species. Habitat characteristics based on collection site for all field-collected species

Section	Species (no.) ^a	Habitat	Daily temperature: July (°C)	Average rainfall (mm)	Rainy season	Growth form	Dissection index
Reniformia	 <i>Pelargonium reniforme</i> (8)	(33 17'S, 26 31'E) Karoid shrubland, shale and quartzitic sandstone, well drained, 600 m	4–18	380	All year	Sub-shrub	4.7
	 <i>Pelargonium ionidiflorum</i> (6)	(33 10'S, 26 35'E) Karoid shrubland, well drained, between rocks in karoo or karroid vegetation, 600–1,000 m	4–18	300–760	All year	Sub-shrub	8.1
Pelargonium	 <i>Pelargonium cucullatum</i> (8)	(34 25'S, 19 10'E) Fynbos, Table Mountain derived sandstone, chronically nutrient poor, well drained, 2 m	8–17	760–890	Winter	Shrub	6.3
	 <i>Pelargonium citronellum</i> (9)	(33 55'S, 21 33'E), Little karoo, alluvial Table Mountain sandstone, well drained, 300 m	4–18	200–300	Winter	Shrub	14.5
Hoarea	 <i>Pelargonium oblongatum</i> ^b (20)	Richtersveld and Northern Namaqualand, Gariep Centre, succulent karoo, gravelly areas, 500–1000 m	4–20	50–180	winter	Ephemeral geophyte	6.5
	 <i>Pelargonium incrassatum</i> (10)	(30 23'S, 17 54'E), Namaqualand broken veld, decomposed granite, well drained, 800 m	6–20	150–250	Winter	Ephemeral geophyte	12.2
Otidia	 <i>Pelargonium klinghardtense</i> ^c (8)	(28 30'S, 16 30'E to 27 35'S, 16 40'E), Gariep Centre—succulent karoo, granite, schist or dolomite hills, 200–1,000 m	4–20	25–75, also fog	Winter	Stem succulent	6.4
	 <i>Pelargonium crithmifolium</i> (2)	(31 50'S, 18 38'E), Fynbos, decomposed granite, well drained 100 m	6–20	125	Winter	Stem succulent	20.1

^a Number of plants used for gas exchange measurements *in parentheses*

^b Seeds sourced from collections growing at University of Connecticut, habitat description based on naturally occurring populations

^c Seed sourced commercially, habitat description based on naturally occurring populations

replication, plants were moved monthly between the two compartments. Bench positions were maintained through the experiment. Moving the plants limited glasshouse

aspect/light effects so the two growth temperature treatments had the same air, light, and soil treatments. Only temperature differed.

Each plant was watered by an individual dripper on a stake in the pot at soil level. Water regimes were controlled by a Water-Pro vapour pressure deficit (VPD) control system (MicroGrow Greenhouse Systems, Temecula, Calif.). Watering events were triggered when pre-set VPD targets were reached. The low water VPD target was set 33% higher than the high water target; high water plants received ~150 ml per watering whereas low water plants received ~70 ml per watering. Watering occurred roughly once per week for low water plants and every 2–3 days for high water plants, depending on VPD in the glasshouse. If low and high water plants had been supplied with water at the same level, regardless of growth temperature, plants in the cooler house would effectively have had higher water availability than those in the warmer house. Instead, the VPD controlled system calculated VPD and delivered water at set VPD targets for each treatment and compartment of the house. Watering events for a given water treatment occurred at an equivalent target, not the same time, in the two houses. The higher water level was set to provide ample water without water-logging; the lower level was set at the minimum supported by the system and did not cause obvious wilting or leaf drop. Average relative humidity was significantly higher in the cool than hot temperature compartments (*t*-test, *df* 193, $P \leq 0.0001$, $58 \pm 13\%$ and $40 \pm 12\%$, respectively). Average VPD was 0.87 and 2.28 KPa in the cool and hot houses, respectively.

Plants were fertilized biweekly with the commercially available water soluble fertilizer MiracleGro (N:P:K, 15:13.1:12.4). Initially, low water plants each received 50 ml nutrient solution at $0.5 \text{ g fertilizer l}^{-1} \text{ water}$; high water plants each received 100 ml of $0.25 \text{ g l}^{-1} \text{ solution}$. In mid-August plants had grown substantially and the concentration of fertilizer was increased by 50% to support the larger plants. No salt problems were apparent at any time.

Leaf morphology

The youngest fully expanded leaf was removed from each of ten plants per species per treatment in September 2003. Leaves were scanned on a flatbed scanner, their area determined using ImageJ (Rasband 1997–2005), and then dried at 70°C to constant weight. Specific leaf area (SLA; area/mass) was determined for each leaf. These were not the same leaves, or, in some cases, the same individuals, used for gas exchange analyses.

Gas exchange measurements

Thermal response of photosynthesis was measured with a LiCor 6400 portable photosynthesis system (LiCor,

Lincoln, Neb.) between 24 July and 27 November 2003 on 71 plants. Experimental plants ($n = 1\text{--}5$ per species per treatment) were placed in the cool glasshouse (18°C) to acclimate for ~1 h before the youngest fully expanded leaf was placed in the infra-red gas analyser (IRGA) chamber for 10 min at 8°C . Light level was maintained at saturating levels, $1,200 \mu\text{mol m}^{-2} \text{ s}^{-1}$. Flow was set at $250 \mu\text{mol s}^{-1}$ and CO_2 at $400 \mu\text{mol}$. Every effort was made to moderate VPD_1 , but as temperature increased, so did VPD_1 . Across all measurements average VPD_1 ($\pm\text{SD}$) was $2.3 \pm 1.6 \text{ KPa}$; at temperatures above 40°C , average VPD_1 was $5.31 \pm 1.52 \text{ KPa}$. All measurements were taken between sunrise and 2 p.m. Measurements were taken at 4°C intervals between 8 and 48°C allowing a maximum wait period of 6 min for stability (total coefficient of variation $< 0.5\%$) to be reached. While some *Pelargonium* species show low-level or facultative CAM photosynthesis (Jones et al. 2003), the study species have not been shown to use CAM and all were fixing diurnally.

Curve fitting

The temperature response of photosynthesis was assessed by fitting curves to the photosynthetic data for each plant in SPlus using the following model:

$$\text{Photosynthesis} = (B - C \times \text{Leaf Temp}) \exp(\log(D) \times \text{Leaf Temp}) \quad (1)$$

The shapes of the fitted curves and fit of the data to the curves were good and were deemed appropriate for the data (Battaglia et al. 1996). Individual r^2 -values exceeded 0.88 for all curves (median = 0.991). The parameters *B*, *C* and *D* were then recorded for each plant. These parameters do not directly describe plant traits, rather they were used to determine the biologically relevant parameters as described below. The temperature at which photosynthesis reached its maximum rate (A_{max}) was determined by setting the derivative of Eq. (1) equal to zero;

$$T_{\text{opt}} = B/C - \log(D) \quad (2)$$

At T_{opt} , the maximum rate of photosynthesis (A_{max}) is

$$A_{\text{max}} = C/\log(D) \times \exp[B/C \times \log(D) - 1] \quad (3)$$

T_{opt} and A_{max} were calculated for each plant. Photosynthetic rates at 18 and 28°C were determined using Eq. (1).

The values of leaf temperature marking the endpoints of the range over which photosynthesis reaches 90% of its maximum value were numerically determined from the fitted curve for each plant. The difference between these values ($90\% T_{\text{range}}$) was calculated as an indication of the breadth of the temperature range over which the highest rates of photosynthesis are maintained.

The shapes of the transpiration and conductance responses to change in leaf temperature varied so we could not fit a single parametric curve to all plants. A smoothing spline with 8° of freedom was therefore fitted to the data from each plant, and used to interpolate values of transpiration, conductance and water use efficiency (WUE) at 18°C, T_{opt} , 28°C and at A_{max} .

Analysis

In the analyses we wished to focus on the effects of leaf shape, growth water and growth temperature treatments while analytically allowing or incorporating the differences between PICs or plants within PICs. Because our focus was on shape contrasts and not the particular species, or pairs, we treated species and PICs as random effects in all analyses. Plants were grown in two glasshouse compartments, one for each growth temperature; plants were blocked by PIC in matching positions in the two greenhouse compartments. Although the temperature treatments were not replicated in separate glasshouses, it was assumed for analyses that the individual plants were true replicates.

To assess whether leaf shape, growth water treatment and growth temperature treatment affected SLA, photosynthetic and water use traits, linear models were fitted to the key variables for each plant using GenStat (version 6; Numerical Algorithms Group, Oxford). Leaf shape, water treatment and temperature treatment were included as fixed effects, and PIC and replicate-nested-within-PIC as random effects. The full model was fitted first and residuals checked. One *Pelargonium reniforme* plant showed little temperature response and was excluded from analysis of T_{opt} , 90% T_{range} and WUE_{growth} , where its inclusion exerted high leverage and affected the fit of the model. One *Pelargonium crithmifolium* plant, a more-dissected species, had very high photosynthetic rates and was excluded for

the sake of conservatism from A_{max} analysis. In two cases, transpiration values suggested thermocouple problems and WUE at growth temperature could not be calculated; these were missing values in that analysis. Full models were reduced by dropping non-significant terms starting with highest-order terms. Non-significant main effects were not dropped if they were significant in interaction terms.

We examined pairwise simple correlations among rainfall, latitude, gas exchange traits and an average dissection index [perimeter/square root(area), cf. McLellan and Endler 1998] for each species. For species whose seed was produced in cultivation, average rainfall and latitude from their native ranges were used. Dissection index was averaged across treatments for a given species using leaves ($n \sim 20$ per species) from the larger glasshouse experiment. Pairwise correlations were judged significant if $r > 0.38$, where $P < 0.001$. Initial assessments included temperature variables as well, but there was little variance among sites in minimum, maximum and mean July temperatures (peak growing season for these winter growers), so these were not included in the final analysis.

Results

Temperature shifts

When considered across the range of measurement temperatures, the photosynthetic temperature response showed the expected pattern, increasing from low temperatures, flattening, and decreasing once leaf temperature exceeded $\sim 33^\circ\text{C}$. As predicted, plants grown at 28/18°C reached their maximum rate of photosynthesis at higher temperatures, approximately 2°C higher than plants grown at 18/8°C (Fig. 1a, Table 2). The T_{opt} were in the range of 33–36°C; however, these were higher than growth temperatures for both temperature treatments. These patterns held

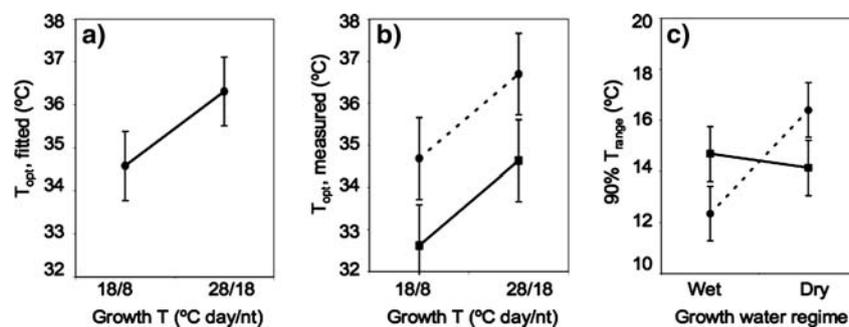


Fig. 1a–c Effects of growth treatments and leaf shape on leaf thermal traits. *Dashed lines* represent more-dissected species and *solid lines* the less-dissected species. **a** Optimal temperature (T_{opt}) for

photosynthesis fitted from curve, **b** T_{opt} from nearest direct measurement; and **c** 90% T_{range} . Values are adjusted means (\pm SE) for significant treatment effects only (see Table 2). *nt* Night

Table 2 Results of linear mixed models. Wald statistic and *P*-value presented for all terms included in models; *df* for all effects is 1. *Empty cells* represent terms dropped from models. *n* Number of units in the analysis, *WUE* water use efficiency, *SLA* specific leaf area

	<i>n</i>	Leaf shape		Water treatment		Temperature treatment		Leaf shape × Water		Leaf shape × Temperature		Leaf shape × Water × Temperature	
		Wald	<i>P</i>	Wald	<i>P</i>	Wald	<i>P</i>	Wald	<i>P</i>	Wald	<i>P</i>	Wald	<i>P</i>
Temperature ^a (<i>T</i>)													
<i>T</i> _{opt} , fitted	70					4.29	0.038						
<i>T</i> _{opt} , measured	71	3.03	0.034			4.51	0.035						
<i>T</i> _{range}	70	0.52	0.473	2.39	0.122			9.12	0.003				
Photosynthesis ^a (<i>A</i>)													
<i>A</i> ₁₈	71	17.65	0.001	4.44	0.035	11.32	0.001						
<i>A</i> ₂₈	71	30.24	0.001			7.82	0.005						
<i>A</i> _{growth}	71	17.71	0.001	4.13	0.042								
<i>A</i> _{max} , fitted	70	36.97	0.001	6.37	0.066	7.10	0.008				5.42	0.020	
<i>A</i> _{max} , measured	70	38.29	0.005	3.85	0.050	7.90	0.005				6.30	0.012	
Water use ^a													
Conductance ₁₈	71	6.45	0.011	9.49	0.002			3.90	0.048				
Conductance ₂₈	71	9.80	0.002	6.71	0.010								
Conductance _{growth}	71	9.20	0.002	8.77	0.002			5.06	0.024				
Conductance _{MP}	71	2.09	0.149	2.01	0.157			3.87	0.050				
Transpiration ₁₈	71	6.15	0.013	4.89	0.027								
Transpiration ₂₈	71	12.39	0.001										
Transpiration _{growth}	71	7.34	0.007			41.27	0.001						
Transpiration _{MP}	71	9.36	0.002										
WUE ₁₈	71												
WUE ₂₈	71					5.03	0.025						
WUE _{growth}	69					36.07	0.001						
WUE _{MP}	70					8.42	0.004						
SLA	257	8.18	0.004	12.43	0.001	14.27	0.001			6.23	0.013		

^a *opt* Optimum, *18* 18°C, *28* 28°C, *growth* growth temperature, *max* maximum, *MP* temperature at which *A*_{max} was measured

for both fitted values from the thermal response curves and measured temperatures for maximal rates of photosynthesis. When measured as opposed to fitted values were considered, species with more-dissected leaves had optimal temperatures 2°C higher than those with less-dissected leaves (Fig. 1b, Table 2).

The breadth of the temperature range at which plants are able to maintain ≥90% of maximum photosynthetic rate (90% *T*_{range}) may be indicative of the breadth of temperatures over which the plant can maintain optimal rates of photosynthesis. We found that the 90% *T*_{range} was unaffected by growth temperature, but there were leaf shape effects that were dependent on water conditions. Compared to less-dissected species, those with more-dissected leaves had larger 90% *T*_{range} when grown at high water, and narrower ranges when grown at low water (Fig. 1c; Table 2). Species with less-dissected leaves showed no effect of growth water treatment on 90% *T*_{range}. This same pattern emerged when ranges on either side of optimal were considered independently (results not shown).

Photosynthetic rates

In general, plants grown under wetter conditions, at cool temperatures and with more-dissected leaves had higher rates of photosynthesis than their dry, hot or less-dissected counterparts (Table 2). This pattern held for measurement temperatures of 18 or 28°C, regardless of growth temperature (Fig. 2a, b). When photosynthetic rate was compared at growth temperature, there was no main effect of growth temperature because plants grown at 28°C achieved similar rates of photosynthesis at 28°C as 18°C-grown plants at 18°C (Table 2, cf. Fig. 2a, b). However, even when compared at growth temperatures, plants with more-dissected leaves and those grown under wet conditions had significantly higher rates of photosynthesis than less-dissected or dry-grown plants (Fig. 2c).

For maximum photosynthetic rate there was a significant three-way (shape by temperature by water treatment) interaction (Table 2, Fig. 2d). More-dissected leafed plants had higher maximal rates, and plants grown at cool

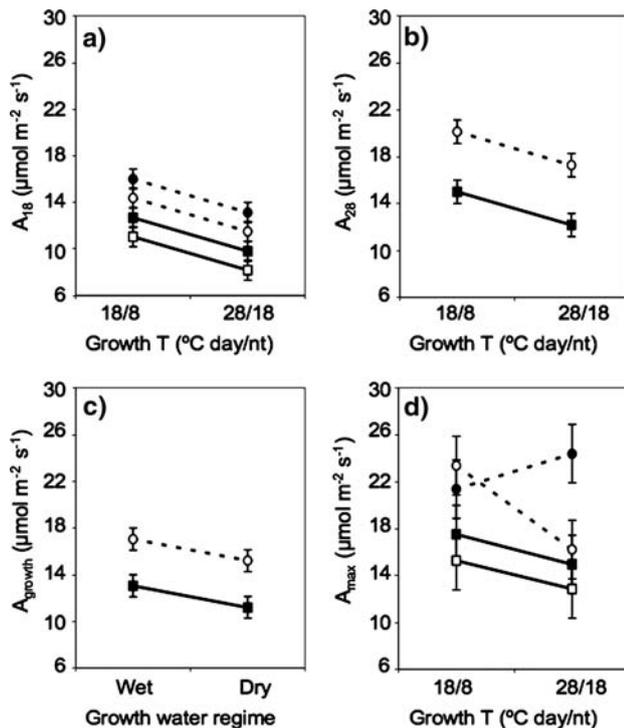


Fig. 2 Effects of growth treatments and leaf shape on photosynthetic rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$) at **a** 18°C, **b** at 28°C, **c** compared at growth temperature, and **d** compared at modelled maximum rate of photosynthesis (A_{max}). **a–c** Dashed lines represent more-dissected species and solid lines the less-dissected species. **a, d** Dashed lines are as above, and open and closed symbols represent low and high water, respectively. Values are adjusted means (\pm SE) for significant treatment effects only (see Table 2). For an explanation of subscripts, see Table 2

temperatures had higher maximal rates in all cases except for more-dissected leafed plants grown under wet conditions, where hot-grown plants had significantly higher maximal rates. Plants grown at low water had lower rates of photosynthesis than plants grown at high water, although dissected-leafed species grown at cool temperatures did not significantly differ in rate between water treatments. These results for maximum photosynthetic rates were identical whether measured or fitted values were analysed.

Water use

Stomatal conductance to water was affected by growth water level and leaf shape in a similar manner to photosynthetic rate: plants with more-dissected leaves and those grown at high water had higher conductance than their counterparts (Table 2). At 18°C and at growth temperature, a significant water by shape interaction resulted; the water effect on conductance was only significant among the more-dissected leafed plants (Fig. 3a, c). At 28°C, more-dissected leafed species had higher conductance than those

with less-dissected leaves, and wet-grown plants had higher conductance than dry-grown ones; there were no significant interactions (Fig. 3b). In contrast, when compared at the temperature at which maximum photosynthetic rate was reached, conductance was lower and plants with more-dissected leaves had relatively higher conductance than less-dissected leafed species, but only under dry conditions (Fig. 3d). There were no significant direct effects or interaction effects of growth temperature on conductance.

Transpiration, like conductance, was higher in more-dissected leaf species than less-dissected leaves (Table 2, Fig. 3e). Wet-grown plants had significantly higher transpiration rates than dry-grown plants but only when compared at 18°C (Table 2). Transpiration also increased with leaf temperature such that, when compared at growth temperature, hot-grown plants had higher transpiration rates than cool-grown plants (Table 2). Given that plants grown at 18/28°C day/night had higher rates of photosynthesis, and lower water use, it follows that they also had higher instantaneous WUE (Table 2, Fig. 3f).

Specific leaf area

SLA was significantly higher at low water than high (Table 2, Fig. 4a). Leaf shape and growth temperature had interactive effects on SLA, with more-dissected leaves having significantly lower SLA than less-dissected leaves when grown at cool temperatures. This difference was not apparent at high temperatures, nor was there an effect of temperature on SLA in the less-dissected leaves (Fig. 4b).

Other explanatory variables

Analysis of correlations among gas exchange traits revealed expected patterns (Fig. 5). As was the case using the two-state descriptor of leaf shape (less- versus more-dissected), the correlation analysis also supported the result that species with higher degrees of dissection had higher photosynthetic rates and transpiration rates at maximum photosynthetic rate ($r = 0.52$ and 0.38 , respectively, $n = 71$, both $P < 0.001$). Rainfall was significantly lower at more northern latitudes ($r = 0.66$, $n = 70$, $P < 0.001$) and plants from further north had significantly higher maximal photosynthetic rates and conductance at maximum photosynthesis ($r = -0.40$ and -0.50 , respectively, $n = 71$, $P < 0.001$). However, neither T_{opt} nor leaf dissection index was correlated with rainfall or latitude so the relationship between these traits and carbon gain are likely to hold within climatic regions as well as among them.

Fig. 3a–d Effects of growth treatments and leaf shape on water use traits. **a** Stomatal conductance ($\text{mol m}^{-2} \text{s}^{-1}$) at 18°C , **b** at 28°C , **c** at growth temperatures, and **d** when compared at A_{max} . **e** Transpiration ($\text{mol m}^{-2} \text{s}^{-1}$) rate and **f** water use efficiency (WUE) are compared at growth temperature. **a–e** Dashed lines represent more-dissected species and solid lines the less-dissected species. Values are adjusted means ($\pm\text{SE}$) for significant treatment effects only (see Table 2). For an explanation of subscripts, see Table 2; for abbreviations, see Figs. 1 and 2

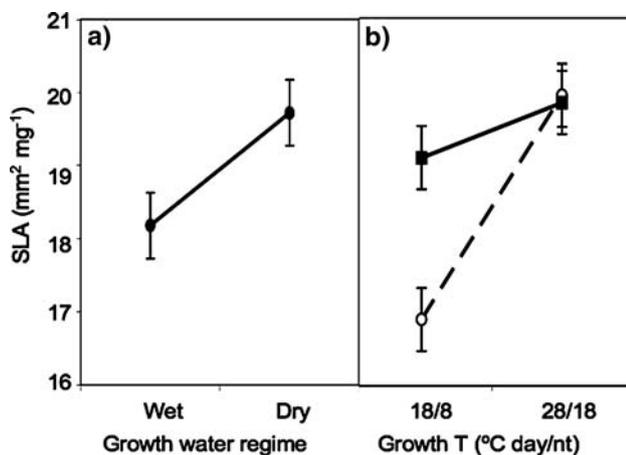
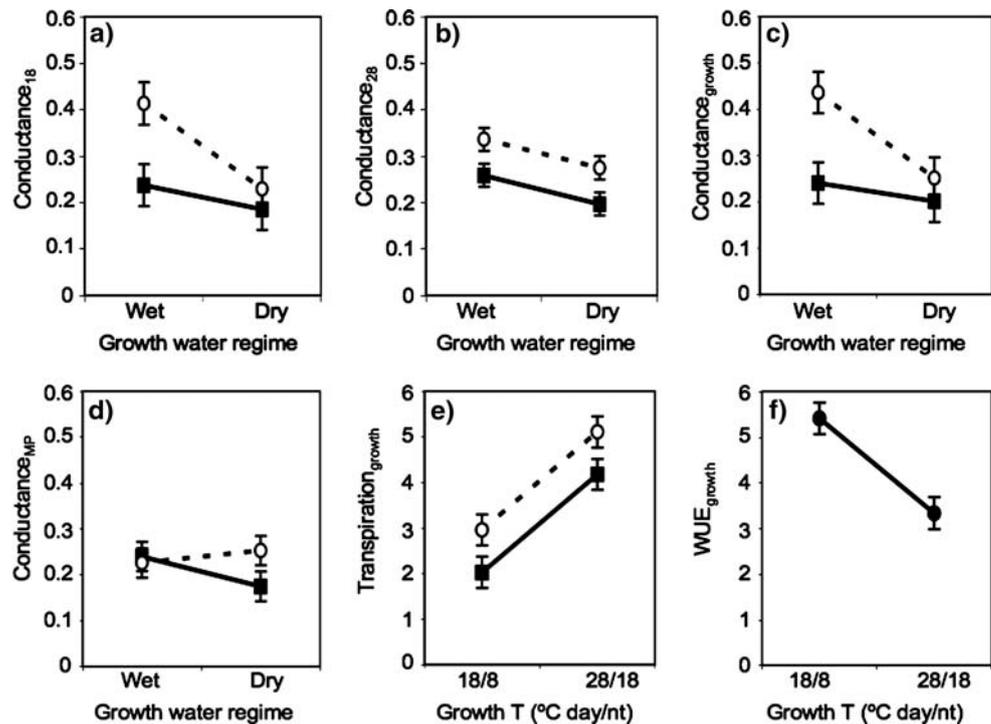


Fig. 4 Effect of **a** growth water regime and **b** growth temperature on specific leaf area (SLA; $\text{mm}^2 \text{mg}^{-1}$). **b** Dashed lines represent more-dissected species and solid lines the less-dissected species. Values are adjusted means ($\pm\text{SE}$) for significant treatment effects only (see Table 2)

Discussion

As predicted, *Pelargonium* species showed plasticity in photosynthetic capacity in response to differences in both water and temperature regimes. Plants grown at $28/18^\circ\text{C}$ achieved their photosynthetic optima at higher temperatures, but attained lower photosynthetic rates than plants grown at $18/8^\circ\text{C}$. Wet-grown plants also had higher photosynthetic rates, but water level had no effect on the optimum temperature for photosynthesis. Such a tradeoff

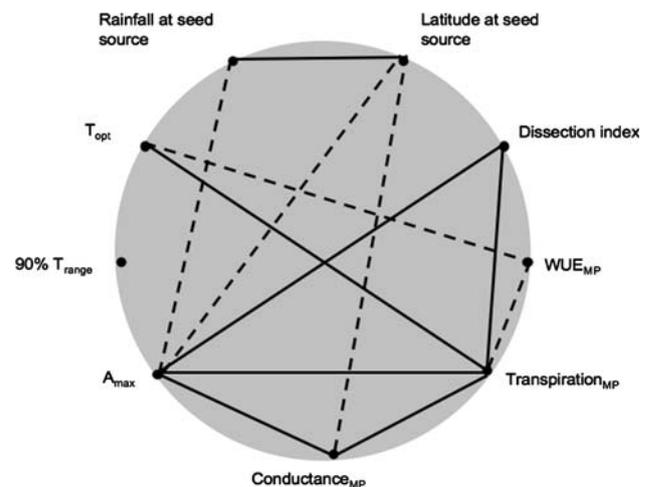


Fig. 5 Significant correlations ($r > 0.38$, $P < 0.001$, $df = 71$), between rainfall and latitude at the source seed site, gas exchange traits and dissection index as a species average across treatments. Positive correlations are presented as solid lines, negative correlations as dashed lines. For an explanation of subscripts, see Table 2; for abbreviations, see Figs. 1, 2 and 4

between increased thermal optima and decreased photosynthetic capacity is well known (Berry and Björkman 1980).

In some species, growth at high temperature results in a sharply reduced 90% T_{range} (Turnbull et al. 2002). In our study, thermal optima were quite high across treatments, and the temperature ranges at which 90% or more of photosynthetic maxima were maintained were broad. The

breadth of this range may be indicative of species or ecotypes from more variable environments (Battaglia et al. 1996; Cunningham and Read 2002), but among *Pelargonium* species there seems to be little differentiation. While rainfall varies both between years and among the habitats of these species, temperature regimes during the growing seasons are very similar across the range. Rainfall variation may explain the breadth of the 90% T_{range} and the similarity of temperature regimes may explain the concordance of the 90% T_{range} of the *Pelargonium* species studied here.

Although previous studies have demonstrated growth temperature effects on stomatal function (Ogle and Reynolds 2002, and references therein), our results showed no evidence of a growth temperature effect on conductance or transpiration at 18 or 28°C or at maximum photosynthetic rate. Even though we did not find direct effects of growth temperature on conductance, transpiration is inevitably higher at higher temperatures, and when coupled with the decline in photosynthetic capacity in plants grown at high temperatures results in a significant reduction in WUE at the higher growth temperature.

Stomatal closure is generally the first response to drought and is the dominant limitation to photosynthesis at mild and moderate drought (Flexas and Medrano 2002). We note that stomatal conductance in the plants in the present experiment was seldom lower than $0.100 \text{ mol m}^{-2} \text{ s}^{-1}$, suggesting that while we elicited growth water effects, we did not dramatically water stress these arid-adapted species. Neither did we find clear indications of greater thermotolerance in the low water plants (Havaux 1992; Valladares et al. 1997; Ladjal et al. 2000). Some species of *Pelargonium* are capable of low level CAM cycling under extreme water stress (Jones et al. 2003). As such, we are curious to assess how *Pelargoniums* in general behave under more severe water stress, and whether those capable of CAM cycling differ in thermotolerance and stomatal responses.

Our most intriguing results were the significant effects of leaf shape: species with more-dissected leaves had higher thermal optima and greater rates of carbon gain and water loss. A more dissected leaf shape will result in thinner boundary layer and more effective convective heat exchange with the environment. So under natural conditions, more-dissected leaves will track ambient conditions more closely and potentially show smaller leaf to air temperature differences than a less divided leaf (Nobel 1983; and see references in Schuepp 1993). We did not measure leaf temperatures directly, and so cannot say whether the more-dissected species operated on average at lower temperatures than the less-dissected species. Recent work demonstrates that effects of leaf shape on canopy temperature may be less than effects of overall canopy architecture and density (Leuzinger and Körner 2007), so it

is hard to predict what the actual photosynthetic operating temperatures of these *Pelargonium* species would be. However, if the more-dissected species did operate at lower leaf temperatures on average as predicted by boundary layer theory, it is all the more striking that they have higher thermal optima at a given growth temperature than their less-dissected counterparts.

Our results are one of only a few demonstrations of physiological divergence between closely related taxa differing in leaf shape. Previous studies have all focused on variation within a species, and have not demonstrated consistent patterns. Gurevitch (1988, 1992) reported that ecotypes of *Achillea millefolium* that differed genetically in leaf dissection also differed in assimilation rates; the less-dissected form had higher rates. In contrast, a dissected leaf form of *Ranunculus repens* was found to have higher rates of both photosynthesis and conductance than the typical broad-leaf form (Lynn and Waldren 2002). And finally, Stiller et al. (2004 and references therein) found that okra-leaf cultivars (a dissected leaf) of cotton (*Gossypium hirsutum*) had higher photosynthetic rates and also higher WUE.

The correlations we demonstrate here, between leaf dissection and high rates of carbon gain and water loss across species, could reflect direct causal links between shape and carbon gain, or instead represent the outcome of correlated evolutionary responses to selective conditions. As such, one argument is that the higher rates of photosynthesis we observed are a result of boundary layer differences between the leaf types. We reject this explanation as a primary cause of the difference in photosynthetic rates because all of our measurements were made in a closed IRGA chamber with a fast chamber fan and moderately high rates of air-flow through the IRGA. These conditions would have effectively removed the boundary around all leaves during the gas exchange measurements, regardless of shape.

We prefer the alternative argument, that selection favours a more dissected leaf shape in hot dry conditions, and that these same conditions are those that favour the evolution of high photosynthetic rates, high leaf nitrogen content and opportunistic use of water when available (Wright et al. 2001, 2004; Heschel and Riginos 2005; Donovan et al. 2007). Analysis of SLA supports this interpretation; the more-dissected leaves also had lower SLA than less-dissected leaves (when grown at 18/8°C). The lower SLA would most likely result from veins making up a relatively greater proportion of the mass of more-than less-dissected leaves, and may lead to equivalent photosynthetic rates on a mass basis. Further investigations may more effectively reveal functional correlates of leaf shape, where other factors can be intentionally held constant.

Global climate change, and in particular increased temperatures, especially in a context of water stress, will lead to marked decreases in productivity (Sharkey 2005). South African species like the *Pelargoniums* studied here may be particularly susceptible to shifts in environmental parameters because the group exhibits high levels of endemism and often very narrow geographic ranges (Midgley et al. 2002; Midgley et al. 2003; Hannah et al. 2005, Thuiller et al 2006). Our work suggests that in predicting responses to increases in temperature and drought, links between leaf shape, morphology and thermal tolerance, in response to both long- and short-term changes in conditions, should be considered (Sharkey 2005). To understand those responses, one would have to understand heating and cooling dynamics of these leaves and how those dynamics scale to the canopy level, in partnership with dynamic measurements of photosynthetic and transpiration responses to temperature changes.

Given that natural growth temperatures of these *Pelargonium* species are generally less than 30°C (Table 1), it seems likely that direct effects of leaf temperature on photosynthetic machinery will be small (Martin et al. 1999; Ward et al. 2001). At least from the data on this collection of eight species, indications are that leaf temperature effects may be more important as determinants of water loss, than as determinants of photosynthetic rates, therefore indirectly affecting productivity, rather than directly inhibiting photosynthetic processes themselves. Stomatal responses to temperature are likely to be critical mediators of the effect of temperature on realized carbon gain. In the *Pelargoniums*, the combination of dissected leaves, higher photosynthetic rates, and relatively low WUE may have evolved as a strategy to optimise water delivery and carbon gain during short periods of high soil moisture. The correlation between higher temperature and leaf dissection may reflect selection pressure to protect photosynthetic machinery against excessive leaf temperatures when stomata close in response to water stress.

Acknowledgements We thank Christine Donnelly for help with data analysis, Susanne von Caemmerer for insightful discussion of the results, and Rachel Saunders (<http://www.silverhillseeds.co.za>) for seed collection. Two anonymous reviewers provided constructive comments on a previous version of the manuscript. This work was supported by an Australian Research Council grant to A. B. N., C. S. J. and C. D. S.

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