

RESEARCH PAPER

Smaller, faster stomata: scaling of stomatal size, rate of response, and stomatal conductance

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Abstract

Maximum and minimum stomatal conductance, as well as stomatal size and rate of response, are known to vary widely across plant species, but the functional relationship between these static and dynamic stomatal properties is unknown. The objective of this study was to test three hypotheses: (i) operating stomatal conductance under standard conditions (g_{op}) correlates with minimum stomatal conductance prior to morning light [$g_{min(dawn)}$]; (ii) stomatal size (S) is negatively correlated with g_{op} and the maximum rate of stomatal opening in response to light, $(dg/dt)_{max}$; and (iii) g_{op} correlates negatively with instantaneous water-use efficiency (WUE) despite positive correlations with maximum rate of carboxylation ($V_{c,max}$) and light-saturated rate of electron transport (J_{max}). Using five closely related species of the genus *Banksia*, the above variables were measured, and it was found that all three hypotheses were supported by the results. Overall, this indicates that leaves built for higher rates of gas exchange have smaller stomata and faster dynamic characteristics. With the aid of a stomatal control model, it is demonstrated that higher g_{op} can potentially expose plants to larger tissue water potential gradients, and that faster stomatal response times can help offset this risk.

Key words: Maximum stomatal conductance, night-time conductance, stomatal control, stomatal size, transpiration, water-use efficiency.

Introduction

Plants regulate stomatal conductance to optimize carbon uptake with respect to water loss (Cowan, 1977; Farquhar *et al.*, 1980a). An important limitation in this process is the rate at which stomata open in the light or close under darkness or water deficit (Cowan, 1977; Hetherington and Woodward, 2003; Franks and Farquhar, 2007; Brodribb *et al.*, 2009; Lawson *et al.*, 2011; Vico *et al.*, 2011). However, although stomatal response times are known to vary widely across species (Assmann and Grantz, 1990; Franks and Farquhar, 2007; Vico *et al.*, 2011), the biophysical factors governing the rate of response are not well understood.

Plant photosynthetic productivity and water-use efficiency (WUE) are also linked to the dynamic range of stomatal conductance. Under favourable conditions of low evaporative demand and high light, the upper limit of the CO₂ assimilation rate is determined by the maximum operating stomatal conductance, g_{op} (assuming the biochemical limitations to CO₂ assimilation rate are fixed). Under severe water deficits resulting from high evaporative demand and/or dry soil, plants rely upon full stomatal closure and a highly water-impermeable leaf cuticle to minimize water loss (Hinckley *et al.*, 1980; McDowell *et al.*, 2008). Across plant taxa there

is a wide range of operating and minimum stomatal conductances (Jones, 1992; Schulze *et al.*, 1994; Körner, 1995). However, it is not known if maximum and minimum stomatal conductance typically scale with one another.

Commonly defined as the minimum stomatal conductance in darkness, g_{\min} for a given leaf may differ on account of the time of day or other physiological circumstances. For example, stomata typically close in response to darkness and remain closed for much of the night, but often the closure is not complete. In fact the night-time or ‘nocturnal’ conductance can be sufficient to allow significant transpiration (Ehrler, 1971; Benyon, 1999; Snyder *et al.*, 2003; Barbour *et al.*, 2005; Bucci *et al.*, 2005; Daley and Phillips, 2006; Dawson *et al.*, 2007), and growth conditions may produce stomata that cannot close completely even when fully deflated at zero turgor (Franks and Farquhar, 2007). Night-time transpiration rates are typically between 5% and 15% of daytime transpiration, but in rare cases can be >30% (Caird *et al.*, 2007; Novick *et al.*, 2009). Such high rates of water loss at times of little or no carbon gain are inconsistent with the general role of stomata as a water-conserving apparatus, but little is known about the mechanism of nocturnally elevated stomatal conductance or its relationship to the minimum conductance in darkness at other times of the day and under desiccation. There is some evidence that nocturnal transpiration assists with nutrient uptake and sustains carbohydrate export, particularly in fast growing trees (Marks and Lechowicz, 2007). Here three different conductance minima are distinguished according to the circumstances in which they are promoted: (i) $g_{\min(\text{dawn})}$, referring to the minimum stomatal conductance to water vapour at the end of the nocturnal dark phase; (ii) $g_{\min(\text{day})}$, referring to the minimum stomatal conductance to water vapour attained when the leaf is exposed to a period of darkness during normal daylight hours; and (iii) the absolute minimum conductance to water vapour, $g_{\min(\text{abs})}$, occurring when the guard cells are fully deflated as a result of complete turgor loss (Fig. 1). The quantities g_{op} , $g_{\min(\text{dawn})}$, $g_{\min(\text{day})}$, and $g_{\min(\text{abs})}$ all comprise a stomatal component in parallel with a cuticular component, although $g_{\min(\text{abs})}$ may closely approximate cuticular conductance. Common empirical stomatal models do not adequately account for elevated minimum conductance at night or its environmental sensitivities (Barbour and Buckley, 2007), but few studies have measured all of these conductances together so the relationship between them is obscure.

The operating stomatal conductance, g_{op} , is also known to scale with other leaf gas exchange and water transport attributes, such as CO_2 assimilation rate and leaf hydraulic conductance (Meinzer, 2003; Brodribb *et al.*, 2007). However, non-linearities in some of these relationships result in trade-offs. For example, increased CO_2 assimilation rate accompanying higher g_{op} may be associated with lower WUE (Franks and Farquhar, 1999) and higher leaf water potential gradients (Franks, 2006). Improved stomatal dynamic properties with increased g_{op} could potentially help to offset these counterproductive properties.

The operating conductance g_{op} is constrained by the maximum stomatal conductance, g_{max} , which in turn is determined

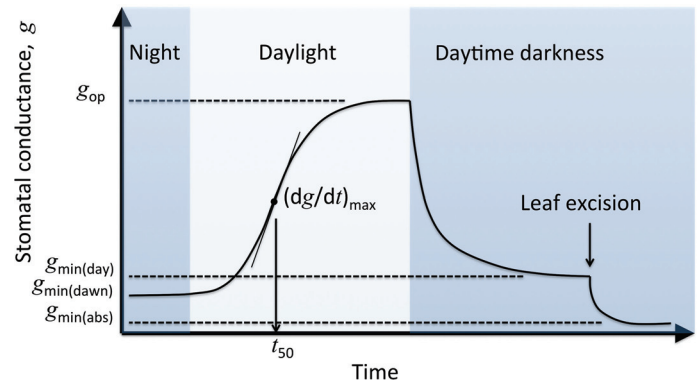


Fig. 1. The different phases of stomatal conductance examined in this study: g_{\min} , steady-state stomatal conductance in darkness, either at dawn [$g_{\min(\text{dawn})}$] or after suddenly induced darkness [$g_{\min(\text{day})}$]; $(dg/dt)_{\max}$, the maximum rate of change of g during light-induced stomatal opening; g_{op} , steady-state operating stomatal conductance under standardized ideal conditions (see the Materials and methods); t_{50} , time taken to reach 50% of the range between g_{\min} and g_{op} ; $g_{\min(\text{abs})}$, the absolute minimum steady-state stomatal conductance after leaf excision, assumed to result from zero turgor in stomatal guard cells.

by two physical attributes of stomata: (i) their size (S) and (ii) their density (D), or number per unit area. A distinction is made between g_{max} and g_{op} because g_{max} relates to stomata opened to their widest possible apertures (e.g. under 100% relative humidity and low ambient CO_2 concentration), whereas under typical operating conditions (<100% relative humidity and normal ambient CO_2 concentration) stomatal apertures will be less than fully open. It has been shown that across broad geological time scales and evolutionary lineages, higher g_{max} and g_{op} are associated with smaller stomatal size and higher density, and that S is negatively correlated with D (Hetherington and Woodward, 2003; Franks and Beerling, 2009). This relationship has also been found to apply within a single species across environmental gradients (Franks *et al.*, 2009), and also across a group of six tree species of different genera (Aasama *et al.*, 2001). Smaller stomata, due to their greater membrane surface area to volume ratio, may have faster response times compared with larger stomata, and this in combination with high stomatal density may allow the leaf to attain high g_{op} rapidly under favourable conditions, and to reduce conductance rapidly when conditions are unfavourable. In such a system, the rate of stomatal response would be positively correlated with g_{op} and negatively correlated with stomatal size. However, to date, these functional relationships have not been confirmed.

Within plant functional groups there is a strong positive correlation between g_{op} , the operating rate of photosynthesis, A , and photosynthetic capacity (maximum rate of carboxylation, $V_{c_{\text{max}}}$, and light-saturated rate of electron transport, J_{max}) (Wullschleger, 1993; Kattge *et al.*, 2009; Cernusak, 2011). For constant atmospheric CO_2 concentration and leaf evaporative potential [leaf-to-air water vapour pressure difference (VPD)], WUE (the ratio of the rate of CO_2

assimilation to transpiration) is proportional to the relative draw-down in CO_2 concentration from the atmosphere to the leaf interior ($1 - c_i/c_a$), where c_i/c_a is the ratio of atmospheric to leaf internal CO_2 concentration. If the correlation between A and g_{op} is linear across species, then this would imply that under constant environmental conditions, WUE is relatively constant. However, there is evidence to suggest that the correlation is non-linear, with g_{op} increasing proportionally more than A across species under constant environmental conditions (Franks and Farquhar, 1999; Franks, 2006). This non-linearity implies that g_{op} is negatively correlated with instantaneous WUE across species, despite increasing photosynthetic capacity.

The objective of this study was to test three hypotheses: (i) operating stomatal conductance under standard conditions (g_{op}) correlates with minimum stomatal conductance prior to morning light [$g_{\text{min(dawn)}}$]; (ii) stomatal size (S) is negatively correlated with g_{op} and the maximum rate of stomatal opening in response to light $(dg/dt)_{\text{max}}$; and (iii) g_{op} correlates negatively with instantaneous WUE despite positive correlations with $V_{c_{\text{max}}}$ and J_{max} . To test these hypotheses, the above traits were measured in a closely related group of *Banksia* species that are distributed across a broad hydrological environment from wetlands to dune crests (Fig. 2) (Groom, 2002,

2004). Restricting the study to a single genus ensured minimal genetic variability while offering a broad range of g_{op} , stomatal size, and stomatal density traits for analysis. The results are assessed in terms of their implications for plant water balance and fitness under the differing hydrological habitats of the study species.

Materials and methods

Plant material

Five *Banksia* species, endemic to the *Banksia* woodland of south-western Australia ($31^{\circ}45'S$, $115^{\circ}57'E$), were selected for study. The species were as follow: *Banksia attenuata* R.Br., *Banksia menziesii* R.Br., *Banksia ilicifolia* R.Br., *Banksia prionotes* Lindl., and *Banksia littoralis* R.Br. Figure 2, based on the natural geographical range of south-west Australian banksias, is an idealized representation of the distribution of the species across five distinct habitats as defined by the depth of groundwater from the natural surface (Table 1).

Four plants from each species were grown from seed in a glasshouse in 10 litre pots. Plants were allowed to develop in 70:30 coarse sand:humus and fertilized with 33.38 ± 0.24 g (mean \pm SE) of slow release fertilizer (Osmocote™). All plants were well watered throughout development and maintained under a day/night temperature regime of 24/15 °C. When leaves had fully matured under these conditions, each plant was periodically transferred to a laboratory (air temperature range = 23 ± 3 °C), rewatered, and allowed

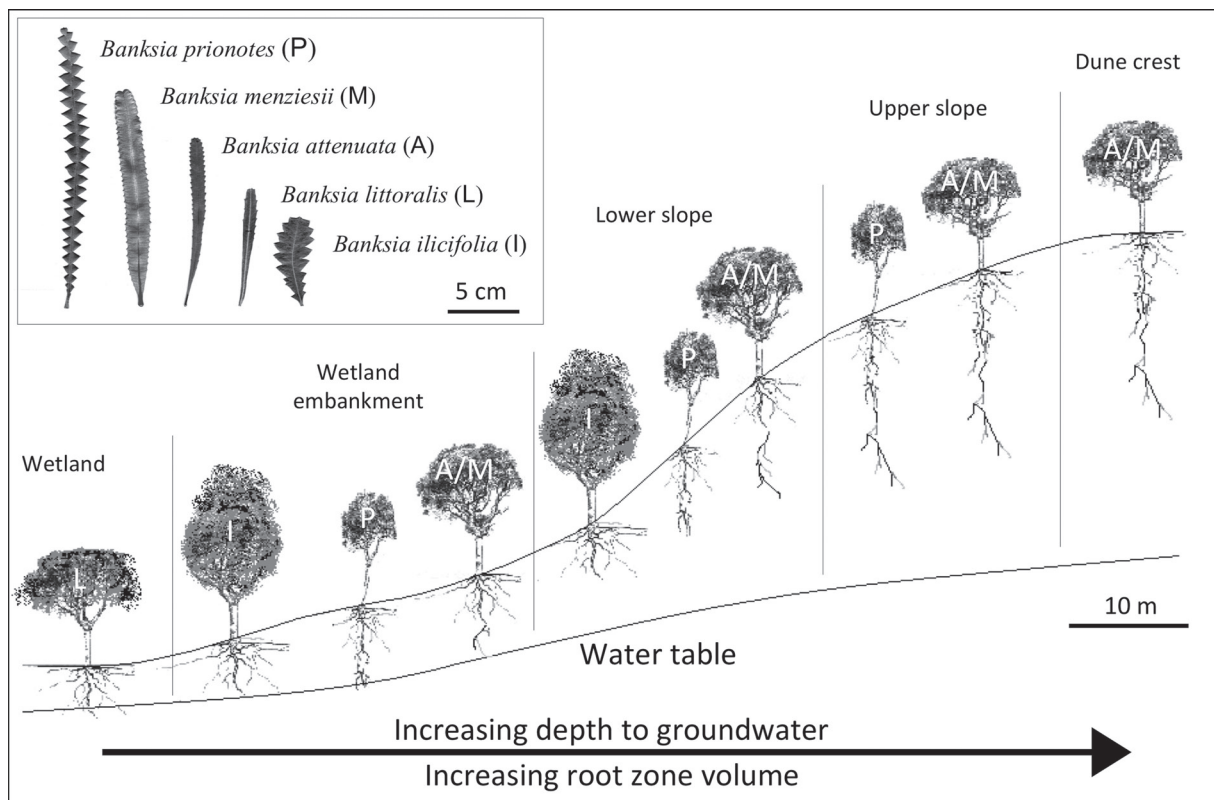


Fig. 2. Idealized distribution of *Banksia* species on the Gnangara Groundwater Mound with respect to depth to groundwater (see Table 1) and unsaturated soil volume. *Banksia littoralis* occurs only in association with watercourses and wetland habitats, and is excluded from dune crests occupied by *Banksia attenuata* and *Banksia menziesii*. Accordingly, *B. littoralis* has a highly restricted geographical distribution, while *B. attenuata* and *B. menziesii* have a more extensive geographical distribution encompassing several hydrological habitats. Adapted from Lam et al. (2004) with kind permission of R.H. Froend. Inset: illustrating the range of leaf size and shape across the study species.

Table 1. Approximate range in groundwater depth of the study species.

Species	Approximate range of groundwater depth (m)	Source
<i>Banksia attenuata</i>	3 to >30	Zencich <i>et al.</i> (2002); Lam <i>et al.</i> (2004)
<i>Banksia menziesii</i>	3 to >30	Lam <i>et al.</i> (2004)
<i>Banksia prionotes</i>	1.5 to 10	Dawson and Pate (1996); Pate <i>et al.</i> (1995)
<i>Banksia illicifolia</i>	<10	Groom <i>et al.</i> (2001); Zencich <i>et al.</i> (2002)
<i>Banksia littoralis</i>	<5	Groom <i>et al.</i> (2001)

to equilibrate overnight in preparation for the following day's gas exchange measurements.

Gas exchange

Leaf gas exchange properties were measured in the laboratory with an open-flow portable photosynthesis system (Model Li 6400, Li-Cor Inc., Lincoln, NE, USA) on one leaf per plant ($n=4$ plants per species). All experiments were initiated early in the morning (07:30–08:30 h local standard time) and were concluded within the natural daylight photoperiod. Plants were kept well watered throughout measurements. Measurements were made on fully expanded leaves (three or four leaves back from a branch apex). Throughout experiments, the ambient mole fraction of CO_2 (c_a) was maintained at $350 \mu\text{mol CO}_2 \text{ mol}^{-1}$ air [except for relationships between assimilation rate (A) and intercellular mole fraction of CO_2 (c_i)], leaf temperature was set at 20°C , and leaf-to-air water VPD regulated to 1 kPa .

In the morning, minimum steady-state stomatal conductance to water vapour prior to light exposure [$g_{\text{min(dawn)}}$, $\text{mol H}_2\text{O m}^{-2} \text{ leaf s}^{-1}$] was determined with the leaf in darkness. A stomatal opening phase, comprising the transition from $g_{\text{min(dawn)}}$ to a maximum steady-state or operating stomatal conductance to water vapour (g_{op} , $\text{mol H}_2\text{O m}^{-2} \text{ leaf s}^{-1}$), was then recorded by exposing leaves to a photosynthetically active radiation (PAR) of $1500 \mu\text{mol m}^{-2} \text{ s}^{-1}$ (while keeping the other chamber conditions constant) and logging instantaneous stomatal conductance (g) at 60 s intervals. This opening phase took ~ 120 min to reach a steady-state g_{op} for each species. It is noted that this opening phase following prolonged darkness is likely to be slower than that following only a brief period of darkness because of reduced photosynthetic induction (Percy *et al.*, 1997). After ensuring that all transient stomatal opening had ceased, the maximum steady-state CO_2 assimilation rate (A_{op} , $\mu\text{mol m}^{-2} \text{ leaf s}^{-1}$) and corresponding intercellular CO_2 mole fraction [$c_{i(\text{op})}$, $\mu\text{mol CO}_2 \text{ mol}^{-1}$ air] and steady-state transpiration rate (E_{op} , $\text{mmol H}_2\text{O m}^{-2} \text{ leaf s}^{-1}$) were also recorded. Also at this point, the relationship between instantaneous CO_2 assimilation rate A and leaf intercellular CO_2 concentration c_i was obtained (see below). PAR was then returned to zero, and the subsequent stomatal closing phase, to a minimum steady-state value, $g_{\text{min(day)}}$, was recorded by logging stomatal conductance at 60 s intervals. The time frame for stomatal closure varied across species, ranging from ~ 100 min to 250 min. The leaf was then excised from the plant and any further decline in stomatal conductance recorded, with the final minimum conductance for excised leaves measured as the absolute minimum, $g_{\text{min(abs)}}$.

The relationship between A and c_i was obtained for each plant by manipulating c_a over the range $50 \mu\text{mol CO}_2 \text{ mol}^{-1}$ air to $2000 \mu\text{mol CO}_2 \text{ mol}^{-1}$ air, beginning with the steady-state conditions at $350 \mu\text{mol CO}_2 \text{ mol}^{-1}$ air, then stepping c_a down to 300, 200, 100, and 50, and then up to 400, 600, 800, 1000, 1400, 1800, and $2000 \mu\text{mol CO}_2 \text{ mol}^{-1}$ air. The relationship was characterized according to the model proposed by Farquhar *et al.* (1980b) and subsequently modified by

van Caemmerer and Farquhar (1981), Sharkey (1985), and Harley *et al.* (1992). Undertaking this mechanistic analysis of the relationship between A and c_i yielded estimates for the maximum rate of carboxylation ($V_{c_{\text{max}}}$, $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ leaf s}^{-1}$) and the light-saturated rate of electron transport (J_{max} , $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ leaf s}^{-1}$).

Deriving the maximum rate of stomatal opening

Plots of instantaneous stomatal conductance (g) versus time elapsed since the start of measurements (t , s) obtained during the stomatal opening phase were described by Boltzmann sigmoidal models:

$$g = \frac{a_1 - a_2}{1 + e^{(t-t_0)/\Delta t}} + a_2 \quad (1)$$

where a_1 ($\text{mol m}^{-2} \text{ s}^{-1}$) is the left horizontal asymptote, a_2 ($\text{mol m}^{-2} \text{ s}^{-1}$) is the right horizontal asymptote, t_0 (s) is the point of inflection, and Δt (s) is the change in time that corresponds to the greatest change in g . Using an iterative least squares fit approach, values for a_1 , a_2 , t_0 , and Δt were determined for each plant. The instantaneous rates of stomatal opening (dg/dt , $\text{mol m}^{-2} \text{ s}^{-2}$) across the entire range of t were then calculated by taking the derivative of Equation 1:

$$\frac{dg}{dt} = \frac{e^{(t_0+t)/\Delta t} (a_2 - a_1)}{(e^{t_0/\Delta t} + e^{t/\Delta t})^2 \Delta t} \quad (2)$$

and the maximum rate of stomatal opening (dg/dt_{max} , $\text{mol m}^{-2} \text{ s}^{-2}$) determined for each plant as dg/dt when $t=t_0$.

This procedure was repeated after converting g to a relative value, g_{relative} :

$$g_{\text{relative}} = \frac{g - g_{\text{min(dawn)}}}{g_{\text{op}} - g_{\text{min(dawn)}}} \quad (3)$$

and the time taken to reach 50% of g_{relative} (t_{50} , minutes) determined.

Stomatal morphology

A tissue sample was obtained halfway from the leaf tip to the base from each leaf that was analysed for gas exchange properties and stored in 70% ethanol. For all species except *B. littoralis*, stomata were concentrated within crypts on the abaxial surface. Stomata of *B. littoralis* also only occurred on the abaxial surface, but no crypts were observed. The leaf epidermal surface of each species was also comprised of thickly intertwined trichomes. To obtain a clear view of stomata amidst these surface features, each sample was first rehydrated by rinsing under tap water then embedded in paraffin wax. Planar (through the epidermis) and transverse sections were then cut to $10 \mu\text{m}$ thickness with a rotary microtome (Leica model RM 2125, Leica Microsystems, Wetzlar, Germany). The sections were then positioned on slides that were dipped in 2% gelatin immediately prior to mounting. Slides were then placed in a coplin jar with filter paper soaked in formaldehyde to allow vapour fixation (of the section to gelatin). The coplin jar was covered with a lid and the sections allowed to dry at room temperature for 12 h. Sections were then stained in 0.1% aqueous toluidine blue, examined under a compound light microscope, and images captured with a digital camera.

Stomatal morphological parameters [guard cell length L (μm) and guard cell pair width W (μm)] were measured from images obtained from planar sections as the mean of 20 stomatal complexes (guard cell pairs) for each species. Stomatal size (S) is reported as the product of L and W (μm^2).

For each species, stomatal density [i.e. number of stomata per unit epidermal area (D , mm^{-2})] was calculated from transverse sections.

For each section, the number of stomata (n_s) intercepted by the microtome during cutting was counted along a known length of epidermis (l , μm , $n=12$ lengths per species). The length of epidermis ranged from ~ 450 μm to 4400 μm . Assuming each transect captured an area of epidermis of width (w_e) approximately equal to the average of the length and width of a stoma, the stomatal density was calculated as $D=n_s/(l \times w_e)$.

Results

The operating stomatal conductance g_{op} was positively correlated with $g_{\min(\text{dawn})}$ [Fig. 3A; $y=0.844-0.562e^{-(x-0.004)/0.024}$, $r^2=0.70$] and with $(dg/dt)_{\max}$ (Fig. 3B; $y=-0.09+3.40x$, $r^2=0.71$, $P < 0.001$). Across species, there was a 3-fold variation in $(dg/dt)_{\max}$, ranging from 0.07 $\text{mmol m}^{-2} \text{s}^{-2}$ to 0.25 $\text{mmol m}^{-2} \text{s}^{-2}$. $g_{\min(\text{dawn})}$ was also positively correlated with $(dg/dt)_{\max}$ [Fig. 3C; $y=5.08 \times 10^{-6}e^{(x/0.03)}+0.01$, $r^2=0.78$]. These results indicate that higher maximum and minimum stomatal conductance is linked to faster absolute rates of response of stomatal conductance to leaf irradiance.

Across species, $(dg/dt)_{\max}$ was negatively correlated with stomatal size (S) [Fig. 4A; $y=1187 \times e^{(-x/37)}+0.13$, $r^2=0.94$, $P < 0.01$] and positively correlated with stomatal density D (Fig. 4B; $y=0.00047x$, $r^2=0.71$, $P < 0.05$), indicating that leaves with smaller and more numerous stomata exhibit faster absolute rates of response of stomatal conductance to water vapour. The positive relationship between t_{50} and S (Fig. 4C; $y=16.63+0.05x$, $r^2=0.34$, $P < 0.05$) further indicates that smaller stomata exhibited a faster response in relative terms.

Stomatal opening in response to a step increase in light followed a similar pattern in all species, resembling the typical dynamic response of a second-order dynamic system with near-critical damping (Fig. 5A–E). For each species, the stomatal opening phase was accompanied by an increase in CO_2 assimilation rate (A) to a maximum steady-state value (A_{op}), although A_{op} was established prior to g_{op} (Fig. 5F–J).

Across species, mean g_{op} varied by ~ 2 -fold and mean $g_{\min(\text{dawn})}$ varied by 7-fold (Table 2). The mean absolute minimum stomatal conductance $g_{\min(\text{abs})}$ ranged from 6.0 $\text{mmol m}^{-2} \text{s}^{-1}$ to 20 $\text{mmol m}^{-2} \text{s}^{-1}$, which compares favourably with the range of minimum stomatal conductance reported for deciduous and evergreen plants using leaf drying curves (1.0 – 20 $\text{mmol m}^{-2} \text{s}^{-1}$) (Burghardt and Riederer, 2003).

Over the dynamic range of stomatal opening, CO_2 assimilation rate increased with stomatal conductance in the usual saturating fashion (Fig. 6A). Steady-state instantaneous WUE, defined as A_{op}/E_{op} at 1 kPa VPD (see the controlled, standardized environmental conditions in the Materials and methods) ranged from 2.5 mmol mol^{-1} to 6.5 mmol mol^{-1} , and all of the species reached a peak in WUE when A was ~ 5 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Fig. 6B). *Banksia attenuata* and *B. menziesii* had the highest WUE and *B. littoralis* had the lowest WUE (Fig. 6B). WUE was negatively correlated with g_{op} (Fig. 6C; $y=6.49-4.51x$; $r^2=0.52$, $P < 0.001$).

The maximum rate of carboxylation ($V_{c_{\max}}$) ranged from 23.90 $\mu\text{mol m}^{-2} \text{s}^{-1}$ to 47.11 $\mu\text{mol m}^{-2} \text{s}^{-1}$, and the light-saturated rate of electron transport (J_{\max}) ranged from 64.2 $\mu\text{mol m}^{-2} \text{s}^{-1}$ to 131 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The average value of $V_{c_{\max}}$ and

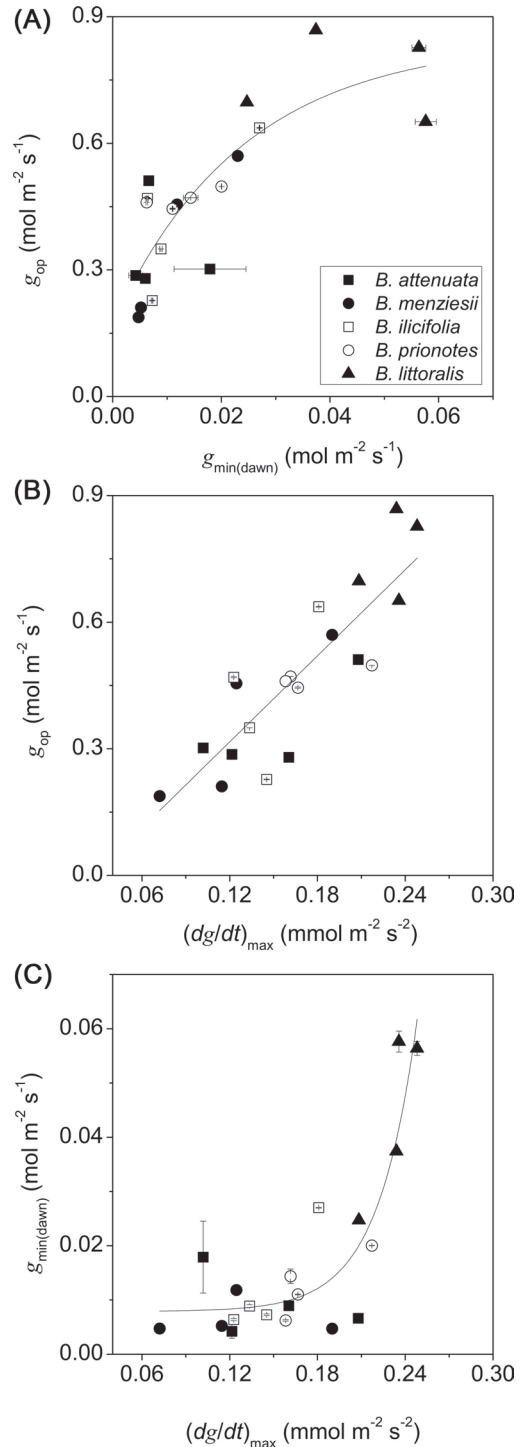


Fig. 3. Relationship between g_{op} , $g_{\min(\text{dawn})}$, and $(dg/dt)_{\max}$. (A) Across individuals, g_{op} was positively correlated with $g_{\min(\text{dawn})}$. Each point represents the mean \pm SE of $n=6$ consecutive steady-state records for an individual plant. The maximum rate of stomatal opening $(dg/dt)_{\max}$ was positively correlated with maximum steady-state stomatal conductance, g_{op} (B) and minimum stomatal conductance induced by darkness, $g_{\min(\text{day})}$ (C).

J_{\max} was 37.22 ± 1.47 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and 103.74 ± 4.24 $\mu\text{mol m}^{-2} \text{s}^{-1}$, respectively. This is lower than the average values reported by Wullschleger (1993) for sclerophyllous shrubs

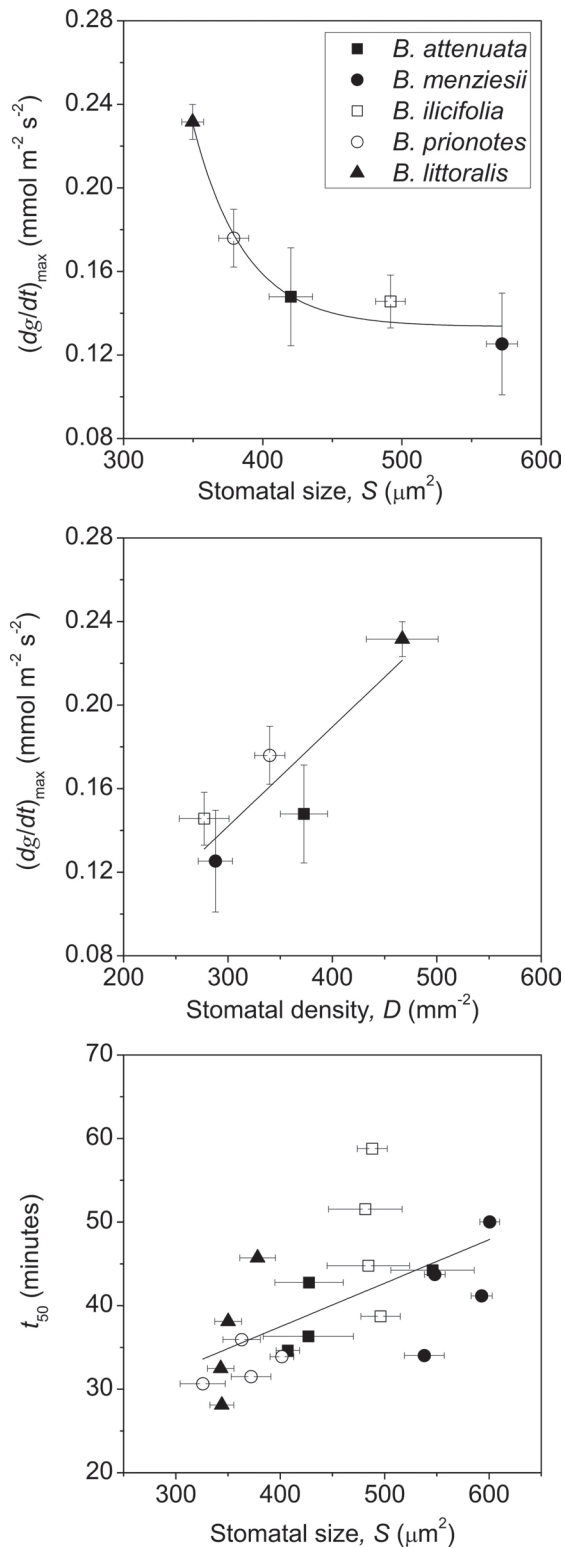


Fig. 4. Smaller, faster stomata. The maximum rate of stomatal opening $(dg/dt)_{max}$ was negatively correlated with maximum stomatal size, S (A) and positively correlated with stomatal density, D (B). The time to reach 50% of the range between $g_{min(dawn)}$ and g_{op} (t_{50}) was positively correlated with stomatal size (C).

($53 \pm 15 \mu\text{mol m}^{-2} \text{s}^{-1}$ and $122 \pm 31 \mu\text{mol m}^{-2} \text{s}^{-1}$ for $V_{c_{max}}$ and J_{max} , respectively), but is similar to the values for temperate forest hardwoods ($47 \pm 33 \mu\text{mol m}^{-2} \text{s}^{-1}$ and $104 \pm 67 \mu\text{mol}$

$\text{m}^{-2} \text{s}^{-1}$ for $V_{c_{max}}$ and J_{max} , respectively). Across individual plants $A_{(op)}$ (defined here as the maximum operating CO_2 assimilation rate under standard conditions, as distinct from the maximum ribulose biphosphate regeneration-limited rate induced under elevated CO_2 concentration) was positively correlated with $V_{c_{max}}$ (Fig. 7A) and J_{max} (Fig. 7B) [$y=0.47x-1.39$, $r^2=0.81$, $P < 0.001$ for $A_{(op)(max)}$ versus $V_{c_{max}}$, and $y=0.14x+1.14$, $r^2=0.64$, $P < 0.001$ for $A_{(op)(max)}$ versus J_{max}]. There was no apparent species grouping within either correlation.

Stomatal closure in response to darkening of leaves followed a similar pattern across species, but the minimum steady conductance during this mid-day darkening of leaves, $g_{min(day)}$, was considerably higher than $g_{min(dawn)}$ (Fig. 8). The average percentage decline in g after mid-day darkening, with respect to the illuminated steady-state conductance (g_{op}), was 59.23, 61.80, 64.36, 65.57, and 86.08 % for *B. menziesii*, *B. prionotes*, *B. ilicifolia*, *B. attenuata*, and *B. littoralis*, respectively. After excising leaves, a further decline in g was noted. The average percentage decline in g with leaf excision, relative to $g_{min(day)}$, was 95.00, 93.28, 95.20, 94.87, and 79.93 for *B. attenuata*, *B. menziesii*, *B. ilicifolia*, *B. prionotes*, and *B. littoralis*, respectively. On this occasion *B. littoralis*, the species restricted to sites with high soil moisture, showed the least relative decline in g .

Discussion

In support of hypotheses (i) and (ii), g_{op} correlated with $g_{min(dawn)}$ and with the maximum rate of stomatal response to light $(dg/dt)_{max}$ (Fig. 3A, B). The results suggest that the day and night-time stomatal conductances are positively correlated across these *Banksia* species and that a functional connection exists between these traits and the dynamic behaviour of stomata. Enhanced dynamic response with higher operational stomatal conductance has implications for improved long-term WUE and lower risk of disruption of the leaf hydraulic system.

The positive correlation between g_{op} and $g_{min(dawn)}$ (Fig. 3A) suggests that there is a trade-off in which leaves built for higher rates of leaf gas exchange maintain higher stomatal conductance at night. The positive correlation also between g_{op} and $(dg/dt)_{max}$ (Fig. 3B) suggests that the water losses due to the accompanying elevated night-time stomatal conductance and, consequently, the elevated night-time transpiration rates are offset by better dynamic control of stomata during the day. The role of night-time stomatal conductance remains elusive, and the mechanism of its control is poorly understood (Barbour and Buckley, 2007). However, the scaling relationships identified in this study provide important mechanistic foundations for predicting the dynamic range of stomatal control and for improved modelling of stomatal control through day–night cycles.

Higher g_{op} , faster $(dg/dt)_{max}$, and shorter t_{50} were associated with smaller and more numerous stomata (Fig. 4A–C). Investment in stomatal infrastructure to facilitate high gas exchange capacity is constrained by the availability of space

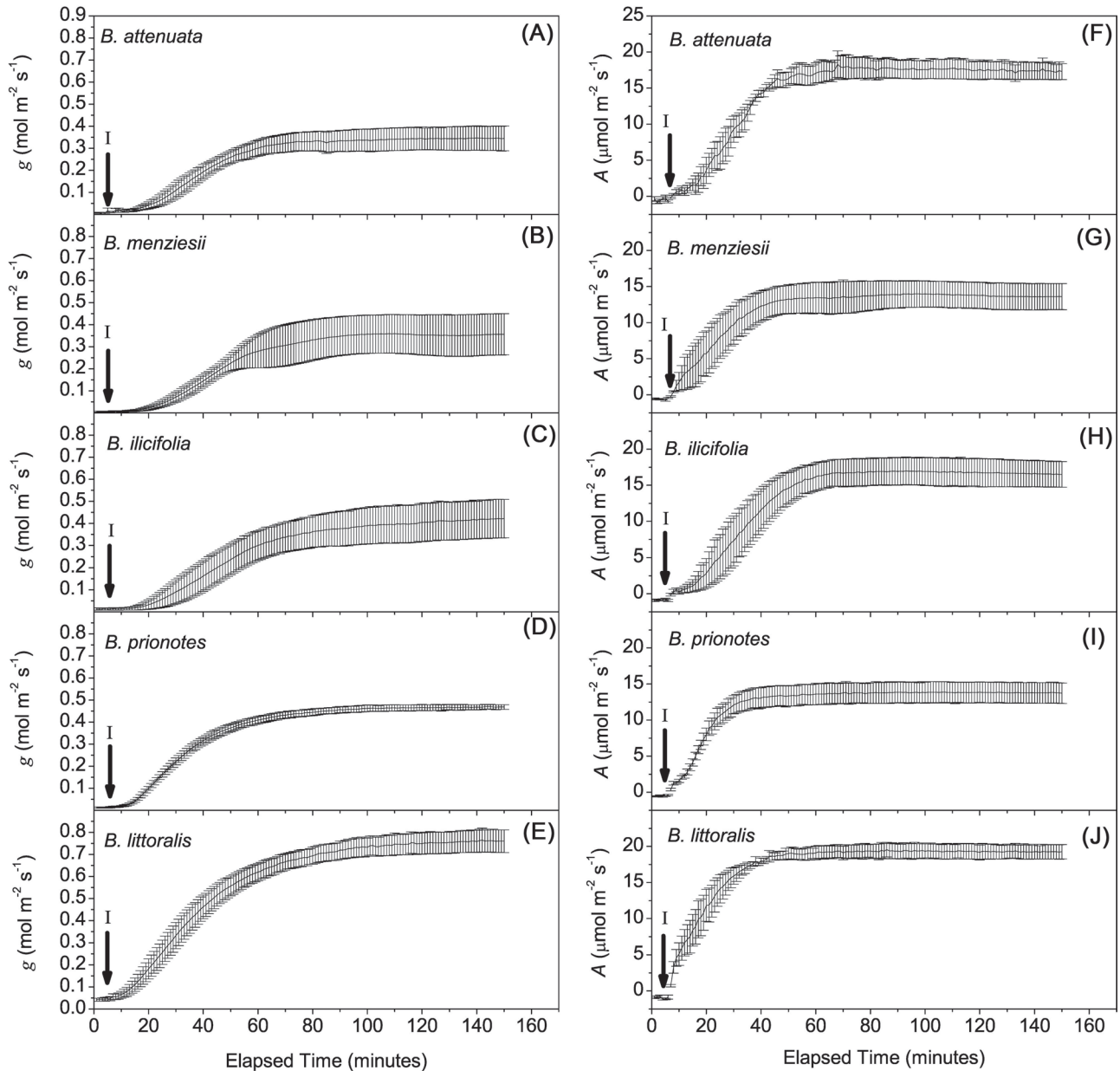


Fig. 5. Time-series of stomatal opening and CO₂ assimilation rate in response to light. Each point is the mean \pm SE stomatal conductance (g ; A–E) and assimilation rate (A ; F–J) measured at discrete time intervals ($n=4$ plants per species). The letter ‘I’ in each graph indicates the start of the illumination phase, when leaves were exposed to a PAR of $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$. Prior to this point, leaves were darkened (PAR = $0 \mu\text{mol m}^{-2} \text{s}^{-1}$).

Table 2. Comparison of stomatal conductances to water vapour ($\text{mmol m}^{-2} \text{s}^{-1}$) in the five *Banksia* species studied. Numbers are means with standard error in parentheses.

Species	$g_{\text{min(dawn)}}$	g_{op}	$g_{\text{min(day)}}$	$g_{\text{min(abs)}}$
<i>B. attenuata</i>	9.42 (1.9)	345 (20)	120 (19)	5.7 (0.8)
<i>B. menziesii</i>	6.03 (0.7)	356 (34)	135 (25)	9.5 (0.8)
<i>B. ilicifolia</i>	12.4 (1.8)	421 (32)	143 (23)	8.5 (2.7)
<i>B. prionotes</i>	12.9 (1.1)	469 (4)	171 (20)	8.8 (0.7)
<i>B. littoralis</i>	44.0 (2.9)	761 (19)	95 (4)	20 (0.7)

$g_{\text{min(dawn)}}$, prior to morning light exposure; g_{op} , at full stomatal opening under ideal conditions; $g_{\text{min(day)}}$, following closure in response to leaf darkening at midday; $g_{\text{min(abs)}}$, after leaf excision.

on the leaf surface and the total metabolic energy required to actively regulate stomatal pore size in a given number of stomata (Franks and Farquhar, 2007; Franks *et al.*, 2009). The present study suggests that the inherently faster stomatal response of leaves with high g_{op} and smaller stomata could provide enhanced water balance in dynamic light environments in addition to the higher assimilation rates accompanying high g_{op} . However, the interaction between the dynamic response of stomata and the frequency of light fluctuations is complex, with frequency dramatically influencing the average stomatal response (Cardon *et al.*, 1994).

Despite the advantages of faster stomatal response (i.e. compared with leaves with the same g_{op} but slower stomatal

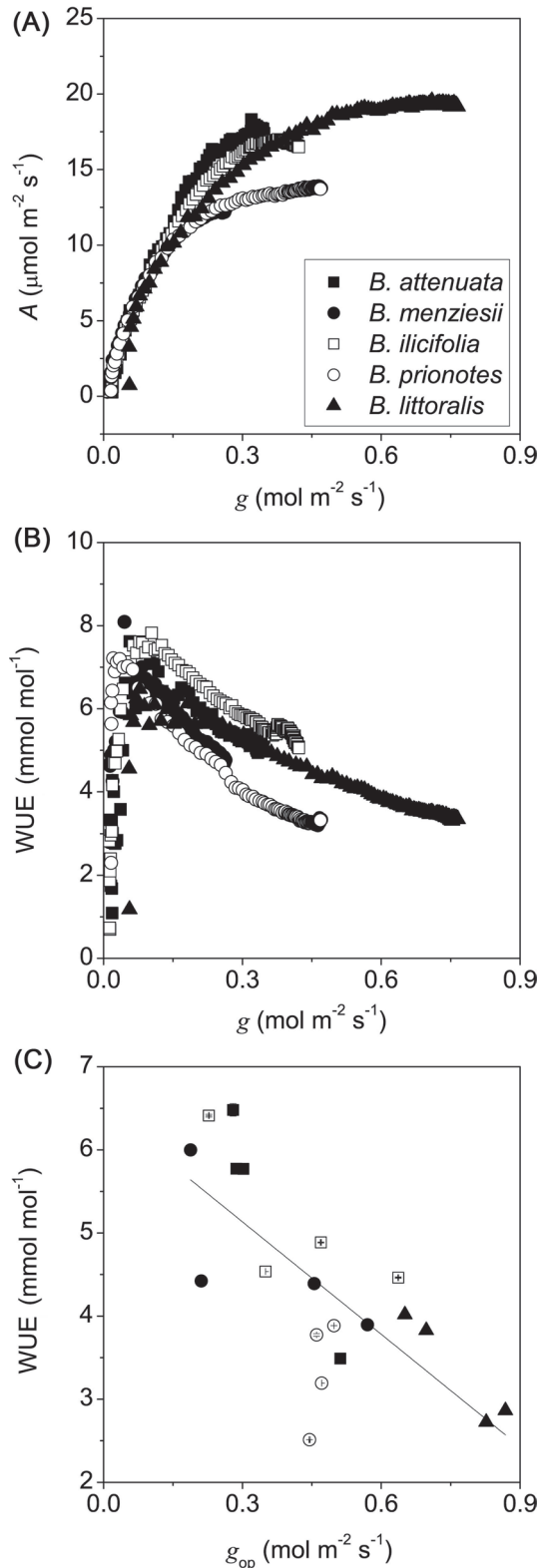


Fig. 6. Relationship between CO₂ assimilation rate (A), stomatal conductance (g), and instantaneous water-use efficiency (WUE). (A) Instantaneous A versus instantaneous g; (B) instantaneous WUE versus g. Note the peak in WUE at around the same A for all species (~5 μmol m⁻² s⁻¹). (C) Negative correlation between WUE and steady-state operating stomatal conductance, g_{op}.

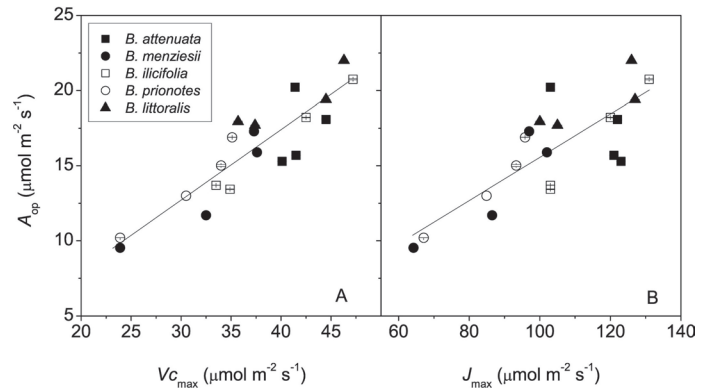


Fig. 7. The maximum (operating) photosynthetic rate A_{op} was positively correlated with the maximum rate of carboxylation, $V_{c,max}$ (A) and the light-saturated rate of electron transport, J_{max} (B).

response), greater overall WUE may still be more strongly associated with lower g_{op} , as indicated by the negative correlation between WUE and g_{op} across species (Fig. 6C). However, the faster response times associated with higher g_{op} (Figs 3B, 4C) help to compensate for this. WUE trended towards higher values in species that occur naturally in areas with a large depth to groundwater (Table 1) and therefore higher probability of water deficit. Assuming these qualities are genetically conserved and the observed differences translate qualitatively to these species in their natural environment, the results help to explain why the species with higher photosynthetic capacity prefer damp habitats while those with lower capacity occupy seasonally dry habitats (Fig. 2). Similarly, Anderson et al. (1996) showed that the WUE of commonly grown *Eucalyptus* species correlated negatively with the rainfall of their respective native habitat, suggesting genetic conservation of gas exchange traits that have been optimized to local conditions. Faster stomatal response improves WUE in environments with fluctuating light and evaporative demand, so higher $(dg/dt)_{max}$ associated with higher g_{op} will help to counteract reduced WUE in leaves with high g_{op} .

The correlation between g_{op} and $(dg/dt)_{max}$ is consistent with selection for a stomatal control mechanism that minimizes exposure to excessive water potential gradients. With increasing g_{op} , the plant is more exposed to potentially damaging water potential gradients arising from sudden changes in evaporation potential. Faster stomatal closure in response to these changes will reduce the risks associated with such exposure, including formation of air embolisms in the xylem. Stomatal response to light and VPD (or transpiration rate) have similar kinetics (Grantz and Zeiger, 1986), so it may be useful to compare species on the basis of them having generally ‘faster’ or ‘slower’ stomatal mechanisms. In Fig. 9 the value of faster response times for plants with higher g_{op} is illustrated. The simulations use the data and model in Franks (2006) for plants with different gas exchange and hydraulic capacities. It is shown that, for a step increase in VPD from 1 kPa to 1.5 kPa, plants operating with higher g_{op} at 1 kPa VPD are exposed to higher leaf water potential gradients ($\Delta\Psi_{leaf}$)

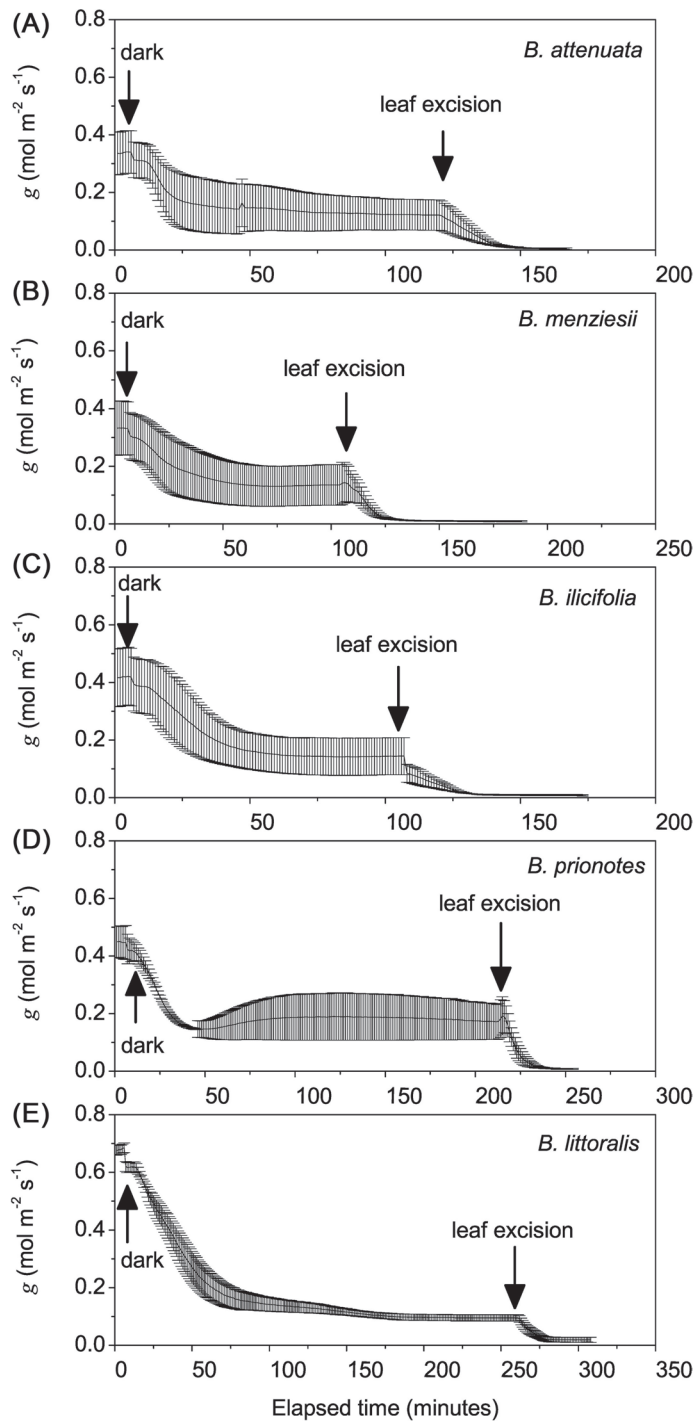


Fig. 8. Incomplete stomatal closure in the dark. Following a sudden transition from 1500 to 0 PAR (indicated by the arrow labelled 'dark'), stomatal conductance (g) declined to a steady-state minimum [$g_{\min(\text{day})}$, see Fig. 1]. Further reduction in g occurred after leaf excision (indicated by the arrow), reaching the absolute minimum conductance [$g_{\min(\text{abs})}$] after desiccation induced the complete loss of guard cell turgor. (A–E) The time-series of g for each species (mean \pm SE, $n=4$ plants per species).

immediately after the change, and may therefore benefit from a faster rate of reduction of stomatal conductance to the new steady rate at 1.5 kPa VPD.

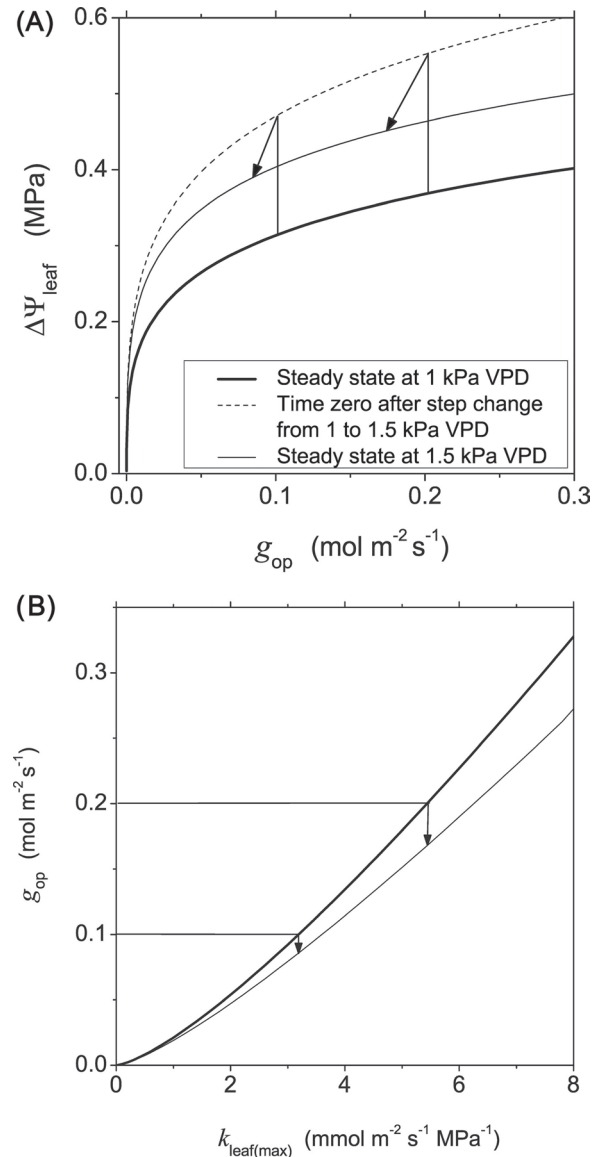


Fig. 9. Simulations based on the data and model in Franks (2006) show that following an increase in evaporative demand (leaf-to-air vapour pressure difference, VPD), plants that operate with higher stomatal conductance (g_{op}) are exposed to larger water potential gradients (shown here for leaves, $\Delta\Psi_{\text{leaf}}$; A), even though they have inherently larger maximum leaf hydraulic conductance, $k_{\text{leaf(max)}}$ (B). For illustrative purposes, two operating stomatal conductances are contrasted with one another ($0.10 \text{ mol m}^{-2} \text{ s}^{-1}$ and $0.20 \text{ mol m}^{-2} \text{ s}^{-1}$ at 1 kPa VPD), with their initial and final values indicated by the start and end point (respectively) of the arrows. A faster response time reduces the duration of exposure to excessive water potential gradients.

Conclusions

Although several studies have demonstrated scaling of stomatal conductance with static indicators of plant gas exchange capacity (Wong *et al.*, 1979; Field and Mooney, 1986; Meinzer, 2003), the present results show scaling with a dynamic performance characteristic, $(dg/dt)_{\text{max}}$, and this dynamic attribute also scaled with stomatal size and stomatal

density. Maximum daytime operating stomatal conductance, g_{op} , and pre-dawn minimum stomatal conductance, $g_{min(dawn)}$, were positively correlated with the rate of stomatal response to light. Leaves with higher g_{op} have lower instantaneous WUE and are exposed to larger transient water potential gradients. Faster stomatal response times in such leaves may improve long-term WUE and reduce exposure to transient water potential gradients. Smaller stomata with faster dynamic characteristics may therefore be integral to selection for high stomatal conductances accompanying higher photosynthetic capacity. This principle may also be applied in the selection for plants with improved agricultural qualities.

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