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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. Our objective 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, $(dg/dt)_{max}$; (iii) g_{op} correlates negatively with instantaneous water-use efficiency (WUE) despite positive correlations with maximum rate of carboxylation (V_{cmax}) and light-saturated rate of electron-transport (J_{max}). Using five closely related species of the genus *Banksia*, we measured the above variables and found that all three hypotheses were supported by the results. Overall, this suggests that leaves built for higher rates of gas exchange have smaller stomata and faster dynamic characteristics. With the aid of a stomatal control model we demonstrate 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: stomatal size, maximum stomatal conductance, night-time conductance, transpiration, stomatal control, water-use efficiency

26 INTRODUCTION

27

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

36 Plant photosynthetic productivity and water-use efficiency also are linked to
37 the dynamic range of stomatal conductance. Under favourable conditions of low
38 evaporative demand and high light, productivity is constrained by the maximum
39 operating stomatal conductance, g_{op} , and under severe water deficits resulting from
40 high evaporative demand and/or dry soil, plants rely upon full stomatal closure and a
41 highly water-impermeable leaf cuticle to minimise water loss (Hinckley *et al.*, 1980;
42 McDowell *et al.*, 2008). Across plant taxa there is a wide range of operating and
43 minimum stomatal conductances (Jones, 1992; Schulze *et al.*, 1994; Körner, 1995).
44 However, it is not known if maximum and minimum stomatal conductance typically
45 scale with one another.

46 Commonly defined as the minimum stomatal conductance in darkness, g_{min} for
47 a given leaf may differ on account of the time of day or other physiological
48 circumstances. For example, stomata typically close in response to darkness and
49 remain so for much of the night, but often the closure is not complete. In fact the
50 night-time or 'nocturnal' conductance can be sufficient to allow significant
51 transpiration (Ehrler, 1971; Benyon, 1999; Snyder, *et al.*, 2003; Barbour *et al.*, 2005;
52 Bucci *et al.*, 2005; Daley and Phillips, 2006; Dawson *et al.*, 2007), and growth
53 conditions may produce stomata that cannot close completely even when fully
54 deflated at zero turgor (Franks and Farquhar, 2007). Night-time transpiration rates
55 are typically between 5% to 15% of daytime transpiration, but in rare cases can be
56 more than 30% (Caird *et al.*, 2007; Novick *et al.*, 2009). Such high rates of water loss
57 at times of little or no carbon gain are inconsistent with the general role of stomata as
58 a water conserving apparatus, but little is known about the mechanism of nocturnally
59 elevated stomatal conductance or its relationship to the minimum conductance in

60 darkness at other times of the day and under desiccation. Here we distinguish
61 between three different conductance minima according to the circumstances in which
62 they are promoted: (i) $g_{\min(\text{dawn})}$, referring to the minimum stomatal conductance to
63 water vapour at the end of the nocturnal dark phase; (ii) $g_{\min(\text{day})}$, referring to the
64 minimum stomatal conductance to water vapour attained when the leaf is exposed to a
65 period of darkness during to normal daylight hours; (iii) the absolute minimum
66 conductance to water vapour, $g_{\min(\text{abs})}$, occurring when the guard cells are fully
67 deflated as a result of complete turgor loss (Fig. 1). The quantities g_{op} , $g_{\min(\text{dawn})}$,
68 $g_{\min(\text{day})}$, and $g_{\min(\text{abs})}$ all comprise a stomatal component in parallel with a cuticular
69 component, although $g_{\min(\text{abs})}$ may closely approximate cuticular conductance.
70 Common empirical stomatal models do not adequately account for elevated minimum
71 conductance at night or its environmental sensitivities (Barbour and Buckley, 2007)
72 but few studies have measured all of these conductances together so the relationship
73 between them is obscure.

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

82 The operating conductance g_{op} is constrained by the maximum stomatal
83 conductance, g_{max} , which in turn is determined by two physical attributes of stomata,
84 (i) their size (S) and (ii) their density (D), or number per unit area. We distinguish
85 between g_{max} and g_{op} because g_{max} relates to stomata opened to their widest possible
86 apertures (e.g. under 100% relative humidity and low ambient CO_2 concentration),
87 whereas under typical operating conditions (less than 100% relative humidity and
88 normal ambient CO_2 concentration) stomatal apertures will be less than fully open. It
89 has been shown that across broad geological timescales and evolutionary lineages
90 higher g_{max} and g_{op} are associated with smaller stomatal size and higher density, and
91 that S is negatively correlated with D (Hetherington and Woodward, 2003; Franks and
92 Beerling, 2009). This relationship has also been found to apply within a single
93 species across environmental gradients (Franks *et al.*, 2009), and also across a group

94 of six tree species of different genus (Aasama *et al.*, 2001). Smaller stomata, due to
95 their greater membrane surface area to volume ratio, may have faster response times
96 compared to larger stomata, and this in combination with high stomatal density may
97 allow the leaf to attain high g_{op} rapidly under favorable conditions, and to rapidly
98 reduce conductance when conditions are unfavorable. In such a system, the rate of
99 stomatal response would be positively correlated with g_{op} and negatively correlated
100 with stomatal size. However, to date, these functional relationships have not been
101 confirmed.

102 Our objective was to test three hypotheses: (i) operating stomatal conductance
103 under standard conditions (g_{op}) correlates with minimum stomatal conductance prior
104 to morning light, ($g_{min(dawn)}$); (ii) stomatal size (S) is negatively correlated with g_{op}
105 and the maximum rate of stomatal opening in response to light, $(dg/dt)_{max}$; (iii) g_{op}
106 correlates negatively with instantaneous water-use efficiency (WUE) despite positive
107 correlations with maximum rate of carboxylation (V_{cmax}) and light-saturated rate of
108 electron-transport (J_{max}). To test our hypotheses we measured the above traits in a
109 closely related group of *Banksia* species that are distributed across a broad
110 hydrological environment from wetlands to dune crests (Fig. 2) (Groom, 2002;
111 Groom, 2004). Restricting the study to a single genus ensured minimal genetic
112 variability while offering a broad range of g_{op} , stomatal size and stomatal density
113 traits for analysis. The results are assessed in terms of their implications for plant
114 water balance and fitness under the differing hydrological habitats of the study
115 species.

116

117 MATERIALS AND METHODS

118

119 *Plant material*

120

121 Five *Banksia* species, endemic to the *Banksia* woodland of south-western Australia
122 (31°45' S, 115°57' E), were selected for study. The species were as follows: *Banksia*
123 *attenuata* R.Br., *Banksia menziesii* R.Br., *Banksia ilicifolia* R.Br., *Banksia prionotes*
124 Lindl. and *Banksia littoralis* R.Br. Figure 2, based on the natural geographical range
125 of south-west Australian banksias, is an idealised representation of the distribution of
126 the species across five distinct habitats as defined by the depth of groundwater from
127 the natural surface (Table 1).

128 Four plants from each species were grown from seed in a glasshouse in 10 L
129 pots. Plants were allowed to develop in 70:30 coarse sand:humus and fertilized with
130 33.38 ± 0.24 grams (mean \pm SE) of slow release fertilizer (Osmocote™). All plants
131 were well watered throughout development and maintained under a day/night
132 temperature regime of 24/15°C. When leaves had fully matured under these
133 conditions, each plant was periodically transferred to a laboratory (air temperature
134 range = $23 \pm 3^\circ\text{C}$), rewatered and allowed to equilibrate overnight in preparation for
135 the following day's gas exchange measurements.

136

137 *Gas exchange*

138

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

150 In the morning, minimum steady-state stomatal conductance to water vapour
151 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
152 in darkness. A stomatal opening phase, comprising the transition from $g_{\text{min(dawn)}}$ to a
153 maximum steady-state or operating stomatal conductance to water vapour (g_{op} , mol
154 $\text{H}_2\text{O m}^{-2} \text{ leaf s}^{-1}$), was then recorded by exposing leaves to a photosynthetically active
155 radiation (PAR) of $1500 \mu\text{mol m}^{-2} \text{ s}^{-1}$ (while keeping the other chamber conditions
156 constant) and logging instantaneous stomatal conductance (g) at 60 second intervals.
157 This opening phase took approximately 120 minutes to reach a steady-state g_{op} for
158 each species. After ensuring that all transient stomatal opening had ceased, the
159 maximum steady-state CO_2 assimilation rate (A_{op} , $\mu\text{mol m}^{-2} \text{ leaf s}^{-1}$) and
160 corresponding intercellular CO_2 mole fraction ($c_{i(\text{op})}$, $\mu\text{mol CO}_2 \text{ mol}^{-1}$ air) and steady-

161 state transpiration rate (E_{op} , $\text{mmol H}_2\text{O m}^{-2} \text{ leaf s}^{-1}$) were also recorded. Also at this
 162 point, the relationship between instantaneous CO_2 assimilation rate A and leaf
 163 intercellular CO_2 concentration c_i was obtained (see below). Photosynthetically
 164 active radiation was then returned to zero and the subsequent stomatal closing phase,
 165 to a minimum steady-state value, $g_{\min(\text{day})}$, was recorded by logging stomatal
 166 conductance at 60 second intervals. The timeframe for stomatal closure varied across
 167 species, ranging from approximately 100 to 250 minutes. The leaf was then excised
 168 from the plant and any further decline in stomatal conductance recorded, with the
 169 final minimum conductance for excised leaves measured as the absolute minimum,
 170 $g_{\min(\text{abs})}$.

171 The relationship between A and c_i was obtained for each plant by manipulating
 172 c_a over the range $50 \mu\text{mol CO}_2 \text{ mol}^{-1} \text{ air}$ to $2000 \mu\text{mol CO}_2 \text{ mol}^{-1} \text{ air}$, beginning with
 173 the steady state conditions at $350 \mu\text{mol CO}_2 \text{ mol}^{-1} \text{ air}$, then stepping c_a down to 300,
 174 200, 100, 50 and then up to 400, 600, 800, 1000, 1400, 1800 and $2000 \mu\text{mol CO}_2 \text{ mol}^{-1}$
 175 air . We characterised the relationship according to the model proposed by Farquhar
 176 *et al.* (1980) and subsequently modified by von Caemmerer and Farquhar (1981),
 177 Sharkey (1985) and Harley *et al.* (1992). Undertaking this mechanistic analysis of the
 178 relationship between A and c_i yielded estimates for the maximum rate of
 179 carboxylation ($V_{c_{\max}}$, $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ leaf s}^{-1}$) and the light saturated rate of electron
 180 transport (J_{\max} , $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ leaf s}^{-1}$).

181

182 *Deriving the maximum rate of stomatal opening*

183

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

187

$$188 \quad g = \frac{a_1 - a_2}{1 + e^{-t-t_0/dt'}} + a_2 \quad (1)$$

189

190 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
 191 horizontal asymptote, t_0 (seconds) is the point of inflection and dt' (seconds) is the
 192 change in time that corresponds to the greatest change in g . Using an iterative least

193 squares fit approach, values for a_1 , a_2 , t_0 and dt' were determined for each plant. The
194 instantaneous rates of stomatal opening (dg/dt , mol m⁻² s⁻²) across the entire range of t
195 were then calculated by taking the derivative of Equation 1:

$$\frac{dg}{dt} = \frac{e^{(t_0+t)/dt'} (a_2 - a_1)}{(e^{t_0/dt'} + e^{t/dt'})^2 dt'}$$

197 (2)

198
199 and the maximum rate of stomatal opening (dg/dt_{\max} , mol m⁻² s⁻²) determined for each
200 plant as dg/dt when $t = t_0$.

201
202 This procedure was repeated after converting g to a relative value, g_{relative} :

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

203 (3)

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

205

206 *Stomatal morphology*

207

208 A tissue sample was obtained halfway from the leaf tip to the base from each leaf that
209 was analysed for gas exchange properties and stored in 70% ethanol. For all species
210 except *B. littoralis*, stomata were concentrated within crypts on the abaxial surface.
211 Stomata of *B. littoralis* also only occurred on the abaxial surface but no crypts were
212 observed. The leaf epidermal surface of each species was also comprised of thickly
213 intertwined trichomes. To obtain a clear view of stomata amidst these surface
214 features, each sample was first rehydrated by rinsing under tap water then embedded
215 in paraffin wax. Planar (through the epidermis) and transverse sections were then cut
216 to 10 μm thickness with a rotary microtome (Leica model RM 2125, Leica
217 Microsystems, Wetzlar, Germany). The sections were then positioned on slides that
218 were dipped in 2% gelatin immediately prior to mounting. Slides were then placed in
219 a coplin jar with filter paper soaked in formaldehyde to allow vapour fixation (of
220 section to gelatin). The coplin jar was covered with a lid and the sections allowed to
221 dry at room temperature for 12 hours. Sections were then stained in 0.1% aqueous

222 toluidine blue, examined under a compound light microscope and images captured
223 with a digital camera.

224 Stomatal morphological parameters (guard cell length L (μm) and guard cell
225 pair width W (μm)) were measured from images obtained from planar sections as the
226 mean of 20 stomatal complexes (guard cell pairs) for each species. We report stomatal
227 size (S) as the product of L and W (μm^2).

228 For each species stomatal density, i.e. number of stomata per unit epidermal
229 area (D , mm^{-2}) was calculated from transverse sections. For each section, the number
230 of stomata (n_s) intercepted by the microtome during cutting was counted along a
231 known length of epidermis (l , μm , $n = 12$ lengths per species). The length of
232 epidermis ranged from approximately 450 μm to 4400 μm . Assuming each transect
233 captured an area of epidermis of width (w_e) approximately equal to the average of the
234 length and width of a stoma, the stomatal density was calculated as $D = n_s/(l \times w_e)$.

235

236 RESULTS

237

238 The operating stomatal conductance g_{op} was positively correlated with $g_{\min(\text{dawn})}$ ($y =$
239 $0.844 - 0.562e^{-(x - 0.004)/0.024}$, $r^2 = 0.70$, Fig. 3A) and with $(dg/dt)_{\max}$ (Fig. 3B, $y = -0.09$
240 $+ 3.40x$, $r^2 = 0.71$, $P < 0.001$). Across species there was a three-fold variation in
241 $(dg/dt)_{\max}$, 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
242 positively correlated with $(dg/dt)_{\max}$ (Fig 3C, $y = 5.08 \times 10^{-6}e^{(x/0.03)} + 0.01$, $r^2 = 0.78$).
243 These results indicate that higher maximum and minimum stomatal conductance is
244 linked to faster absolute rates of response of stomatal stomatal conductance to leaf
245 irradiance.

246 Stomatal size (S) was negatively correlated with $(dg/dt)_{\max}$ across species (Fig.
247 4A, $y = 15877.84 \times e^{(-x/0.03)} + 340.52$, $r^2 = 0.88$, $P < 0.01$) and stomatal density D was
248 positively correlated with $(dg/dt)_{\max}$ (Fig. 4B, $y = 77.43 + 1643.06x$, $r^2 = 0.71$, $P <$
249 0.05), indicating that leaves with smaller and more numerous stomata exhibit faster
250 absolute rates of response of stomatal conductance to water vapour. The positive
251 relationship between t_{50} and S (Fig. 4C, $y = 16.63 + 0.05x$, $r^2 = 0.34$, $P < 0.05$) further
252 indicates that smaller stomata exhibited a faster response in relative terms.

253 Stomatal opening in response to a step increase in light followed a similar
254 pattern in all species, resembling the typical dynamic response of a second order

255 dynamic system with near-critical damping (Fig 5A-E). For each species the stomatal
256 opening phase was accompanied by an increase in CO₂ assimilation rate (*A*) to a
257 maximum steady-state value (*A*_{op}), although *A*_{op} was established prior to *g*_{op} (Fig 5F-
258 J).

259 Although *g*_{op} varied by about five fold across species, *g*_{min(dawn)} varied by 15
260 fold (Table 2.). Across the five species, there was well over a two-fold range between
261 highest and lowest mean species *g*_{op} measured under controlled laboratory conditions.
262 The mean absolute minimum stomatal conductance *g*_{min(abs)} ranged from 6.0 to 20
263 mmol m⁻² s⁻¹, which compares favourably to the range of minimum stomatal
264 conductance reported for deciduous and evergreen plants using leaf drying curves (1.0
265 to 20 mmol m⁻² s⁻¹) (Burghardt and Riederer, 2003).

266 Over the dynamic range of stomatal opening, CO₂ assimilation rate increased
267 with stomatal conductance in the usual saturating fashion (Fig. 6A). Steady-state
268 instantaneous water use-efficiency (WUE_i), defined as *A*_{op}/*E*_{op} at 1 kPa VPD (see the
269 controlled, standardised environmental conditions in Methods) ranged from 2.5 to 6.5
270 mmol mol⁻¹ and all of the species reached a peak in WUE_i when *A* was about 5 μmol
271 m⁻² s⁻¹ (Fig. 6B). *B. attenuata* and *B. menzeisii* had the highest WUE_i and *B. littoralis*
272 had the lowest WUE_i (Fig. 6B). Also, WUE_i was negatively correlated with *g*_{op} (Fig.
273 6C; $y = 6.49 - 4.51x$; $r^2 = 0.52$, $P < 0.001$).

274 The maximum rate of carboxylation (*V*_{C_{max}}) ranged from 23.90 μmol m⁻² s⁻¹ to
275 47.11 μmol m⁻² s⁻¹ and the light saturated rate of electron transport (*J*_{max}) ranged from
276 64.2 μmol m⁻² s⁻¹ to 131 μmol m⁻² s⁻¹. The average value of *V*_{C_{max}} and *J*_{max} was 37.22
277 ± 1.47 μmol m⁻² s⁻¹ and 103.74 ± 4.24 μmol m⁻² s⁻¹ respectively. This is lower than
278 the average values reported by (Wullschleger SD, 1993) for sclerophyllous shrubs (53
279 ± 15 μmol m⁻² s⁻¹ and 122 ± 31 μmol m⁻² s⁻¹ for *V*_{C_{max}} and *J*_{max} respectively), but is
280 similar to the values for temperate forest hardwoods (47 ± 33 μmol m⁻² s⁻¹ and 104 ±
281 67 μmol m⁻² s⁻¹ for *V*_{C_{max}} and *J*_{max} respectively). Across individual plants *A*_(op)
282 (defined here as the maximum operating CO₂ assimilation rate under standard
283 conditions, as distinct from the maximum ribulose bisphosphate regeneration limited
284 rate induced under elevated CO₂ concentration) was positively correlated with *V*_{C_{max}}
285 (Fig. 7A) and *J*_{max} (Fig. 7B) ($y = 0.47x - 1.39$, $r^2 = 0.81$, $P < 0.001$ for *A*_{(op)(max)} versus
286 *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
287 apparent species grouping within either correlation.

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

299

300 DISCUSSION

301

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

309 The positive correlation between g_{op} and $g_{\min(\text{dawn})}$ (Fig. 3A) suggests that there
310 is a trade-off in which leaves built for higher rates of leaf gas exchange maintain
311 higher stomatal conductance at night. The positive correlation also between g_{op} and
312 $(dg/dt)_{\text{max}}$ (Fig. 3B) suggests that the water losses due to the accompanying elevated
313 night-time stomatal conductance and, consequently, the elevated night-time
314 transpiration rates are offset by better dynamic control of stomata during the day.
315 The role of night-time stomatal conductance remains elusive and the mechanism of its
316 control is poorly understood (Barbour and Buckley, 2007). However, the scaling
317 relationships identified in our study provide important mechanistic foundations for
318 predicting the dynamic range of stomatal control and for improved modelling of
319 stomatal control through day-night cycles.

320 Higher g_{op} , faster $(dg/dt)_{\text{max}}$ and shorter t_{50} were associated with smaller and
321 more numerous stomata (Fig. 4A-C). Investment in stomatal infrastructure to

322 facilitate high gas exchange capacity is constrained by the availability of space on the
323 leaf surface and the total metabolic energy required to actively regulate stomatal pore
324 size in a given number of stomata (Franks and Farquhar, 2007; Franks *et al.*, 2009).
325 Our study suggests that the inherently faster stomatal response of leaves with high g_{op}
326 and smaller stomata could provide enhanced water balance in dynamic light
327 environments in addition to the higher assimilation rates accompanying high g_{op} .
328 However, the interaction between the dynamic response of stomata and the frequency
329 of light fluctuations is complex, with frequency dramatically influencing the average
330 stomatal response (Cardon *et al.*, 1994).

331 Despite the advantages of faster stomatal response (i.e. compared to leaves
332 with the same g_{op} but slower stomatal response), greater overall water-use efficiency
333 may still be more strongly associated with lower g_{op} , as suggested by the negative
334 correlation between WUE_i and g_{op} across species (Fig 6C). However, the faster
335 response times associated with higher g_{op} (Fig 3B, Fig 4C) help to compensate for
336 this. WUE_i trended towards higher values in species that occur naturally in areas with
337 a large depth to groundwater (Table 1) and therefore higher probability of water
338 deficit. Assuming these qualities are genetically conserved and the observed
339 differences translate qualitatively to these species in their natural environment, the
340 results help to explain why the species with higher photosynthetic capacity prefer
341 damp habitats while those with lower capacity occupy seasonally dry habitats (Fig. 2).
342 Similarly, Anderson *et al.* (1996) showed that the water-use efficiency of commonly
343 grown *Eucalyptus* species correlated negatively with the rainfall of their respective
344 native habitat, suggesting genetic conservation of gas exchange traits that have been
345 optimised to local conditions. Faster stomatal response improves water-use efficiency
346 in environments with fluctuating light and evaporative demand, so higher $(dg/dt)_{max}$
347 associated with higher g_{op} will help to counteract reduced WUE_i in leaves with high
348 g_{op} .

349 The correlation between g_{op} and $(dg/dt)_{max}$ is consistent with selection for a
350 stomatal control mechanism that minimizes exposure to excessive water potential
351 gradients. With increasing g_{op} the plant is more exposed to potentially damaging
352 water potential gradients arising from sudden changes in evaporation potential. Faster
353 stomatal closure in response to these changes will reduce the risks associated with
354 such exposure, including formation of air embolisms in the xylem. Stomatal response
355 to light and VPD (or transpiration rate) have similar kinetics (Grantz and Zeiger,

356 1986), so it may be useful to compare species on the basis of them having generally
357 'faster' or 'slower' stomatal mechanisms. In Fig. 9 we illustrate the value of faster
358 response times for plants with higher g_{op} . The simulations use the data and model in
359 Franks (2006) for plants with different gas exchange and hydraulic capacities. It is
360 shown that, for a step increase in VPD from 1 to 1.5 kPa, plants operating with higher
361 g_{op} at 1 kPa VPD are exposed to higher leaf water potential gradients ($\Delta\Psi_{leaf}$)
362 immediately after the change, and may therefore benefit from a faster rate of
363 reduction of stomatal conductance to the new steady rate at 1.5 kPa VPD.

364

365 *Conclusions*

366 Although several studies have demonstrated scaling of stomatal conductance with
367 static indicators of plant gas exchange capacity (Wong et al.1979; Field and Mooney,
368 1986; Meinzer, 2003), our results show scaling with a dynamic performance
369 characteristic, $(dg/dt)_{max}$, and this dynamic attribute also scaled with stomatal size and
370 stomatal density. Maximum daytime operating stomatal conductance, g_{op} , and pre-
371 dawn minimum stomatal conductance, $g_{min(dawn)}$, were positively correlated with the
372 rate of stomatal response to light. Leaves with higher g_{op} have lower instantaneous
373 water-use efficiency and are exposed to larger transient water potential gradients.
374 Faster stomatal response times in such leaves may improve long-term water-use
375 efficiency and reduce exposure to transient water potential gradients. Smaller stomata
376 with faster dynamic characteristics may therefore be integral to selection for high
377 stomatal conductances accompanying higher photosynthetic capacity. This principle
378 may also be applied in the selection for plants with improved agricultural qualities.

379

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381

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REFERENCES

- Aasama K, Söber A, Rahi M. (2001)** Leaf anatomical characteristics associated with shoot hydraulic conductance, stomatal conductance and stomatal sensitivity to changes of leaf water status in temperate deciduous trees. *Australian Journal of Plant Physiology* **28**, 765-774.
- Anderson JE, Williams J, Kriedemann PE, Austin MP, Farquhar GD. 1996.** Correlations between carbon isotope discrimination and climate of native habitats for diverse eucalypt taxa growing in a common garden. *Australian Journal of Plant Physiology* **23**, 311-320.
- Assmann SM, Grantz DA. 1990.** Stomatal response to humidity in sugarcane and soybean: effect of vapour pressure difference on the kinetics of the blue light response. *Plant, Cell and Environment* **13**, 163-169.
- Barbour MM, Cernusak LA, Whitehead D, Griffin KL, Turnbull MH, Tissue DT, Farquhar GD. 2005.** Nocturnal stomatal conductance and implications for modelling delta $\delta^{18}\text{O}$ of leaf-respired CO_2 in temperate tree species. *Functional Plant Biology* **32**, 1107-1121.
- Barbour MM, Buckley TN. 2007.** The stomatal response to evaporative demand persists at night in *Ricinus communis* plants with high nocturnal conductance. *Plant, Cell and Environment* **30**, 711-721.
- Benyon RG. 1999.** Nighttime water use in an irrigated *Eucalyptus grandis* plantation. *Tree Physiology* **19**, 853-859.
- Brodribb TJ, Field TS, Jordan GS. 2007.** Leaf maximum photosynthetic rate and venation are linked by hydraulics. *Plant Physiology* **144**, 1890-1898.
- Brodribb TJ, McAdam S. A. M., Jordan GJ, Field TS. 2009.** Evolution of stomatal responsiveness to CO_2 and optimisation of water-use efficiency among land plants. *New Phytologist*. **183**, 839-847.
- Bucci SJ, Goldstein G, Meinzer FC, Franco AC, Campanello P, Scholz FG. 2005.** Mechanisms contributing to seasonal homeostasis of minimum leaf water potential and predawn disequilibrium between soil and plant water potential in neotropical savanna trees. *Trees-Structure and Function* **19**, 296-304.
- Burghardt M, Riederer M. 2003.** Ecophysiological relevance of cuticular transpiration of deciduous and evergreen plants in relation to stomatal closure and leaf water potential. *Journal of Experimental Botany* **54**, 1941-1949.
- Caird MA, Richards JH, Donovan LA. 2007.** Nighttime stomatal conductance and transpiration in C_3 and C_4 plants. *Plant Physiology* **143**, 4-10.
- Cowan IR. 1977.** Stomatal behaviour and environment. *Advances in Botanical Research* **4**, 117-228.
- Daley MJ, Phillips NG. 2006.** Interspecific variation in nighttime transpiration and stomatal conductance in a mixed New England deciduous forest. *Tree Physiology* **26**, 411-419.
- Dawson TE, Pate JS. 1996.** Seasonal water uptake and movement in the root systems of Australian phreatophytic plants of dimorphic root morphology: A stable isotope investigation. *Oecologia* **107**, 13-20.
- Dawson TE, Burgess SSO, Tu KP, Oliveira RS, Santiago LS, Fisher JB, Simonin KA, Ambrose AR. 2007.** Nighttime transpiration in woody plants from contrasting ecosystems. *Tree Physiology* **27**, 561-575.
- Ehrler WL. 1971.** Periodic nocturnal stomatal opening of citrus in a steady environment. *Physiologia Plantarum* **25**, 488-&.

- Farquhar GD, Caemmerer SV, Berry JA.** 1980. A biochemical model of photosynthetic CO₂ assimilation in leaves of C₃ species. *Planta* **149**, 78-90.
- Farquhar GD, Schulze ED, Kupperts M.** 1980. Responses to humidity by stomata of *Nicotina glauca* L. and *Corylus avellana* L. are consistent with the optimization of carbon dioxide uptake with respect to water loss. *Australian Journal of Plant Physiology* **7**, 315-327.
- Field CB, Mooney HA.** 1986. The photosynthetic-nitrogen relationship in wild plants. In: Givnish TJ, ed. *On the economy of plant form and function*. Cambridge: Cambridge University Press, pp. 22-55.
- Franks PJ.** 2006. Higher rates of leaf gas exchange are associated with higher leaf hydrodynamic pressure gradients. *Plant Cell and Environment* **29**, 584-592.
- Franks PJ, Farquhar GD.** 1999. A relationship between humidity response, growth form and photosynthetic operating point in C₃ plants. *Plant, Cell and Environment* **22**, 1337-1349.
- Franks PJ, Farquhar GD.** 2007. The mechanical diversity of stomata and its significance in gas-exchange control. *Plant Physiology* **143**, 78-87.
- Franks PJ, Beerling DJ.** 2009. CO₂-forced evolution of plant gas exchange capacity and water-use efficiency over the Phanerozoic. *Geobiology* **7**, 227-236.
- Franks PJ, Drake PL, Beerling DJ.** 2009. Plasticity in maximum stomatal conductance constrained by negative correlation between stomatal size and density: an analysis using *Eucalyptus globulus*. *Plant Cell and Environment* **32**, 1737-1748.
- Grantz DA, Zeiger E.** 1986. Stomatal responses to light and leaf-air water vapour pressure differences show similar kinetics in sugarcane and soybean. *Plant Physiology* **81**, 865-868.
- Groom PK, Froend RH, Matiske EM.** 2001. Long-term changes in vigour and distribution of *Banksia* and *Melaleuca* overstorey species on the Swan Coastal Plain. *Journal of the Royal Society of Western Australia* **84**, 63-69.
- Groom PK.** 2002. Seedling water stress response of two sandplain *Banksia* species differing in ability to tolerate drought. *Journal of Mediterranean Ecology* **3**, 3-9.
- Groom PK.** 2004. Seedling growth and physiological response of two sandplain *Banksia* species differing in flood tolerance. *Journal of the Royal Society of Western Australia* **87**, 115-121.
- Harley PC, Thomas RB, Reynolds JF, Strain BR.** 1992. Modelling photosynthesis of cotton grown in elevated CO₂. *Plant, Cell and Environment* **15**, 271-282.
- Hetherington AM, Woodward FI.** 2003. The role of stomata in sensing and driving environmental change. *Nature* **424**, 901-908.
- Hinckley TM, Duhme F, Hinckley AR, Richter H.** 1980. Water relations of drought-hardy shrubs: Osmotic potential and stomatal reactivity. *Plant Cell and Environment* **3**, 131-140.
- Jones HG.** 1992. *Plants and Microclimate*. Cambridge: Cambridge University Press.
- Körner C.** 1995. Leaf diffusive conductances in the major vegetation types of the globe. In: Schulz E-D, Caldwell MM, eds. *Ecophysiology of Photosynthesis*. Berlin: Springer-Verlag, 463-490.
- Lam A, Froend RH, Downes S, Loomes R.** 2004. Water availability and plant response: Identifying the water requirements of *Banksia* woodland on the Gngangara Groundwater Mound. A report to the Water Corporation of Western Australia.
- McDowell N, Pockman WT, Allen CD, Breshears DD, Cobb N, Kolb T, Plaut J, Sperry J, West A, Williams DG, Yezzer EA.** 2008. Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? *New Phytologist* **178**, 719-739.

- Meinzer FC.** 2003. Functional convergence in plant responses to the environment. *Oecologia* **134**, 1-11.
- Novick KA, Oren R, Stoy PC, Siqueira MBS, Katul GG.** 2009. Nocturnal evapotranspiration in eddy-covariance records from three co-located ecosystems in the Southeastern US: Implications for annual fluxes. *Agricultural and Forest Meteorology* **149**, 1491-1504.
- Pate JS, Jeschke WD, Aylward MJ.** 1995. Hydraulic Architecture and Xylem Structure of the Dimorphic Root Systems of South-West Australian Species of Proteaceae. *Journal of Experimental Botany* **46**, 907-915.
- Schulze ED, Kelliher FM, Körner C, Lloyd J, Leuning R.** 1994. Relationships among maximum stomatal conductance, ecosystem surface conductance, carbon assimilation rate, and plant nitrogen nutrition- A global ecology scaling exercise. *Annual Review of Ecology and Systematics* **25**, 629-660.
- Sharkey TD.** 1985. Photosynthesis in intact leaves of C₃ plants: physics, physiology and rate limitations. *The Botanical Review* **51**, 53-105.
- Snyder KA, Richards JH, Donovan LA.** 2003. Night-time conductance in C₃ and C₄ species: do plants lose water at night? *Journal of Experimental Botany* **54**, 861-865.
- Vico G, Manzoni S, Palmroth S, Katul G.** 2011. Effects of stomatal delays on the economics of leaf gas exchange under intermittent light regimes. *New Phytologist* **192**, 640-652.
- von Caemmerer S, Farquhar GD.** 1981. Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. *Planta* **153**, 376-387.
- Wong SC, Cowan IR, Farquhar GD.** 1979. Stomatal conductance correlates with photosynthetic capacity. *Nature* **282**, 424-426.
- Wullschleger SD.** 1993. Biochemical limitations to carbon assimilation in C₃ plants - a retrospective analysis of the A/c_i curves from 109 Species. *Journal of Experimental Botany* **44**, 907-920.
- Zencich SJ, Froend RH, Turner JV, Gailitis V.** 2002. Influence of groundwater depth on the seasonal water sources of water accessed by *Banksia* tree species on a shallow, sandy coastal aquifer. *Oecologia* **131**, 8-19.

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 ilicifolia</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

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

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. illicifolia</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)

FIGURE LEGENDS

Figure 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 standardised ideal conditions (see 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.

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

Figure 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 S.E. 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).

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

Figure 5. Time-series of stomatal opening and CO₂ assimilation rate in response to light. Each point is the mean \pm S.E. stomatal conductance (g , panels A-E) and assimilation rate (A , panels 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 $\mu\text{mol m}^{-2} \text{s}^{-1}$).

Figure 6. Relationship between CO₂ assimilation rate, stomatal conductance and instantaneous water use efficiency. Panel (A), instantaneous CO₂ assimilation rate A versus instantaneous stomatal conductance g ; panel (B), instantaneous water-use efficiency WUE_i versus g . Note the peak in WUE_i at around the same A for all species (approximately 5 $\mu\text{mol m}^{-2} \text{s}^{-1}$); panel (C), negative correlation between WUE_i and steady state operating stomatal conductance, g_{op} .

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

Figure 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 ($g_{min(day)}$, see Fig. 1). Further reduction in g occurred after leaf excision (indicated by the arrow), reaching the absolute minimum conductance ($g_{min(abs)}$) after desiccation induced the complete loss of guard cell turgor. Panels A-E show the time series of g for each species (mean \pm S.E., $n = 4$ plants per species).

Figure 9. Simulations based on the data and model in (Franks PJ, 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, $\Delta\Psi_{leaf}$; A), even though they have inherently larger maximum leaf hydraulic conductance $k_{leaf(max)}$ (B). For illustrative purposes two operating stomatal conductances are contrasted with one another (0.10 and 0.20 mol m⁻² s⁻¹ at 1 kPa VPD), with their initial and final values indicated by the start and end point (respectively) of the arrows. Faster response time reduces the duration of exposure to excessive water potential gradients.

Figure 1

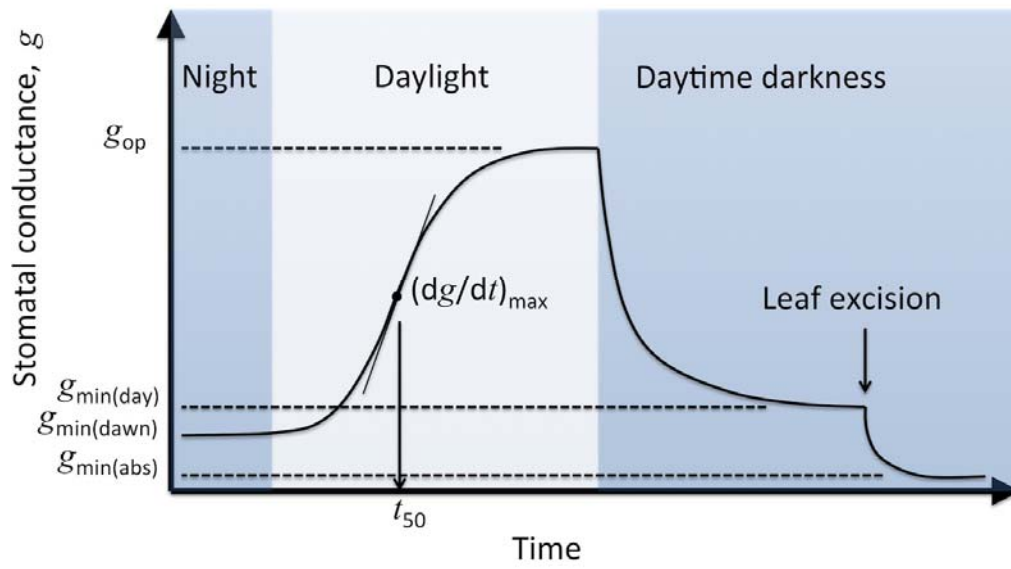


Figure 2

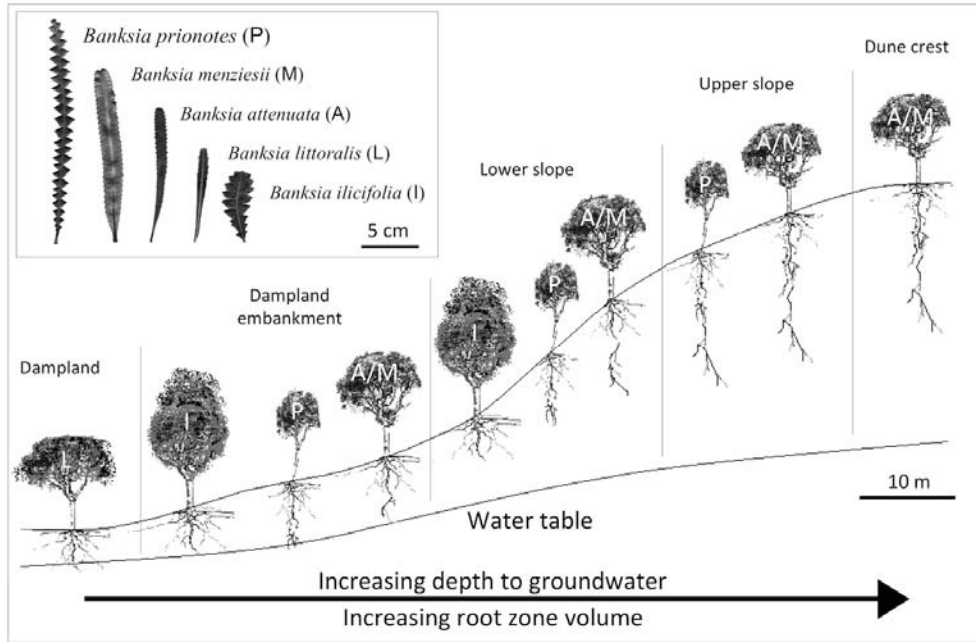


Figure 3

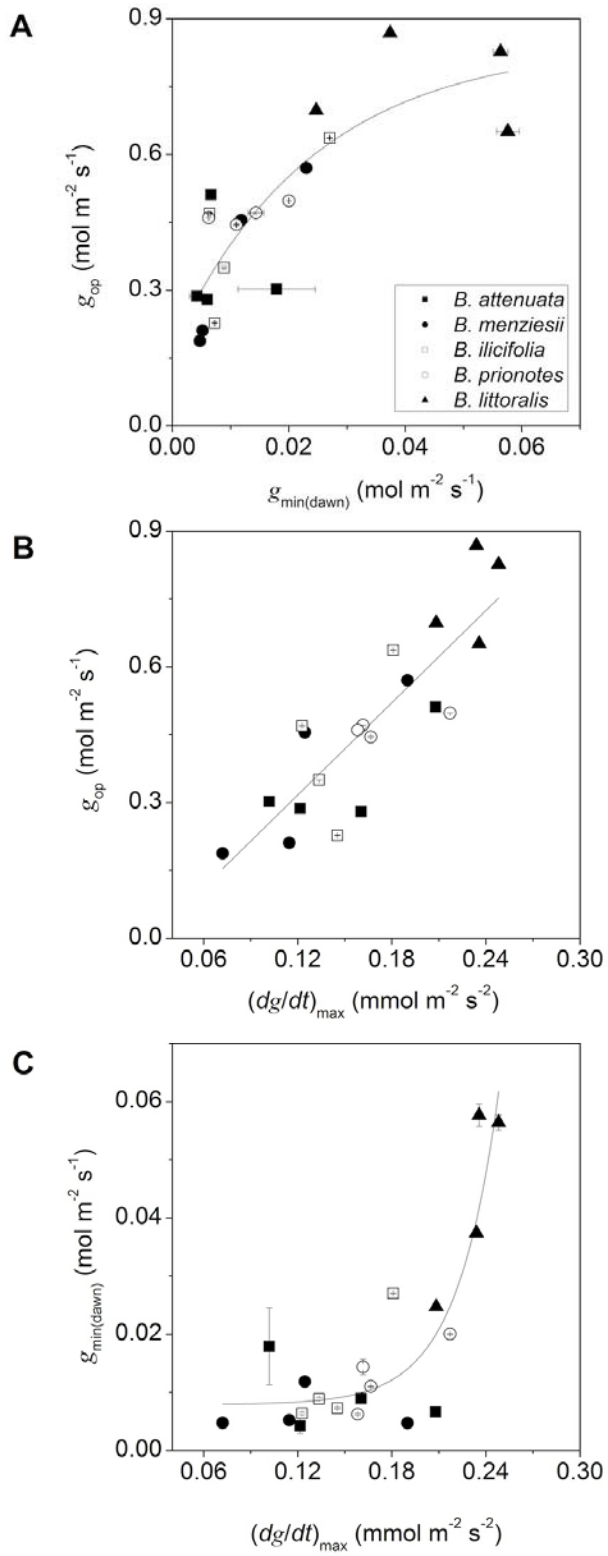


Figure 4

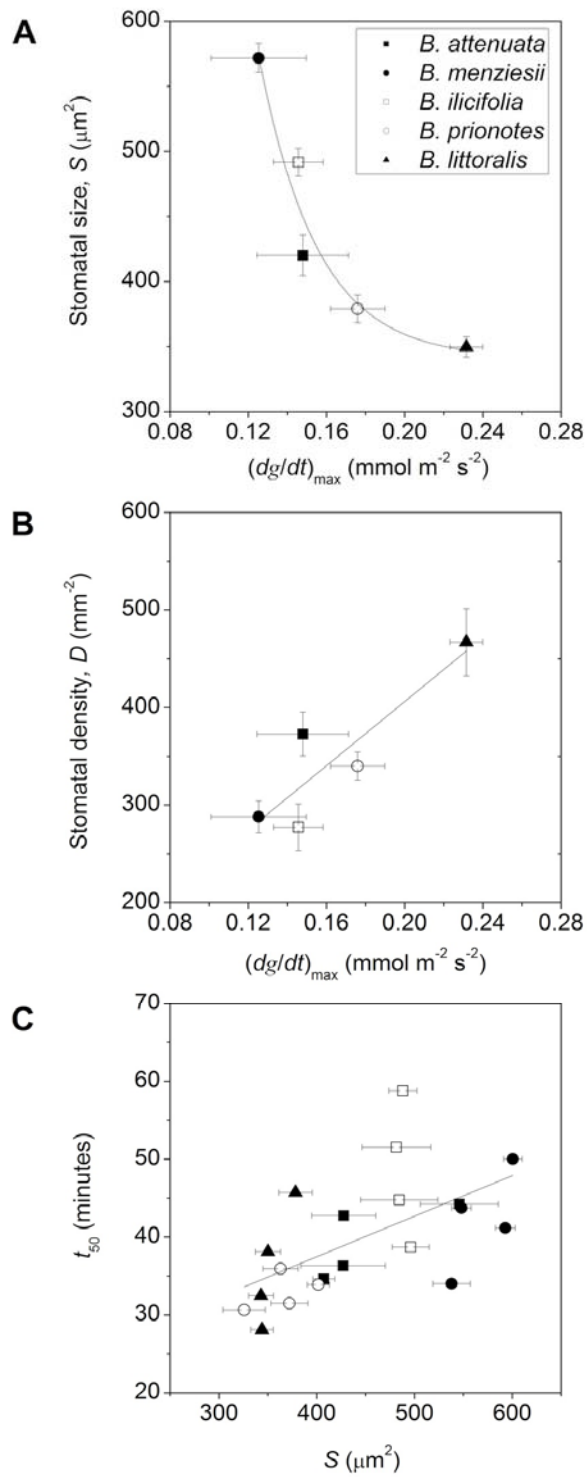


Figure 5

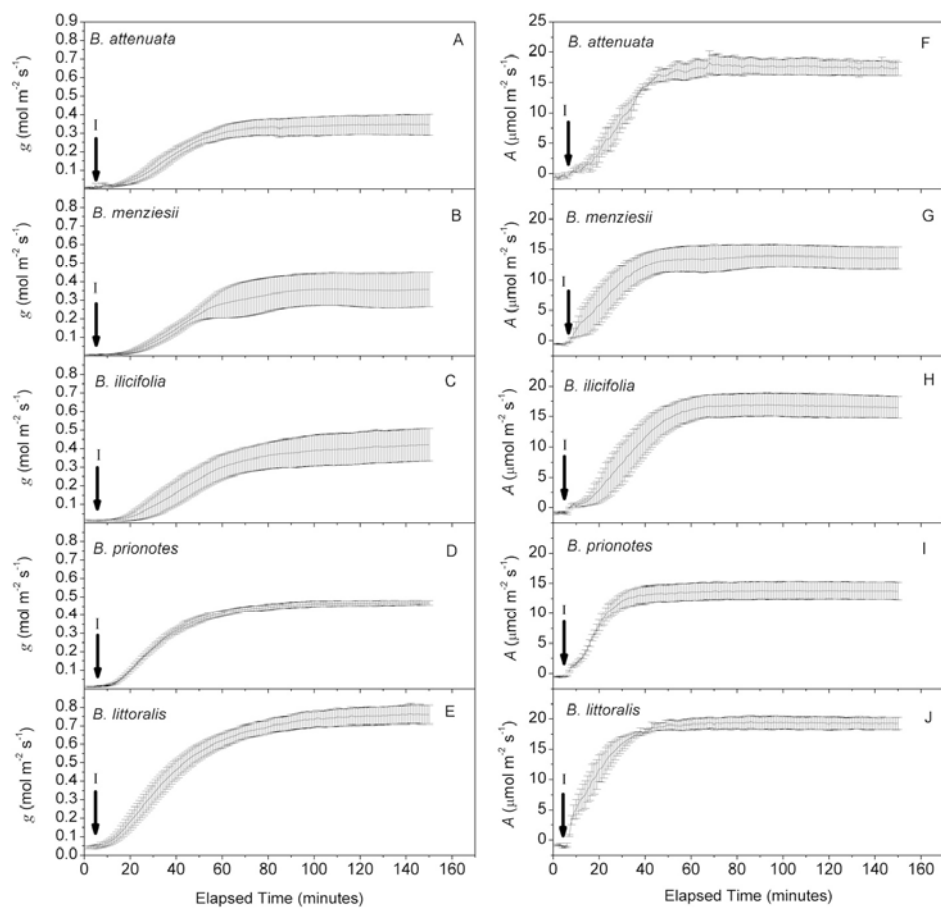


Figure 6

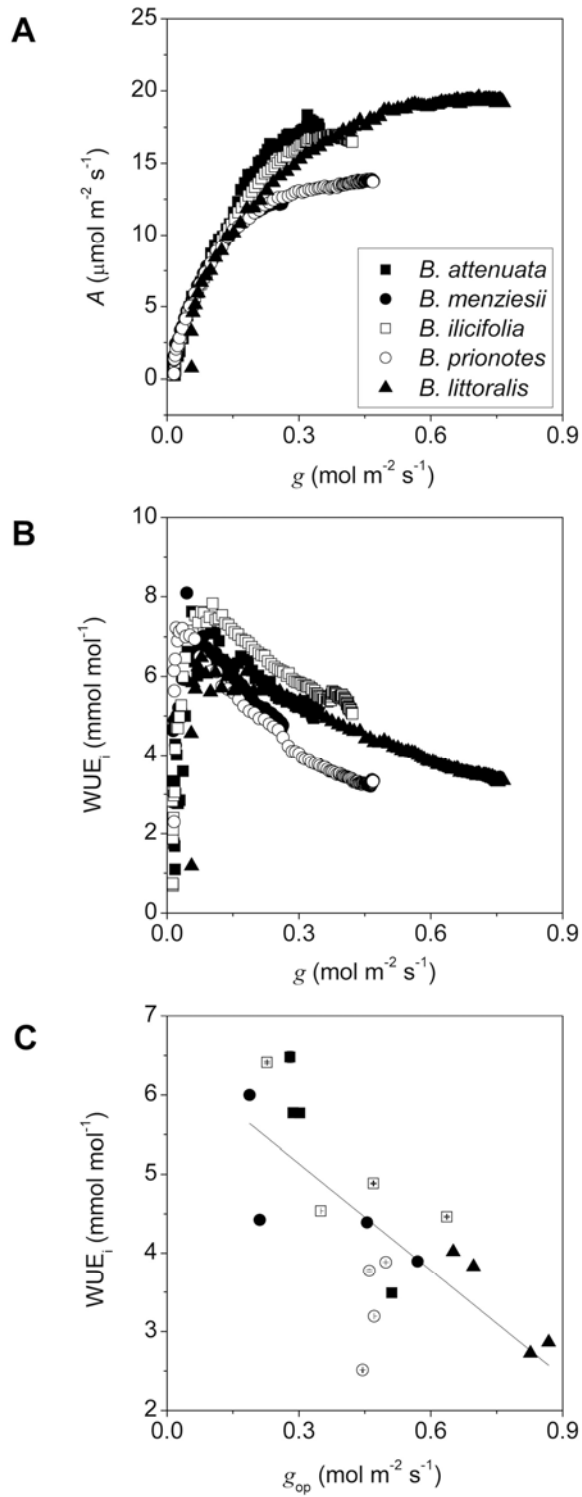


Figure 7

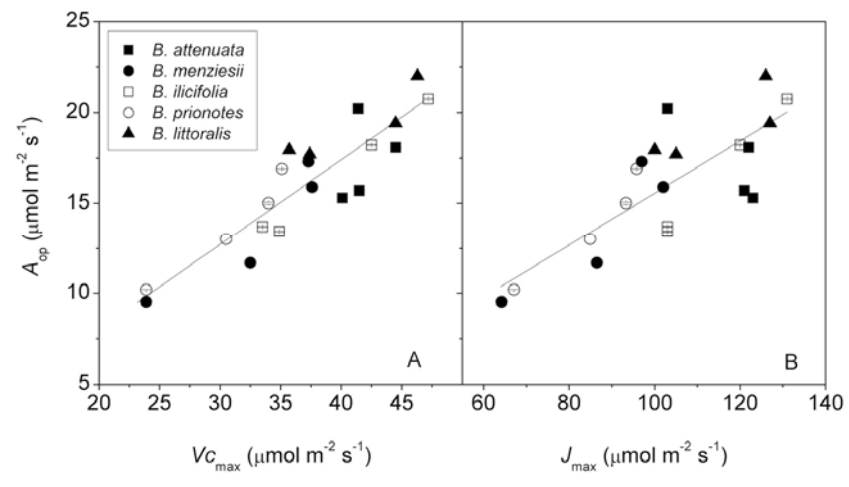


Figure 8

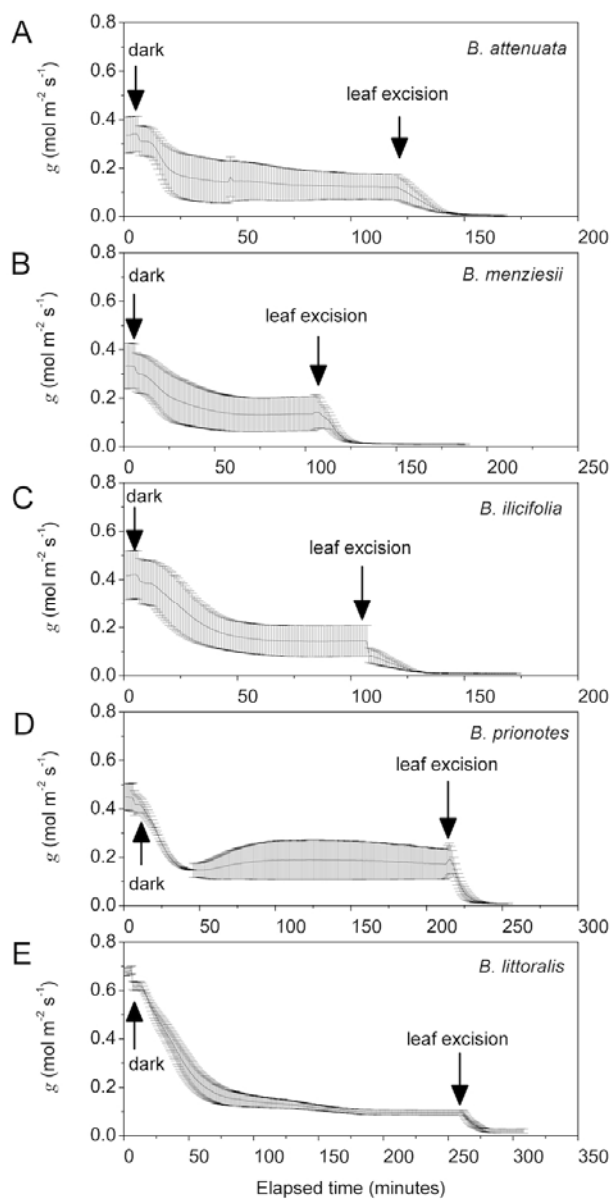


Figure 9

