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The stomatal response to evaporative demand persists at night in *Ricinus communis* plants with high nocturnal conductance

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ABSTRACT

Evidence is building that stomatal conductance to water vapour (g_s) can be quite high in the dark in some species. However, it is unclear whether nocturnal opening reflects a mechanistic limitation (i.e. an inability to close at night) or an adaptive response (i.e. promoting water loss for reasons unrelated to carbon gain). Further, it is unclear if stomatal responses to leaf-air vapour pressure difference (D) persist in the dark. We investigated nocturnal stomatal behaviour in castor bean (*Ricinus communis* L.) by measuring gas exchange and stomatal responses to D in the light and in the dark. Results were compared among eight growth environments [two levels for each of three treatment variables: air saturation deficit (D_a), light and water availability]. In most plants, stomata remained open and sensitive to D at night. g_s was typically lower at night than in the day, whereas leaf osmotic pressure (Π) was higher at night. In well-watered plants grown at low D_a, stomata were less sensitive to D in the dark than in the light, but the reverse was found for plants grown at high D_a. Stomata of droughted plants were less sensitive to D in the dark than in the light regardless of growth D_a. Drought also reduced g_s and elevated Π in both the light and the dark, but had variable effects on stomatal sensitivity to D. These results are interpreted with the aid of models of stomatal conductance.

Key-words: humidity; nocturnal stomatal conductance; osmotic pressure; stomata; stomatal model.

INTRODUCTION

Stomata regulate water loss from leaves in relation to rates of photosynthetic carbon fixation and water supply (Cowan & Farquhar 1977), suggesting that non-CAM plants should close their stomata at night when there is no opportunity for carbon gain. However, there is increasing evidence of significant stomatal water loss in the dark in many species from a range of environments (Benyon 1999; Musselman & Minnick 2000; Barbour *et al*. 2005). Recent work suggests that plants with inherently high daytime stomatal conductance (g_s; see Table 2 for a list of symbols) tend to have high nocturnal g_s (Snyder, Richards & Donovan 2003), that stomata of some species respond to leaf-to-air vapour pressure difference (VPD, D) in the dark (Bucci *et al*. 2004), that early successional, shade-intolerant species have higher nocturnal g_s than later-successional shade-tolerant species (Tobiessen 1982; Daley & Phillips 2006), and that, among temperate tree species, angiosperms tend to have higher nocturnal g_s than conifers (Barbour *et al*. 2005).

Nocturnal water loss in non-CAM plants is clearly maladaptive if one assumes that the plant has a fixed amount of water available to be transpired in a single diurnal cycle, and that the only benefit of water loss is carbon gain. However, transpiration incurs other benefits, associated with continued water movement through the plant. These may include improved nutrient acquisition due to higher total water flux through the plant (Masle, Farquhar & Wong 1992; McDonald, Erickson & Kruger 2002; Snyder *et al*. 2003), nocturnal recovery from xylem cavitation during the day (Snyder *et al*. 2003), prevention of excess leaf turgor at night (Donovan, Linton & Richards 2001) and continuation of O_2 delivery to xylem parenchyma in the stems of larger trees (Gansert 2003; Daley & Phillips 2006). The lost potential for daytime carbon gain may be offset by these benefits, particularly in environments with high soil water availability and/or low air saturation deficits at night, where nocturnal water loss is a small fraction of the diurnal total (Daley & Phillips 2006).

In a recent study of *Quercus rubra*, Barbour *et al*. (2005) found g_s to be significantly higher than estimates of cuticular conductance close to sunrise and sunset. That is, g_s typically increased before sunrise (well before net CO_2 assimilation rate became positive), and stomatal closure lagged behind the photosynthetic response to light near sunset. Stomatal aperture is known to be influenced by circadian rhythms (Raschke 1979; but see Williams & Gorton 1998), and stomatal opening prior to sunrise has been observed in at least two other species (Zeiger, Field & Mooney 1981). Interestingly, Barbour *et al*. (2005) also found that g_s at midnight correlated with g_s at midday on the previous day for all but the uppermost exposed leaves,

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suggesting that the daytime g_s versus night-time g_s relationship found across species (Snyder *et al*. 2003) may also hold within species.

Despite these observations of significant g_s at night in a wide range of species, the underlying mechanism is unknown. Furthermore, it is unknown whether existing models of g_s can predict observed patterns of nocturnal stomatal behaviour. In refining the formal basis of predictive models, it is often informative to study their behaviour under controlled conditions known to produce divergent responses in real plants. Stomatal sensitivity to leaf-to-air vapour pressure difference (VPD or D) varies with growth environment and measurement conditions, both within and among species (Oren *et al*. 1999; Bucci *et al*. 2004; Barbour *et al*. 2005). The purpose of this study was to explore the process basis of nocturnal stomatal regulation by measuring stomatal responses to D in the light and dark for castor bean (*Ricinus communis*) plants grown under a range of conditions, and comparing those observations with the predictions of several stomatal models.

MATERIALS AND METHODS

Growth conditions

Castor bean (*R. communis* L.) plants were grown in 10 L pots with potting mix and a slow-release fertilizer in two controlled-environment growth cabinets, one with high air saturation deficit and one with low. A green shade cloth stretched across half of each cabinet, and extending to the shelf on which the pots sat, reduced the photosynthetically active radiation (PAR) at leaf height by about one-third. The shade cloth was determined to be spectrally neutral by measuring absorption of 10 cloth samples at 2 nm intervals between 400 and 700 nm using a double beam spectrometer (model UV2-100; ATI Unicam, Cambridge, UK). Two experiments were conducted, the first to determine the range of g_s in the dark in *R. communis* and the second, at higher air saturation deficits (D_a) and two levels of soil water availability, to confirm the response of nocturnal g_s both to growth environment and to experimentally applied variation in D. Air temperature and relative humidity were measured every minute (Model 207 sensors; Campbell Scientific, Logan, UT, USA) and hourly averages recorded by data loggers for both cabinets during both experiments.

For the first experiment, average daytime air temperature and D_a within the growth cabinets were 24.4 °C and 1.39 kPa, and 26.0 °C and 0.66 kPa for the high and low D_a cabinets, respectively. Average dark air temperature and D_a were 17.9 °C and 0.50 kPa, and 18.7 °C and 0.17 kPa for the high and low D_a cabinets, respectively. Within the high D_a cabinet, PAR at leaf level was 315 and 540 μmol m^{-2} s^{-1} (for the low and high light environments, respectively). Within the low D_a cabinet, PAR at leaf level was 360 and 500 μmol m^{-2} s^{-1} for the low and high light environments, respectively. Much of the difference in PAR between the two cabinets was related to the height of the plants, as those in the low D_a cabinet were significantly taller than those in the high D_a cabinet. The day length was 13 h. Plants were well watered every second day (until the fourth leaf had expanded), or every day (after the fourth leaf had finished expanding), and grown until the sixth to eighth leaf emerged (depending on growth environment). Five plants were grown in each environment.

After confirming high nocturnal g_s for *R. communis*, a second experiment was conducted at higher D_a. For the second experiment, average daytime air temperature and D_a within the growth cabinets were 26.4 °C and 2.19 kPa, and 26.8 °C and 0.92 kPa for the high and low D_a cabinets, respectively. Average dark air temperature and D_a were 17.9 °C and 1.15 kPa, and 17.6 °C and 0.45 kPa for the high and low D_a cabinets, respectively. Within the high D_a cabinet, PAR at leaf level was 435 and 525 μmol m^{-2} s^{-1} for the low and high light environments, respectively. Within the low D_a cabinet, PAR at leaf level was 370 and 625 μmol m^{-2} s^{-1} for the low and high light environments. Again, most of the difference in PAR between the cabinets was related to differences in plant height. Day length was 14 h (increased by 1 h compared to experiment 1 to reduce the length of time spent on measurements each day). In the second experiment, six plants were grown in each environment and were well watered every day until the third leaf had expanded. Drainage holes in all pots were then sealed and the pots weighted. Three pots per growth environment remained well watered by adding water daily to maintain pot weight. Water was withheld from the remaining three pots in each growth environment for 7 d, after which the pots remained at lower water content by adding water daily to maintain (the lower) pot weight.

Stomatal conductance

Measurements of g_s were made on the youngest fully expanded leaf (leaves 4–6, depending on the growth environment) using portable photosynthesis systems (LI-6400; Li-Cor Inc., Lincoln, NE, USA). The standard 2×3 cm clear-top chamber was used for measurements in the light, and for dark measurements when $g_s > 0.02$ mol m^{-2} s^{-1}. A large custom-built leaf chamber was used when $g_s < 0.02$ mol m^{-2} s^{-1}. The chamber was milled from stainless steel and an area of 80 cm^2 was sealed with a closed cell foam gasket of 1 cm width. The enclosed leaf typically filled two-thirds of the 80 cm^2 area, and approached the maximum size recommended to ensure a well-mixed chamber volume (K. L. Griffin, personal communication). At the flow rates used for measurement, the air within the chamber turned over completely every 10 to 40 s. The large leaf area maximized the difference in vapour pressure between incoming and outgoing chamber air, reducing errors in calculated g_s. A thermocouple was placed within the chamber to measure leaf temperature, and a 30 L buffer volume was used to stabilize the CO_2 concentration of air entering the leaf chamber. In experiment 2, the vapour pressure of air entering the leaf chamber was altered by (1) inclusion of a water bubbler within the buffer volume for measurements at low D, (2) varying the flow rate through

the leaf chamber and (3) varying the flow rate through the LI-6400 vapour trap. This allowed variation of D over a range between 0.1 and 4.2 kPa, depending on the leaf transpiration rate.

The CO_2 and water vapour analysers were calibrated using the zero function and fresh chemicals (non-deliquescent, self-indicating soda lime granules; BDH Laboratory Supplies, Poole, UK; Drierite, WA Hammond, Xenia, OH, USA) prior to the start of measurements each day and night. Air was passed through the vapour trap for at least 50 min prior to zeroing of the analysers, to ensure accurate calibration. Temperature in the controlled-environment growth cabinets was extremely stable during all measurements, so additional analyser calibrations were not necessary (LI-6400 manual).

One system was used during experiment 1 and three systems during experiment 2. For experiment 1, a clear-top leaf chamber was used and g_s was measured under ambient conditions (i.e. temperature, D and CO_2 concentration were not controlled) for one leaf on each of the five plants per growth environment in the light, and for one leaf on each of three plants per growth environment in the dark. g_s was recorded when water and CO_2 fluxes had stabilized, typically 5–10 min after the leaf was placed in the chamber.

The three photosynthesis systems used in experiment 2 allowed the responses of leaves from all three replicate plants per growth environment to be measured simultaneously. Accordingly, g_s response curves in the dark and the light of all plants from a single growth environment were measured over a 15 h period on a single day. Leaf chamber temperature was not controlled by the photosynthesis system, but in the controlled-environment growth cabinets, air temperature was stable and leaf chamber temperature within 2 °C of air temperature. Well-watered plants were measured over the first 4 d after leaf 3 had finished expanding, and droughted plants measured over 4 d from 2 d after the low soil water level had been reached. Response curves took 5 to 6 h in the dark and 2.5 to 3 h in the light (three to five step changes in D).

Sensitivity analysis suggests that g_s measurements become inaccurate when the difference in vapour pressure between incoming and outgoing air ($w_i - w_o$) is less than 0.04 kPa (Barbour *et al*. 2005). Accordingly, the flow rate and leaf area within the custom-built large chamber were controlled to maintain $w_i - w_o > 0.04$ kPa. This allowed accurate measurement of g_s with a precision of at least ± 0.0005 mol m^{-2} s^{-1}.

The relationship between leaf area and boundary layer conductance was measured for the large leaf chamber using wet filter paper, and the procedure outlined in the LI-6400 manual. Two-sided leaf boundary layer conductance (g_b) was found to be related to leaf area within the chamber (L) by

$$g_b = g_{b0} + a \cdot e^{-aL/z}, \quad (1)$$

where $g_{b0} = 0.69$, $a = 0.97$, $z = 27.2$ ($r^2 = 0.999$). The area of each leaf enclosed within the chamber was measured after completion of gas-exchange measurements using an area meter (LI-3100; Li-Cor Inc.).

Lohammar function

To aid the analysis of the observed responses of g_s to D, we fitted them to an empirical function developed by Lohammar *et al*. (1980) and commonly modified as

$$g_s = g_{smin} \times \left(1 + \frac{D - D_{min}}{D_0}\right)^{-1} \quad (2)$$

where D_{min} is the smallest value of D in the data set to which Eqn 2 was fitted, and g_{smin} and D_0 are fitted parameters.

Leaf osmotic pressure

In experiment 2, a vapour pressure osmometer (Wescor 5500; Wescor Inc, Logan, UT, USA) was used to measure leaf osmotic pressure on leaf discs (diameter 6.2 mm) removed from a section of leaf adjacent to that enclosed within the leaf chamber, immediately after the last gas-exchange measurement was made. Values are presented as positive numbers and symbolized Π (MPa). Three discs were measured from each leaf in both the light and the dark. The SD for a single leaf was typically 0.07 MPa (cf. 0.01 MPa for repeated measurements of standard solutions). The osmometer was calibrated prior to the start of measurements on the first day using new standard solutions (300 and 1000 mmol kg^{-1}, Wescor Inc), following procedures outlined in the instrument manual. Subsequent calibrations were deemed unnecessary as the temperature in the laboratory remained stable (within 2 °C) over the measurement period and the instrument remained on during this time.

Soil water potential

Soil water potential (ψ_s) was measured for three pots at each soil water availability using calibrated tensiometers. Measurements were made just prior to daily watering at the end of the experiment, so measured ψ_s values likely represent diurnal minima. ψ_s of well-watered pots was -0.0022 ± 0.0001 MPa, and droughted pots -0.0101 ± 0.0009 MPa.

'Operational' leaf-to-air saturation deficit (D_{op}) and stomatal conductance ($g_{s,op}$)

The air within the leaf chamber tends to be warmer and leaf boundary layer conductances lower than for leaves outside the chamber. This results in higher D within the chamber during measurement than that typically experienced by the leaf. To compare stomatal sensitivities to D under growth conditions, we estimated an 'operational' D (D_{op}) for each leaf. Leaf temperature in the light and the dark was measured over three 15 h periods during the days of D response measurements in experiment 2, using an infrared

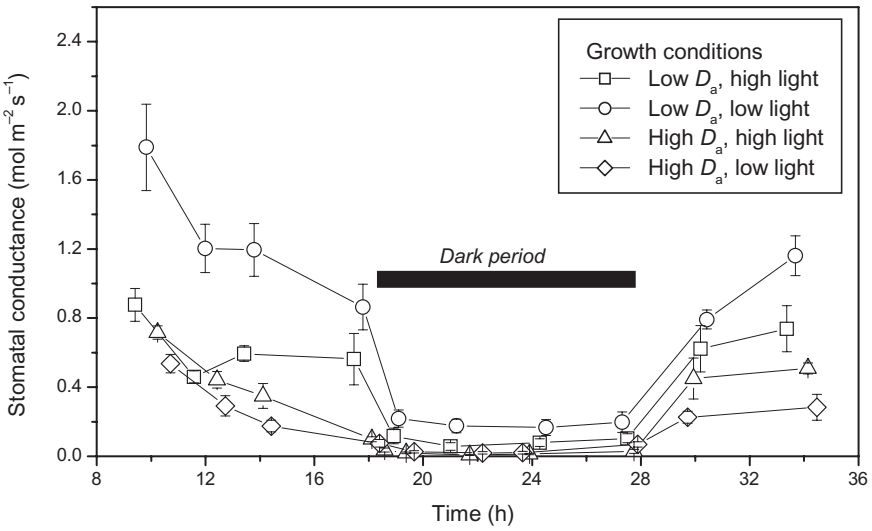

Figure 1. Average stomatal conductance over a 26 h period for *Ricinus communis* plants in four growth environments in experiment 1. Error bars represent the SEs ($n = 3$ or 5 plants). D_a, vapour saturation deficit of air.

thermometer (AG42; Telatemp Corp., Fullerton, CA, USA). Hourly average air temperature and relative humidity within the growth cabinets were combined with average leaf temperature to calculate D_{op} for the leaf used for gas-exchange measurements on each plant. We then applied these values of D_{op} to the fitted Lohammar functions to calculate values of g_s at D_{op} ($g_{s,op}$) for each growth environment in the light and the dark.

RESULTS

A diurnal course of stomatal conductance in experiment 1 confirmed high g_s in both the light and the dark, particularly at low growth D_a. In the dark, g_s varied between 0.006 and 0.218 mol m^{-2} s^{-1} (the lowest for high D_a, low light-grown plants and the highest for low D_a, low light-grown plants, respectively). Stomatal conductance started to increase before the cabinet lights came on for leaves from all growth environments, so that g_s was lowest near the middle of the dark period in all cases (Fig. 1).

Growth environment had significant effects on g_s both in the light and the dark. Plants grown at high D_a had lower g_s, both in the light and in the dark, than plants grown at low D_a. The effect of growth light level differed between plants grown at high and low D_a: among high D_a-grown plants, g_s was slightly greater in high light-grown plants, whereas among low D_a-grown plants, g_s was substantially lower in high light-grown plants (Fig. 1).

Typical stomatal responses to D (i.e. declining in a saturating fashion) were observed at growth light levels for all leaves in experiment 2 (Figs 2 & 3). The responses were not significantly different among well-watered plants

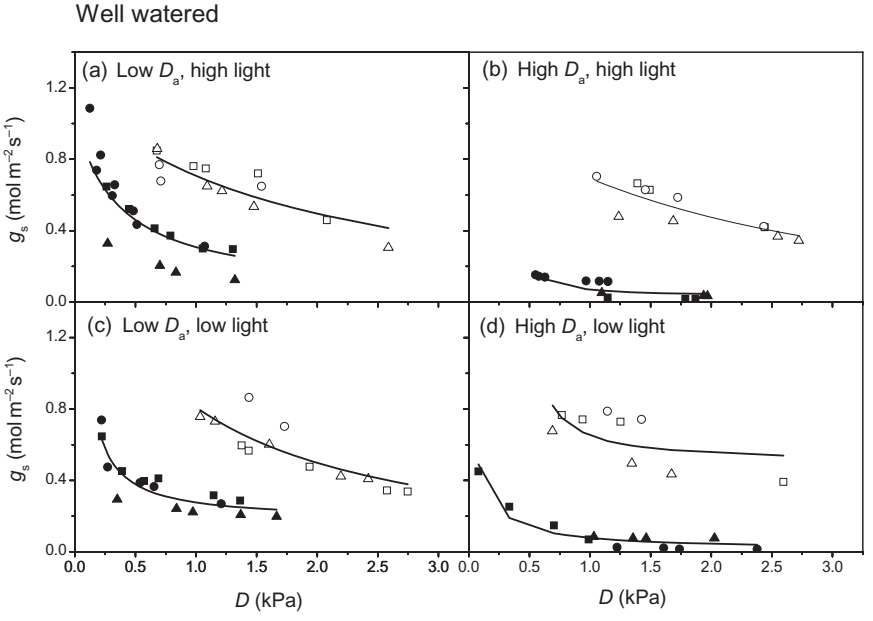

Figure 2. Stomatal response to leaf-air saturation deficit (D) during the day (open symbols) and the night (closed symbols) for well-watered *Ricinus communis* plants grown under four light and air saturation deficit environments: low D_a and high light (a); high D_a and high light (b); low D_a and low light (c); and high D_a and low light (d). Square, circular and triangular symbols represent leaves from different plants within the same growth environment. The fitted functions are modified Lohammar functions (Eqn 2), with parameters given in Table 1. D_a, vapour saturation deficit of air.

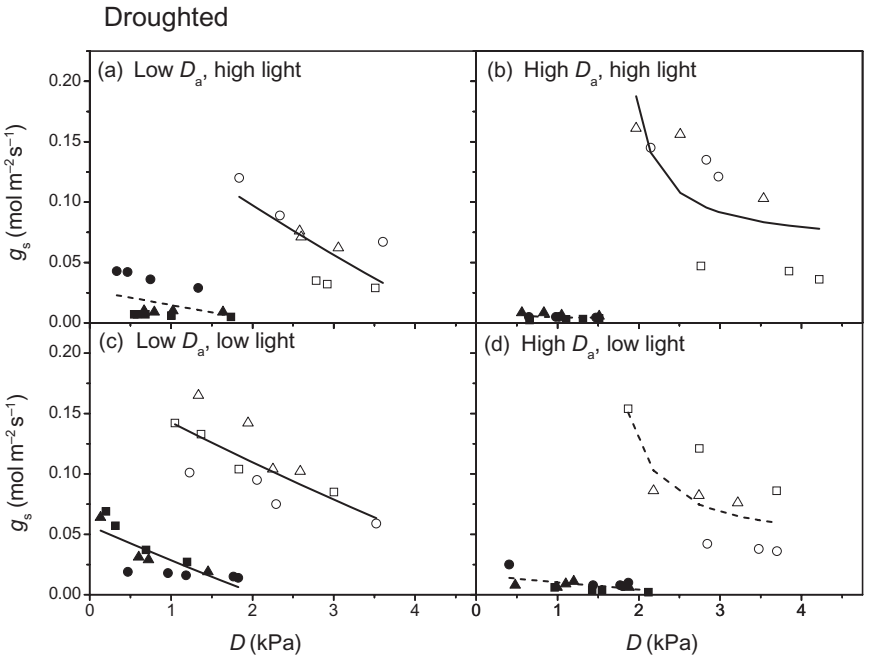

Figure 3. Stomatal response to leaf-air saturation deficit (D) during the day (open symbols) and the night (closed symbols) for droughted *Ricinus communis* plants grown under four light and air saturation deficit environments: low D_a and high light (a); high D_a and high light (b); low D_a and low light (c); and high D_a and low light (d). Square, circular and triangular symbols represent leaves from different plants within the same growth environment. The fitted relationships are modified Lohammar functions (Eqn 2), and fitted parameters are listed in Table 1. D_a, vapour saturation deficit of air.

(from fitted Lohammar parameters), although the fitted Lohammar functions revealed slightly lower values of D_0 in the light for plants grown at low D_a and low light (Table 1). As expected, drought significantly reduced g_s in the light (g_s at 1.5 kPa D was between 0.57 and 0.61 mol m^{-2} s^{-1} for well-watered plants, and between 0.12 and 0.21 mol m^{-2} s^{-1} for droughted plants), although the g_s response to D was retained in the light among droughted plants (Fig. 3).

Stomata were observed to be open in the dark in all growth environments in experiment 2 and significant g_s responses to D were observed in all well-watered plants (Fig. 2). Drought significantly reduced g_s in the dark (g_s at 1.5 kPa D was between 0.05 and 0.24 mol m^{-2} s^{-1} for well-watered plants, and between 0.004 and 0.015 mol m^{-2} s^{-1} for droughted plants), although measured g_s was still significantly higher than values of cuticular conductance (e.g. Šantrůček *et al.* 2004) (Fig. 3). The Lohammar function adequately described the g_s response to D for well-watered plants in the dark (r^2 values between 0.69 and 0.96; Table 1), but for droughted plants only those grown at low D_a and low light were found to show significantly decreasing g_s with increasing D (Table 1, Fig. 3).

Table 1. Modified Lohammar function (Eqn 2) parameters fitted to the stomatal conductance (g_s) response to D in experiment 2

Growth environment			Measured				
D_a	Light	Water status	Light/dark	g_{smin}	D_{min}	D_0	r^2
Low	High	WW	Light	0.89 ± 0.06	0.5	1.89 ± 0.51	0.75
Low	Low	WW	Light	1.25 ± 0.29	0.5	0.99 ± 0.46	0.68
High	High	WW	Light	0.78 ± 0.08	0.8	1.82 ± 0.63	0.70
High	Low	WW	Light	0.89 ± 0.13	0.4	2.14 ± 1.23	0.54
Low	High	WW	Dark	1.15 ± 0.10	0.0	0.39 ± 0.40	0.96
Low	Low	WW	Dark	0.73 ± 0.09	0.0	0.75 ± 0.24	0.81
High	High	WW	Dark	0.22 ± 0.02	0.4	0.40 ± 0.26	0.69
High	Low	WW	Dark	0.73 ± 0.13	0.0	0.13 ± 0.04	0.95
Low	High	D	Light	0.20 ± 0.07	1.5	0.55 ± 0.32	0.67
Low	Low	D	Light	0.16 ± 0.02	0.8	1.77 ± 0.80	0.68
High	High	D	Light	0.26 ± 0.11	1.5	0.87 ± 0.55	0.58
High	Low	D	Light	0.22 ± 0.07	1.5	0.09 ± 0.55	0.55
Low	High	D	Dark	0.57 ± 8.30	0.0	0.02 ± 0.36	0.28
Low	Low	D	Dark	0.16 ± 0.09	0.0	0.15 ± 0.11	0.81
High	High	D	Dark	0.62 ± 1.37	0.4	0.51 ± 1.40	0.54
High	Low	D	Dark	0.02 ± 0.04	0.2	0.61 ± 0.43	0.67

WW, well watered; D, droughted; D_a, vapour saturation deficit of air.

Table 2. List of quantities referenced in this article, with definitions and dimensions

Symbol	Dimensions	Definition
g_s	mol air m^{-2} s^{-1}	Stomatal conductance to H_2O
$g_{s,op}$	mol air m^{-2} s^{-1}	Operational value of g_s
g_b	mol air m^{-2} s^{-1}	Boundary layer conductance to H_2O
D	kPa	Leaf to air vapour pressure difference
D_{op}	kPa	Operational value of D
D_{min}	kPa	Lowest D in each data set fitted to Lohammar model
D_a	kPa	Vapour saturation deficit of air
E	μmol H_2O m^{-2} s^{-1}	Transpiration rate
E_{op}	μmol H_2O m^{-2} s^{-1}	Operational value of E
g_{smin}	mol air m^{-2} s^{-1}	y-Intercept of Lohammar model
D_0	kPa	D sensitivity parameter in Lohammar model
A_n, A_m	mol CO2 m^{-2} s^{-1}	Net and CO_2-saturated CO_2 assimilation rates
c_i, c_a	μmol mol^{-1}	Intercellular and ambient CO_2 mole fractions
Γ	μmol mol^{-1}	Photorespiratory CO_2 compensation point
π_g, π_e, π_a	MPa	Guard cell, epidermal cell and apoplastic osmotic pressure
Π	MPa	Bulk leaf osmotic pressure
ψ_s	MPa	Soil water potential
R, r_{eg}	MPa [mol H_2O m^{-2} s^{-1}]$^{-1}$	Soil-epidermis and epidermis-guard cell hydraulic resistance
f_g	Dimensionless	Fraction of E that occurs directly from guard cells
ρ	Dimensionless	Ratio of $f_g r_{eg}$ to R
B	Dimensionless	Sensitivity of π_g to P_e
M	Dimensionless	Net epidermal mechanical advantage
S	Dimensionless	Relative sensitivity of g_s to D ($-\partial \ln g_s/\partial \ln D$)
$S_{1.5}$, S_{op}	Dimensionless	Values of S at D = 1.5 kPa and $D = D_{op}$, respectively

The average 'operational' leaf-to-air saturation deficit, D_{op}, increased with growth D_a for a given watering regime, and was greater for droughted plants at a given growth D_a (Fig. 4). Stomatal conductance and transpiration rate at D_{op} ($g_{s,op}$ and E_{op}, respectively), calculated by applying D_{op} to the Lohammar function fitted to each leaf, were lower in droughted plants than in well-watered plants under all conditions (Fig. 4). $g_{s,op}$ and E_{op} were also lower in the dark than in the light for all plants [except those grown at low D_a and high light (Fig. 4), where $g_{s,op}$ was similar in the light and dark]. $g_{s,op}$ in the dark tended to increase with increasing $g_{s,op}$ in the light (Fig. 5).

Relative stomatal sensitivity to D [$-\partial \ln g_s/\partial \ln D$, calculated from each leaf's fitted Lohammar parameters at $D = D_{op}$ (S_{op}) and at D = 1.5 kPa ($S_{1.5}$)] was generally higher in plants grown or measured under conditions expected to promote reduced water status (Fig. 6). For example, both $S_{1.5}$ and S_{op} were higher for plants grown at high D_a than at low D_a, for plants grown in droughted soil than in well-watered soil, and for plants measured in the light than in the dark. Two exceptions occurred among well-watered plants: when measured in the light, S_{op} was lower in plants grown at high D_a than at low D_a; and for plants grown at high D_a, both S_{op} and $S_{1.5}$ were higher in the dark than in the light.

Leaf osmotic pressure (Π, Fig. 6) was higher for plants grown in droughted soil or at high D_a, but Π was unaffected by growth lighting regime. Interestingly, among well-watered plants, Π was significantly higher ($P < 0.001$, from Scheffe post hoc analysis) in the dark (8 h after the start of the dark period) than in the light (5 h after the start of the light period). One exception to these trends was the occurrence of lower Π in droughted than in well-watered plants, when grown at low D_a and low light and measured in the dark. Prevailing stomatal conductance ($g_{s,op}$) was typically lower in plants with greater Π (Fig. 7a). High-Π plants also showed reduced stomatal sensitivity to D at D_{op} (S_{op}; $P = 0.024$; Fig. 7b) when measured in the dark, but not when measured in the light.

DISCUSSION

Stomatal response to *D* persists in the dark among well-watered plants

We observed stomata to be open in the dark (i.e. g_s was significantly greater than published estimates of g_c; e.g. Šantrůček *et al*. 2004) for *R. communis* under a wide range of growth conditions. Even when leaf osmotic pressure was increased 1.5- to 2-fold by drought, stomata remained open in the dark. Under conditions of water stress but high nutrient availability, as experienced by the droughted plants in experiment 2, it is difficult to imagine a benefit to herbaceous plants in keeping stomata open in the dark. It seems likely that, in this case, the stomata were simply unable to fully close in the dark.

Stomatal conductance was lower in the dark than in the light, at the same measurement D. The stomatal response to D persisted in the dark for all well-watered plants. The relative sensitivity of that response ($-\partial \ln g_s/\partial \ln D$) was higher in the dark than in the light among well-watered plants grown at high ambient D_a, but lower in the dark than in the light for plants grown at low ambient D_a and at both

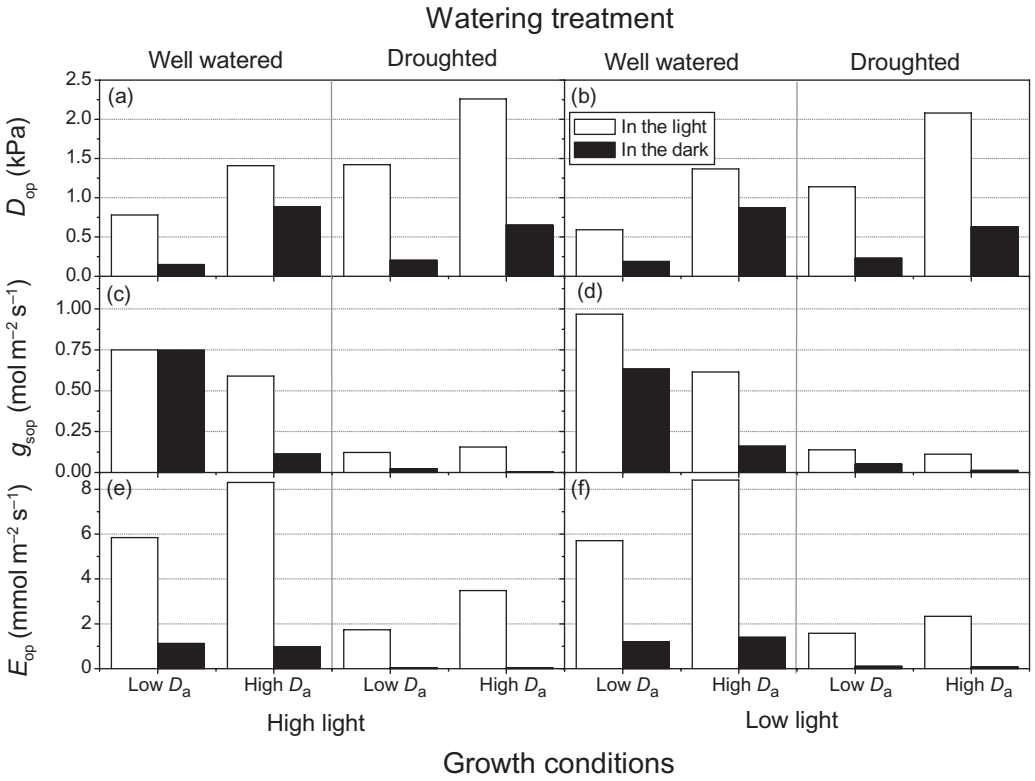

Figure 4. Daytime and nocturnal values under ambient conditions for: the leaf-air saturation deficit (a,b); calculated stomatal conductance (c,d); and calculated transpiration rate, for *Ricinus communis* plants grown under well-watered and droughted conditions, two ambient air saturation deficits and at high (a,c,e) and low (b,d,f) light. E_{op}, operational value of transpiration rate; $g_{s,op}$, operational value of stomatal conductance; D_{op}, operational value of leaf to air vapour pressure difference; D_a, vapour saturation deficit of air.

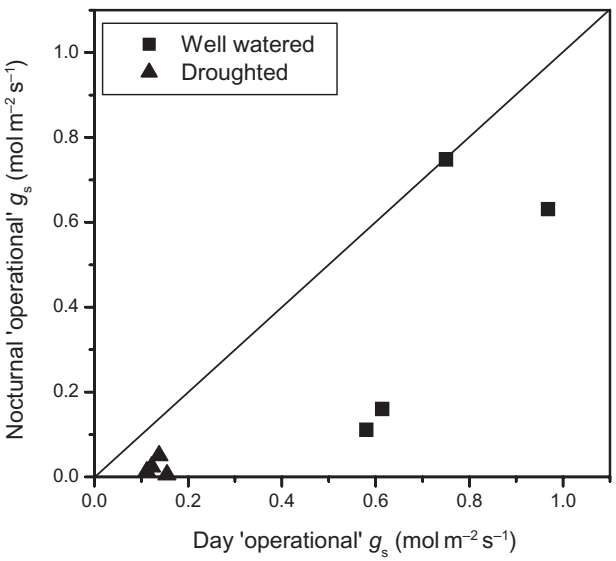

Figure 5. Relationship between stomatal conductance to water vapour (g_s) at ambient leaf-air saturation deficit in the light and the dark for *Ricinus communis* plants grown in eight air saturation deficit, light and water availability environments. The 1:1 line is shown.

high and low ambient D_a when droughted. Although significant g_s responses to D were found among droughted plants in the light, stomata were generally rather unresponsive to D in the dark in droughted plants. The two levels of growth light applied in experiment 2 generally did not result in significantly different responses of stomata to D between treatments, perhaps because the difference in treatment PAR was not large. This result needs to be confirmed with both higher and lower light treatments than those described here.

Do existing stomatal models predict responses to *D* in the dark?

Our results suggest that models of energy, water and carbon exchange between terrestrial vegetation and the atmosphere should include, as a minimum, non-zero g_s in the dark, particularly under conditions of non-zero nocturnal D. Additionally, because the oxygen isotope composition of leaf-respired CO_2 is strongly influenced by g_s (Cernusak *et al*. 2004; Barbour *et al*. 2005), inclusion of a stomatal response to D at night is likely to be of vital importance in models that predict the stable oxygen isotope composition of ecosystem-respired CO_2. Less clear is what model to use. As stomata are generally assumed to be closed at night, models of g_s are often based upon patterns and processes

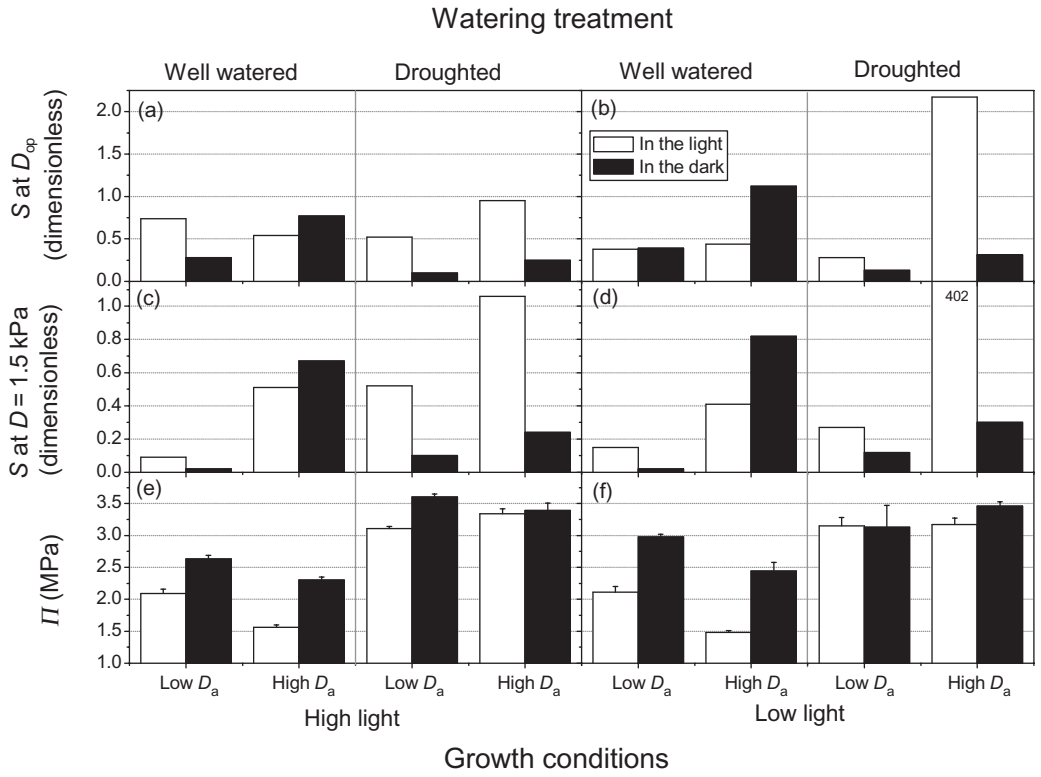

Figure 6. Daytime and nocturnal values for relative sensitivity of g_s to D at operational D (a,b); relative sensitivity to D at the reference D of 1.5 kPa (c,d); and leaf osmotic pressure (e,f), for *Ricinus communis* plants grown under well-watered and droughted conditions, two ambient air saturation deficits and at high (a,c,e) and low (b,d,f) light.

that are known to apply during the daytime. However, those patterns may not be sufficient to predict the behaviour of g_s in the dark. In the next sections we evaluate four models of stomatal conductance with respect to their predictions concerning stomatal behaviour in the dark.

Ball–Berry-Leuning (BBL) model

The model of stomatal functioning developed by Ball, Woodrow & Berry (1987), and later modified by Leuning (1995) (hereafter the BBL model), describes the commonly observed correlation between g_s and net CO_2 assimilation rate (A_n) and stomatal closure in response to increased D:

$$g_s = g_0 + \frac{a\phi A_n}{(c_i - \Gamma)(1 + bD)} \quad (3)$$

where g_0 is the value of g_s at the light compensation point, a and b are empirical coefficients, c_i is intercellular CO_2 concentration, Γ is the CO_2 compensation point and ϕ is a soil limitation factor that depends on soil water potential (ψ_s), allowing g_s to decline as the soil dries (Walcroft *et al.* 1997).

In the dark, A_n is typically negative and much smaller in magnitude than in the light. Although c_i is difficult to measure accurately in practice under these conditions, it must exceed ambient CO_2 if $A_n < 0$, so the quantity $(c_i - \Gamma)$ will be larger in the dark than in the light. Therefore, the quantity $a\phi A_n / (c_i - \Gamma)$ typically reverses signs and becomes much smaller in magnitude in the dark. As a result, the BBL model predicts very small *positive* responses of g_s to D in the dark, provided g_0 is large enough to ensure g_s remains positive (otherwise, the BBL model simply predicts stomatal closure in the dark).

Jarvis–Davies (JD) model

Jarvis & Davies (1998) presented a model (hereafter the JD model) that describes g_s as the product of two quantities, each representing a negative feedback putatively involved in regulating g_s. The first is the 'residual photosynthetic capacity', $A_m - A_n$ (where A_m is the value of A_n in the absence of stomatal diffusion constraints, i.e. $c_i = c_a$, but at ambient light). The second quantity, $G - sE$, describes the negative response of g_s to transpiration rate, E (Mott & Parkhurst 1991), where G and s are empirical coefficients. These assumptions lead to

$$g_s = \frac{G(A_m - A_n)}{1 + sD(A_m - A_n)} \quad (4)$$

Although the JD model's formulation in terms of photosynthetic properties indicates that it was not meant to explain stomatal behaviour in darkness, its empirical validity in darkness can still be assessed. In the dark, $A_n \leq 0$ and $c_i \geq c_a$. Because elevated CO_2 concentration suppresses mitochondrial respiration, A_n will generally be smaller in

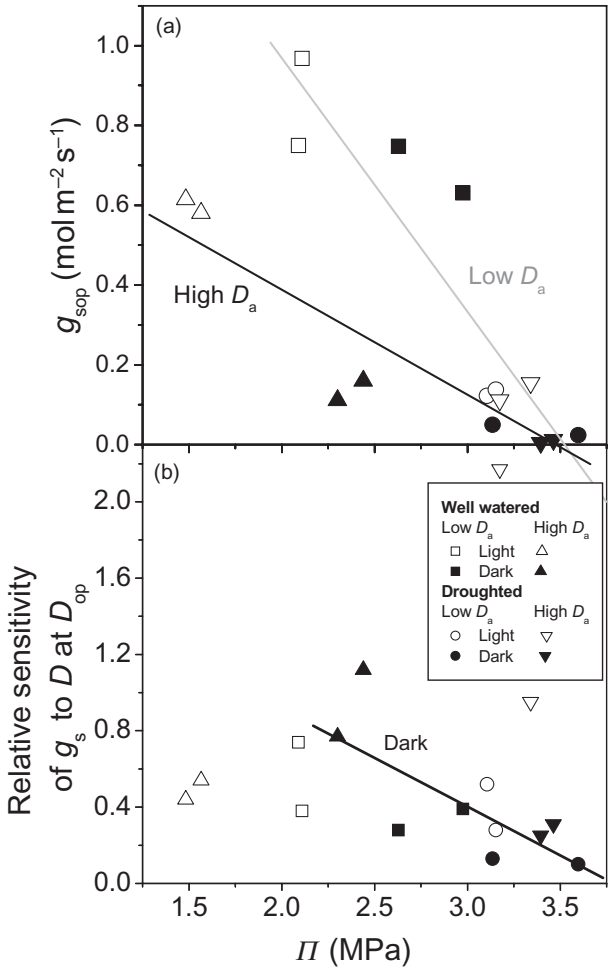

Figure 7. Relationships between leaf osmotic pressure and: stomatal conductance (g_s) at ambient leaf-air saturation deficit (a); and the relative sensitivity of g_s to leaf-air saturation deficit at ambient leaf-air saturation deficit (b) for *Ricinus communis* plants grown in eight air saturation deficit, light and water availability environments. In (a), the black line represents the fitted relationship among plants grown at high air saturation deficit ($g_{s,op} = 0.91–0.26\Pi$, $r^2 = 0.80$, $P = 0.003$), and the grey line the fitted relationship among plants grown at low air saturation deficit ($g_{s,op} = 2.24–0.64\Pi$, $r^2 = 0.79$, $P = 0.003$). In (b), the line represents the fitted relationship among measurements made in the dark ($-\partial \ln g_s / \partial \ln D = 2.08–0.55\Pi$, $r^2 = 0.60$, $P = 0.024$). D_a, vapour saturation deficit of air.

magnitude than it would be if c_i equalled c_a; that is, $A_n \geq A_m$ or $A_m - A_n \leq 0$ in the dark. For this to coincide with stomatal opening ($g_s > 0$), the quantity $A_m - A_n$ must be more negative than $-1/sD$, causing the response to D in darkness to become positive. However, that state cannot be achieved without $A_m - A_n$ first exceeding $-1/sD$ (in which case Eqn 4 predicts $g_s < 0$) and then equalling $-1/sD$ (in which case Eqn 4 is undefined). The JD model therefore cannot predict stomatal opening in darkness without modifying its numerical implementation to exclude periods when $0 > A_m - A_n \geq -1/sD$, and it would predict positive responses to D in the darkness in any event.

Dewar model and Buckley–Mott–Farquhar (BMF) model

Dewar (1995, 2002) and Buckley, Mott & Farquhar (2003) derived models by applying principles of plant water relations to the observed relationship between stomatal aperture and epidermal and guard cell turgor pressures (P_e and P_g, respectively): $g_s = \chi[P_g - (1 + M) \cdot P_e]$, where χ is an empirical scaling factor and M is the net mechanical advantage of the epidermis (most data suggest $M > 0$; e.g. Franks, Cowan & Farquhar 1998). In turn, P_e and P_g depend on g_s via transpiration rate, and on several other biophysical variables: soil water potential (ψ_s), epidermal and guard cell osmotic pressures (π_e and π_g), the hydraulic resistances from soil to epidermis (R) and from epidermal to guard cells (r_{eg}), and the fraction of leaf transpiration that occurs directly from guard cells (f_g).

If $M > 0$, the factors predict a positive response of g_s to increased D, contrary to observations. To resolve the dilemma, Dewar (2002) hypothesized that $M = 0$ and $f_g r_{eg} >> 0$:

$$g_s = \chi \frac{\pi_g - \pi_e}{1 + \chi f_g r_{eg} D} \quad (5)$$

Buckley *et al.* (2003) instead hypothesized, after Haefner, Buckley & Mott (1997), that the guard cell osmotic gradient is directly sensitive to P_e, such that $\pi_g = BP_e + \pi_a$, where π_a is apoplastic osmotic pressure. This implies

$$g_s = \chi \frac{(B - M)(\psi_s + \pi_e) - \pi_e + \pi_a}{1 + \chi RD(B - M + \rho)} \quad (6)$$

where $\rho = f_g r_{eg}/R$. (see Buckley *et al.* 2003 for derivations of Eqns 5 and 6). The Dewar model as given in Eqn 5 is consistent with both nocturnal opening and a persistent negative D response in the dark, provided $\pi_g - \pi_e$ can be substantially positive in the dark. In the BMF model, stomatal opening requires $B > M + (\pi_e - \pi_a) / (\psi_s + \pi_e)$, and a negative response of g_s to D implies $B > M - \rho$. However, $\rho \geq 0$ by definition, and $\psi_s + \pi_e \geq 0$ provided $P_e \geq 0$ (because $P_e = \psi_e + \pi_e \geq 0$ and $\psi_e \leq \psi_s$). Therefore, provided B can be substantially positive in the dark (and assuming $\pi_e > \pi_a$), the BMF model is also consistent with both nocturnal opening and a persistent negative D response in the dark.

Dewar (2002) further suggested that $\pi_g - \pi_e$ should depend on the pool of energy available to drive active solute uptake in guard cells. Similarly, Buckley *et al.* (2003) hypothesized that B is proportional to guard cell ATP content (τ), based on Farquhar & Wong's (1984) derivation of the behaviour of chloroplast-derived ATP in the photosynthesis model of Farquhar, von Caemmerer & Berry (1980). Because mitochondrial respiration represents a large ATP source that persists in the dark, this hypothesis does not preclude opening or negative D-sensitivity in the dark. Dewar (2002) used the ratio of gross photosynthesis rate to c_i as a convenient measure of chloroplastic reducing

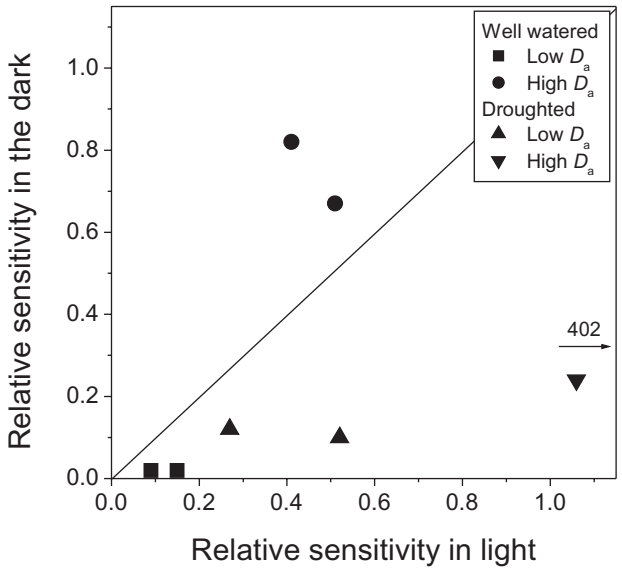

Figure 8. Relationship between the relative sensitivity of stomatal conductance to leaf-air saturation deficit, at the reference D of 1.5 kPa, in the light and the dark for *Ricinus communis* plants grown in eight air saturation deficit, light and water availability environments. The 1:1 line is shown, and the value for high D_a-grown, droughted plants that is off scale is indicated with an arrow. D_a, vapour saturation deficit of air.

power, which cannot predict nocturnal opening. However, if that measure is replaced with τ, the Dewar model remains consistent with our results.

The major distinction between these two models with respect to D responses in the dark therefore lies in the presence of the parameters B and R in the denominator of the BMF model. This predicts direct effects of guard cell osmoregulation and plant hydraulic resistance on the stomatal response to D, whereas the Dewar model predicts no such effects. To examine this more closely, we may compute the relative sensitivity of g_s to D ($S = -\partial \ln g_s / \partial \ln D$), S is given by Eqns 7 and 8 for the Dewar and BMF models, respectively:

$$S = \frac{\chi f_g r_{eg} D}{1 + \chi f_g r_{eg} D} \quad \text{(Dewar)} \tag{7}$$

$$S = \frac{\chi f_g r_{eg} D + \chi R(B - M)D}{1 + \chi f_g r_{eg} D + \chi R(B - M)D} \quad \text{(BMF)} \tag{8}$$

(The product ρR has been expanded as $f_g r_{eg}$ in Eqn 8 to aid comparison with Eqn 7.) We found S to be much larger in the light than in darkness under most conditions (Figs 6 & 8). This is easier to reconcile with Eqn 8 than with Eqn 7, because the parameter B in the former should generally be larger in the light than in the dark. A notable exception to this trend occurred for plants grown under well-watered conditions at high D_a, where sensitivity was somewhat larger in the dark – particularly for plants grown at low light. Intriguingly, the nocturnal elevation of bulk leaf osmotic pressure (Π) was also greatest in those treatments (Fig. 6). The BMF model also predicts greater sensitivity to D in light or darkness when plant hydraulic resistance (R) is elevated, whereas the Dewar model predicts no effect of R on S. These predictions could be tested by measuring S before and after inducing a step increase in R (e.g. by notching the stem or petiole), both in the light and in darkness.

CONCLUSIONS

Stomata remain partially open and sensitive to D in the dark in *R. communis* grown under a range of controlled conditions. Stomatal conductance was lower and stomata were typically less sensitive to D in the dark than in the light. In contrast, g_s was lower but sensitivity greater in plants grown under less favourable hydraulic conditions (at high D_a or in droughted soil), relative to more favourable conditions, all else being equal.

The BBL and JD models of g_s were unable to predict a persistent negative stomatal response to D in darkness, in contrast to the BMF model and the Dewar model (provided the latter is modified to remove its dependence on photosynthetic rate). Predictions of the latter two models diverge with respect to the relative sensitivity of g_s to D (S): whereas the Dewar model predicts no differences in S between light and darkness, the BMF model predicts greater sensitivity in the light than in darkness – consistent with our results under most conditions. Additionally, the BMF model predicts greater S when plant hydraulic resistance is increased, in contrast to the Dewar model. We did not measure R, so the latter prediction awaits further experimental tests.

The demonstration of significant and highly variable stomatal responses to D in dark-opened stomata, together with the conclusion that two common empirical models of stomatal control cannot predict those responses, suggests a need for incorporation of process-based stomatal models in simulations of plant gas exchange. This is particularly relevant when nocturnal dynamics of leaf-air vapour exchange are of interest, as, for example, when examining variations in the oxygen isotopic composition of leaf-respired CO_2.

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