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How do leaf hydraulics limit stomatal conductance at high water vapour pressure deficits?

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ABSTRACT

A reduction in leaf stomatal conductance (g) with increasing leaf-to-air difference in water vapour pressure (D) is nearly ubiquitous. Ecological comparisons of sensitivity have led to the hypothesis that the reduction in g with increasing D serves to maintain leaf water potentials above those that would cause loss of hydraulic conductance. A reduction in leaf water potential is commonly hypothesized to cause stomatal closure at high D. The importance of these particular hydraulic factors was tested by exposing *Abutilon theophrasti*, *Glycine max*, *Gossypium hirsutum* and *Xanthium strumarium* to D high enough to reduce g and then decreasing ambient carbon dioxide concentration ($[CO_2]$), and observing the resulting changes in g, transpiration rate and leaf water potential, and their reversibility. Reducing the $[CO_2]$ at high D increased g and transpiration rate and lowered leaf water potential. The abnormally high transpiration rates did not result in reductions in hydraulic conductance. Results indicate that low water potential effects on g at high D could be overcome by low $[CO_2]$, and that even lower leaf water potentials did not cause a reduction in hydraulic conductance in these well-watered plants. Reduced g at high D in these species resulted primarily from increased stomatal sensitivity to $[CO_2]$ at high D, and this increased sensitivity may mediate stomatal responses to leaf hydraulics at high D.

Key-words: carbon dioxide concentration; hydraulic conductance; leaf water potential; transpiration.

INTRODUCTION

At air levels of carbon dioxide concentration ($[CO_2]$), increasing the leaf-to-air difference in water vapour pressure (D) reduces leaf stomatal conductance (g) in most species. This response limits transpiration rates at high D and affects the energy balance of vegetation. It also limits carbon dioxide supply to the leaf interior and often reduces carbon dioxide assimilation rates. The reduction in g at high D can therefore have important effects on multiple functions of ecosystems. Because increasing D would increase transpiration, it seems logical to assume that the resulting stomatal closure is caused by a reduction in water potential somewhere in the plant. However, the mechanism behind stomatal responses to D remains uncertain.

One possible role of low leaf water potential in reducing g at high D would be by decreasing hydraulic conductance. Sensitivity of conductance to D varies widely among species (Franks & Farquhar 1999). Comparisons among species indicating a correlation between maximum g and the sensitivity of conductance to D (Oren *et al*. 1999), combined with observations that low water potentials may lead to cavitation of xylem and loss of hydraulic conductance (Sperry 2000), have led to the concept that, by limiting transpiration, the reduction in g with increasing D serves to maintain leaf water potentials above those that would cause cavitation of xylem and loss of hydraulic conductance (Oren *et al*. 1999). The reduction in g at high D is often such that steady-state transpiration rates become insensitive to changes in D at high D. This would seem to require a feedback regulation mechanism, and a reduction in leaf water potential is often suggested as the proximate signal that limits g as D increases (Oren *et al*. 1999). In support of these concepts, there are numerous studies indicating that reductions in hydraulic conductance cause a reduction in g (e.g. Salleo *et al*. 2000; Sperry, Alder & Easlack 1993). Observations of midday depressions of g in a tropical tree have suggested that reduced midday hydraulic conductance may cause the midday stomatal closure, with leaf water potential mediating the stomatal response (Brodribb & Holbrook 2004).

In this work, I tested whether higher transpiration rates and lower leaf water potentials than normally occur at high D would reduce hydraulic conductance in four herbaceous species. This was accomplished by exposing plants to *D*-values high enough that g was reduced, and then lowering the ambient $[CO_2]$ to increase g and transpiration rate. Responses of g, transpiration rate, leaf water potential and their reversibility were examined. Because stomatal responses to high D may vary depending on whether only parts of leaves or whole shoots are exposed to high D (e.g. Bunce 2003), and because it was anticipated that leaf water potential responses might also differ with the amount of tissue exposed to high D, parallel experiments were conducted exposing leaves or whole shoots to high D. In all of these experiments, the plant leaves were deliberately

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exposed to values of D higher than those at which they were grown, in order to increase the probability that they would be under water stress at high D. However, all plants were well watered.

There has been a lengthy debate about whether increased transpiration and consequent reduction in bulk leaf water potential cause the observed stomatal closure at high D (cf. Monteith 1995). Observations of decreases in steady-state transpiration rate with increases in D, a 'feedforward' response (Farquhar 1978), have long been cited as evidence against bulk leaf water potential controlling g at high D. However, Franks, Cowan & Farquhar (1997) have recently questioned whether such evidence is conclusive, because the stomatal closure at high D may not be rapidly reversible. Furthermore, because changes in g must lag imposed changes in D, transient reductions in leaf water potential as D is increased are inevitable, and slow recovery of g from temporary reductions in leaf water potential could be mistaken for feedforward responses. Because I observed large increases in g at high D in response to low $[CO_2]$s, I examined whether D affected the sensitivity of g to $[CO_2]$, and also tested whether increased light could also increase g despite high D.

MATERIALS AND METHODS

Plants of *Glycine max* L. cv. Kent, *Gossipyum hirsutum* L. cv. Stoneville 474, and *Abutilon theophrasti* L and *Xanthium strumarium* L. from local Beltsville, MD, USA populations were grown separately in controlled environment chambers. The plants were grown in 20 cm diameter plastic pots filled with vermiculite and flushed daily with a complete nutrient solution containing 14.5 mM nitrogen. Chamber day/night air temperatures were 26/20 °C, dew point temperature was 18 °C, for a daytime D of about 1.3 kPa. There were 14 h d^{-1} of light at a photosynthetic photon flux density (PPFD) of 1000 μmol m^{-2} s^{-1} from a mixture of high pressure sodium and metal halide lamps. The $[CO_2]$ was kept between 370 and 390 μmol mol^{-1} by injection of carbon dioxide or air scrubbed of carbon dioxide, under the control of an absolute infrared carbon dioxide analyser, which sampled chamber air continuously.

Experiments were conducted on plants 3–4 weeks after seeding, with total leaf areas of 450–800 cm^2. Experiments using single leaves were conducted on the most recent fully expanded leaves, which were main stem trifoliolate leaf numbers 3 or 4 in *G. max*, main stem leaf numbers 5 or 6 in *A. theophrasti* and main stem leaf numbers 3 or 4 in the other species.

Initial experiments were conducted exposing parts of leaves to high D and then lowering the $[CO_2]$. The purpose was to determine a range of values of D where g consistently decreased with increasing D, and to determine if g and transpiration rate could be increased by lowering $[CO_2]$ despite high D. For these experiments, a broad leaf cuvette was used with a CIRAS-2 portable photosynthesis system. The cuvette enclosed 2.5 cm^2 of a single leaf, while the rest of the plant was exposed to the daytime growth conditions. The section of leaf in the cuvette was exposed to a PPFD of 1500 μmol m^{-2} s^{-1}, and a leaf temperature of 28 °C. The leaves were initially exposed to an external $[CO_2]$ of 380 μmol mol^{-1} and a D of 1.5 ± 0.3 kPa. The D was then increased in two steps, to 2.3 ± 0.3 kPa, and then to 3.3 ± 0.3 kPa, while keeping leaf temperature, PPFD and external $[CO_2]$ constant. Steady-state values of transpiration, g and CO_2 assimilation rate were recorded at each step in D. While keeping the leaf at the high value of D and constant PPFD and temperature, the external $[CO_2]$ was decreased to 100 μmol mol^{-1}. The $[CO_2]$ was kept at this low level for about 1 h, by which time gas exchange rates had stabilized, and then returned to 380 μmol mol^{-1} to determine the reversibility of gas exchange rates. No measurements of leaf water potential were made in this set of observations. The value of 100 μmol mol^{-1} for the lower $[CO_2]$ was chosen to avoid possible photoinhibition of photosynthesis, which might have occurred at lower $[CO_2]$ and affected g.

The same gas exchange system was also used to determine the sensitivity of g to substomatal $[CO_2]$ (C_i) at high and low D. Measurements of photosynthesis and g were made at 28 °C, 1500 μmol m^{-2} s^{-1} PPFD, a D of 1.5 ± 0.3 kPa at external $[CO_2]$ of 380 and 100 μmol mol^{-1}. D was then increased to 3.3 ± 0.3 kPa and gas exchange rates were determined at the same two values of external $[CO_2]$. C_i was calculated by the system software. The relative stomatal sensitivity to C_i was estimated from $d(\ln g)/d(C_i)$, because the species used here had negative exponential responses of g to C_i (not shown), which is typical (e.g. Morison & Gifford 1983), and because $d(\ln g)/d(C_i) = [d(g)/g]/d(C_i)$, and thus expresses relative stomatal sensitivity (Comstock & Ehleringer 1993).

A different gas exchange system was used for experiments exposing whole leaves to high D. Entire leaves, or terminal leaflets in the case of *G. max*, were enclosed in a water-jacketed acrylic cuvette lined with Teflon film and containing a mixing fan. The leaf petiole was inserted through a groove in a side wall of the cuvette and sealed with caulk. A gas blending system provided air with controlled concentrations of CO_2 and water vapour at a flow rate that was measured with a mass flow meter. Leaf temperature was measured using a miniature thermistor pressed against the lower leaf surface. The g, transpiration rate and net CO_2 assimilation rate were measured using a CIRAS-1 portable photosynthesis system configured for using an external air supply and a leaf temperature probe. Measurements were made on plants in the growth cabinet starting a few hours after lights came on. The leaves were first exposed to an external $[CO_2]$ of 380 ± 10 μmol mol^{-1}, temperature of 28 ± 2 °C, PPFD of 1500 μmol m^{-2} s^{-1} and D of 2.3 ± 0.2 kPa for 1.5 h, by which time gas exchange parameters had stabilized. Water content of the inlet air was then lowered in one step to produce a D of 3.3 ± 0.3 kPa at the same leaf temperature, $[CO_2]$ and PPFD. After at least an hour at the higher D, stable gas exchange parameters were recorded and a 6 mm diameter leaf disc was removed from the leaf in the cuvette for

determination of leaf water potential using a Wescor HR-33 dew point hygrometer (Wescor, Inc., Logan, UT, USA) and a recently calibrated insulated leaf chamber. Leaf water potential was also determined for a leaf not in the cuvette, which had been covered in aluminium foil since the night before. The $[CO_2]$ was then reduced to 100 ± 10 μmol mol^{-1}. After gas exchange rates had been constant for an hour, another leaf disc was taken for determination of leaf water potential, along with a disc from a leaf that had been covered in aluminium foil since the night before. The $[CO_2]$ was then increased to 380 ± 10 μmol mol^{-1} to determine reversibility. After gas exchange rates had again stabilized, another leaf disc was removed for determination of leaf water potential for the leaf in the cuvette as well as from the external leaf covered in foil. Leaf hydraulic conductance (i.e. from the stem to the leaf) was calculated from the transpiration rate and the difference between water potential of the leaf in the cuvette and the leaf outside the cuvette, which had been covered with aluminium foil to prevent transpiration. This assumes that the leaf water potential of the non-transpiring leaf was equal to the water potential of the stem supplying water to the adjacent leaf inside the cuvette. This assumption was not tested, and could be questionable (Fiscus, Parsons & Alberte 1973).

The same sequence of measurements used for whole leaves was also used on whole shoots of each species, except that no leaves were covered with aluminium foil, and leaf water potential was also determined at the lower, initial value of D (2.3 kPa). The whole shoots were enclosed in an acrylic cuvette with an internal radiator and mixing fan and illuminated with the same type of lamps used in the growth cabinet. The stem base was inserted through a slot in the base of the cuvette and sealed with caulk. Temperature of a mature unshaded leaf was monitored with a miniature thermister pressed against the lower surface. Discs for leaf water potential measurement were taken from a mature fully illuminated leaf. Total shoot leaf area was determined after the gas exchange measurements were complete. Whole plant hydraulic conductance (i.e. from rooting medium to leaves) was calculated from the transpiration rate per unit leaf area and the difference between the leaf water potential and osmotic potential of the nutrient solution (–0.03 MPa).

Another means of increasing g and transpiration rate despite high D, increased light, was tested using 2.5 cm^2 sections of leaves and the CIRAS-2 system. In this method, the leaves were equilibrated at 28 °C, 380 μmol mol^{-1} $[CO_2]$, D of 1.5 ± 0.3 kPa and PPFD of 300 μmol m^{-2} s^{-1}. Steady-state gas exchange rates were determined at two higher steps in D, 2.3 ± 0.2 and 3.3 ± 0.3 kPa. While at the highest value of D, the PPFD was increased to 1500 μmol m^{-2} s^{-1} to determine whether g and transpiration rate were increased.

Each type of experiment, low $[CO_2]$ effects on parts of leaves, whole leaves and whole shoots, and increased light on parts of leaves, was conducted on either three or four different individual plants per species. Experiments on parts of leaves, whole leaves and whole shoots were conducted on different individual plants within species.

RESULTS

When D was altered for parts of leaves at constant external $[CO_2]$, values of g decreased with each step of increasing D in all species (Table 1). In each species, there was less than a 10% increase in transpiration rate between the two highest steps in D, while the increase in D was 33%. While at the highest value of D, lowering the external $[CO_2]$ increased g in all species by 51–78%, with a corresponding increase in transpiration rate (Table 1). The values of g returned to very close to the initial values when the external $[CO_2]$ was returned to the higher level. The g was relatively more sensitive to C_i at a D of 3.3 than 1.5 kPa in each species, with relative sensitivity increasing by factors of 1.6–2.6 at the higher D, depending on species (Table 2). The C_i values ranged from about 75–80 μmol mol^{-1} at low external $[CO_2]$ to 220–285 μmol mol^{-1} at higher external $[CO_2]$, depending on species and D (Fig. 1). The ratio of g at a D of 3.3 kPa to that at 1.5 kPa averaged 0.81 at the external $[CO_2]$ of 100 μmol mol^{-1} across species (not shown), compared to 0.63 at the external $[CO_2]$ of 380 μmol mol^{-1} (Table 1).

Table 1. Stomatal conductance (g, in mmol m^{-2} s^{-1}) and transpiration rate (E, in mmol m^{-2} s^{-1}) of 2.5 cm^2 sections of leaves of *Abutilon theophrasti*, *Glycine max*, *Gossypium hirsutum* and *Xanthium strumarium* under sequential changes in leaf-to-air water vapour pressure difference (D) and external carbon dioxide concentration (C_a)

	D (kPa) C_a (μmol mol^{-1})	1.5 380	2.3 380	3.3 380	3.3 100	3.3 380 species
A. theophrasti	g	551 ± 45	416 ± 38	320 ± 25	571 ± 51	336 ± 31
	E	8.0 ± 0.6	9.8 ± 0.8	10.4 ± 1.1	17.6 ± 1.2	11.1 ± 1.2
G. max	g	342 ± 28	294 ± 22	218 ± 19	378 ± 23	207 ± 15
	E	5.2 ± 0.3	7.0 ± 0.5	7.2 ± 0.5	12.0 ± 1.1	6.9 ± 0.5
G. hirsutum	g	512 ± 50	463 ± 35	361 ± 29	544 ± 45	361 ± 32
	E	7.5 ± 0.7	11.1 ± 0.9	11.6 ± 1.0	17.3 ± 1.4	11.5 ± 1.1
X. strumarium	g	750 ± 84	597 ± 67	463 ± 42	793 ± 78	455 ± 37
	E	11.1 ± 1.3	14.1 ± 1.5	15.0 ± 1.4	24.6 ± 2.8	14.7 ± 1.5

Values are means ± SE for $n = 3$ or 4.

Table 2. Relative sensitivity of stomatal conductance (g) to substomatal carbon dioxide concentration (C_i) at low and high leaf-to-air water vapour pressure difference (D) in leaves of four species

	Relative sensitivity (per μmol mol^{-1})	
	D = 1.5 kPa	D = 3.3 kPa
Species		
Abutilon theophrasti	–0.0024 ± 0.0002	–0.0039 ± 0.0004*
Glycine max	–0.0015 ± 0.0001	–0.0039 ± 0.0002*
Gossypium hirsutum	–0.0011 ± 0.0003	–0.0023 ± 0.0005*
Xanthium strumarium	–0.0017 ± 0.0004	–0.0034 ± 0.0006*

C_i values ranged from 75 to 285 μmol mol^{-1}. Relative sensitivity was calculated at d(ln g)/d(C_i). Values are means ± SE for $n = 3$ or 4. *indicates a significant change in relative sensitivity with D within a species, at $P = 0.05$.

Transpiration rates were not higher at a D of 3.3 kPa than at 2.3 kPa in any of the species, when whole leaves were exposed to the change in D, because of a larger reduction in g at high D than what occurred when only parts of leaves were exposed to high D (Table 3). When the external [CO_2] was lowered while leaves were at high D, g and transpiration increased and leaf water potential decreased in all species (Table 3). Increases in g and transpiration rate ranged from about 34 to 90% in the four species, and water potential decreases ranged from about 0.17 to 0.3 MPa. Values of g, transpiration rates and leaf water potentials returned to very nearly the initial values when the external [CO_2] was returned to the initial higher value (Table 3). Water potential of the leaves covered with foil did not change with the D or low [CO_2] treatments (not shown), and the hydraulic conductance from the stem to the leaf was unchanged by the low [CO_2], high transpiration rate treatment (Table 3).

When whole shoots were exposed to increasing D, transpiration rates were increased and leaf water potentials decreased between D-values of 2.3 and 3.3 kPa, in each species (Table 4). Exposure to low [CO_2] at high D increased g and transpiration rate and decreased leaf water potential in each case. These effects were reversible. Whole plant hydraulic conductance was essentially the same at the two values of D at the external [CO_2] of 380 μmol mol^{-1}, but was higher at low [CO_2] (Table 4). Thus, the response of plant hydraulic conductance to the low [CO_2], high transpiration rate treatment was reversible.

The g was also reduced with each step increase in D at the low PPFD of 300 μmol m^{-2} s^{-1} in all species (Table 5). Increasing PPFD while at the highest value of D increased g and transpiration rate by 58–132%, depending on species (Table 5).

DISCUSSION

The measurements on parts of leaves demonstrated that even when D was high enough to reduce g and limit transpiration rate, g and transpiration rate could be increased either by lowering the external [CO_2] or by increasing light. For whole leaves and shoots, lower leaf water potentials and higher transpiration rates were achieved by lowering the [CO_2] despite high D. Furthermore, maintaining the artificially high transpiration rates and low leaf water potentials for as long as an hour did not decrease leaf or plant hydraulic conductance, or subsequent g. Clearly, in these cases, g was reduced at high D without leaf water potential being low enough to prevent stomatal opening at low [CO_2] or to produce xylem cavitation. There were no qualitative differences in this regard among the four species examined, or between experiments exposing parts of leaves, whole leaves or whole shoots to high D. The apparent increase in whole plant hydraulic conductance when transpiration was increased by low [CO_2] is similar to many reports of increasing apparent whole plant hydraulic conductance as transpiration increases, which is often attributed to a change in the partitioning of water between growth and transpiration (Fiscus, Klute & Kaufmann 1983).

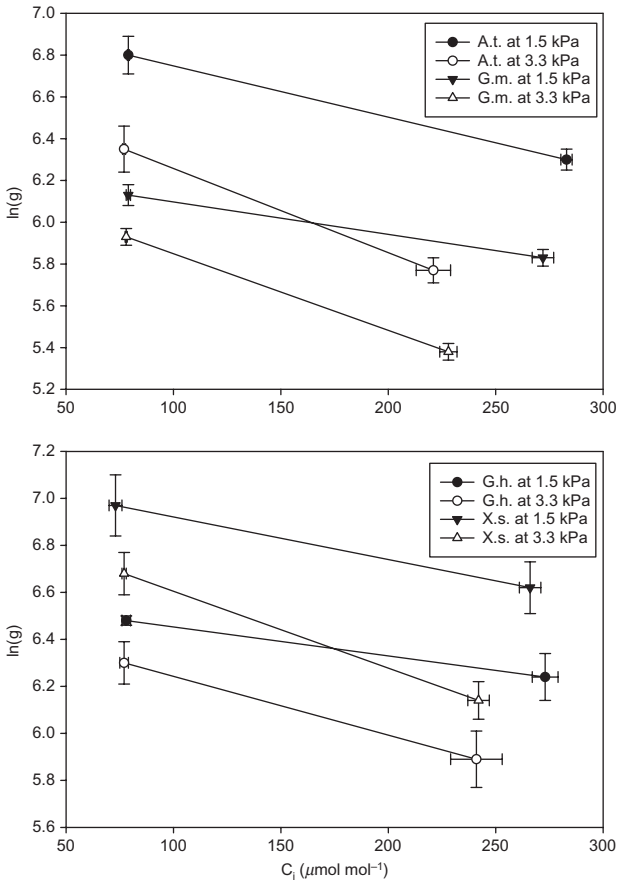

Figure 1. Relationships between the natural log of stomatal conductance (g) and substomatal carbon dioxide concentration (C_i) at two values of leaf-to-air water vapour pressure difference (D) in *Abutilon theophrasti* (A.t.), *Glycine max* (G.m.), *Gossipyum hirsutum* (G.h.) and *Xanthium strumarium* (X.s.). Values of g were in mmol m^{-2} s^{-1}. Bars represent SE values of the mean. Results of statistical tests of effects of D on slopes are given in Table 2.

Table 3. Stomatal conductance (g, in mmol m^{-2} s^{-1}), transpiration rate (E, in mmol m^{-2} s^{-1}), leaf water potential (LWP, in MPa) and leaf hydraulic conductance (K_l, in mmol m^{-2} s^{-1} MPa^{-1}) of whole leaves of four species under sequential changes in leaf-to-air water vapour pressure difference (D) and external carbon dioxide concentration (C_a)

Species	D (kPa) C_a (μmol mol^{-1})	2.3 380	3.3 380	3.3 100	3.3 380
Abutilon theophrasti	g	356 ± 25	235 ± 14	315 ± 25	240 ± 31
	E	8.3 ± 0.6	8.3 ± 0.4	10.4 ± 0.7	8.2 ± 0.6
	LWP	nd	−1.50 ± 0.03	−1.71 ± 0.05	−1.47 ± 0.04
	K_l	nd	11.9 ± 0.7	11.4 ± 0.8	12.2 ± 0.8
Glycine max	g	280 ± 33	193 ± 10	302 ± 12	200 ± 18
	E	6.3 ± 0.3	6.2 ± 0.4	9.4 ± 0.7	6.3 ± 0.5
	LWP	nd	−1.16 ± 0.03	−1.48 ± 0.04	−1.21 ± 0.03
	K_l	nd	11.5 ± 0.4	10.8 ± 0.7	10.9 ± 0.5
Gossypium hirsutum	g	249 ± 40	155 ± 29	295 ± 29	162 ± 25
	E	5.5 ± 0.9	5.2 ± 0.7	9.8 ± 0.8	5.3 ± 0.8
	LWP	nd	−1.25 ± 0.06	−1.58 ± 0.11	−1.28 ± 0.07
	K_l	nd	13.7 ± 1.2	14.1 ± 1.4	12.9 ± 1.2
Xanthium strumarium	g	308 ± 35	195 ± 32	295 ± 16	197 ± 26
	E	6.9 ± 0.8	6.5 ± 1.5	9.7 ± 1.0	6.5 ± 1.2
	LWP	nd	−1.11 ± 0.07	−1.28 ± 0.05	−1.09 ± 0.04
	K_l	nd	20.1 ± 1.4	19.8 ± 1.1	21.7 ± 1.5

Values are means ± SE for $n = 3$ or 4.
nd, not determined

It is difficult to argue with the idea that reductions in g at high D benefit plants by reducing transpiration rate, which maintains higher leaf water potentials. Decreasing g at high D would also serve to protect plants from xylem cavitation caused by low water potentials. Xylem cavitation reduces hydraulic conductance of the xylem, which often reduces g (Sperry *et al*. 1993; Salleo *et al*. 2000; Sperry 2000). There has been considerable interest recently in determining the threshold water potentials for cavitation in relation to usual midday water potentials. In some herbaceous species, even in wet soil, midday leaf water potentials are near the threshold for cavitation (Stiller, Lafitte & Sperry 2003), while in others there may be little risk of cavitation (Cochard 2002). In the species examined here, even exposing whole shoots of plants to midday values of D much higher than those experienced during growth, in combination with artificially high transpiration rates caused by low [CO_2], did not cause a reduction in hydraulic

Table 4. Stomatal conductance (g, in mmol m^{-2} s^{-1}), transpiration rate (E, in mmol m^{-2} s^{-1}), leaf water potential (LWP, in MPa) and plant hydraulic conductance (K_p, in mmol m^{-2} s^{-1} MPa^{-1}) of whole plants of four species under sequential changes in leaf-to-air water vapour pressure difference (D) and external carbon dioxide concentration (C_a)

Species	D (kPa) C_a (μmol mol^{-1})	2.3 380	3.3 380	3.3 100	3.3 380
Abutilon theophrasti	g	145 ± 15	110 ± 9	160 ± 13	118 ± 10
	E	3.3 ± 0.4	3.5 ± 0.2	5.2 ± 0.3	3.5 ± 0.3
	LWP	−1.30 ± 0.08	−1.51 ± 0.05	−1.67 ± 0.06	−1.48 ± 0.05
	Kp	2.54 ± 0.22	2.36 ± 0.18	3.17 ± 0.28	2.55 ± 0.25
Glycine max	g	90 ± 12	70 ± 10	133 ± 16	77 ± 11
	E	2.0 ± 0.3	2.2 ± 0.4	4.2 ± 0.4	2.4 ± 0.3
	LWP	−1.20 ± 0.1	−1.51 ± 0.08	−1.77 ± 0.07	−1.45 ± 0.11
	Kp	1.71 ± 0.12	1.58 ± 0.10	2.36 ± 0.18	1.71 ± 0.15
Gossypium hirsutum	g	169 ± 14	131 ± 10	197 ± 15	127 ± 18
	E	3.6 ± 0.3	4.3 ± 0.2	6.3 ± 0.4	4.1 ± 0.3
	LWP	−1.13 ± 0.05	−1.24 ± 0.07	−1.49 ± 0.09	−1.24 ± 0.07
	Kp	3.27 ± 0.22	3.55 ± 0.25	4.32 ± 0.25	3.39 ± 0.21
Xanthium strumarium	g	193 ± 26	145 ± 11	230 ± 15	150 ± 14
	E	4.3 ± 0.4	4.6 ± 0.3	7.3 ± 0.3	4.8 ± 0.4
	LWP	−0.72 ± 0.10	−0.94 ± 0.05	−1.07 ± 0.04	−0.92 ± 0.06
	Kp	5.81 ± 0.05	5.75 ± 0.06	7.02 ± 0.08	6.02 ± 0.11

Values are means ± SE for $n = 3$ or 4.

Table 5. Stomatal conductance (g, in mmol m^{-2} s^{-1}) and transpiration rate (E, in mmol m^{-2} s^{-1}) of 2.5 cm^2 sections of leaves of four species under sequential changes in leaf-to-air water vapour pressure difference (D) and photosynthetic photon flux density (PPFD)

Species	D (kPa) PPFD (μmol m^{-2} s^{-1})	1.5 300	2.3 300	3.3 300	3.3 1500
Abutilon theophrasti	g	437 ± 17	300 ± 35	206 ± 25	325 ± 33
	E	6.3 ± 0.6	6.7 ± 0.5	6.7 ± 0.6	10.6 ± 0.5
Glycine max	g	178 ± 27	127 ± 16	108 ± 11	251 ± 18
	E	2.8 ± 0.4	3.3 ± 0.3	3.7 ± 0.4	8.0 ± 0.9
Gossypium hirsutum	g	286 ± 45	205 ± 35	161 ± 21	304 ± 55
	E	4.1 ± 0.7	4.6 ± 0.8	5.3 ± 1.0	9.5 ± 1.1
Xanthium strumarium	g	251 ± 44	182 ± 47	155 ± 38	340 ± 48
	E	3.8 ± 0.4	4.2 ± 0.4	5.1 ± 0.7	12.5 ± 1.5

Values are means ± SE for $n = 3$ or 4.

conductance. In these species, values of g at high D were much lower than values that would have threatened either leaf or whole plant hydraulic conductance. Oren *et al*. (2001) recognized that cavitation was not required for all stomatal responses to D, because stomatal responses to D occurred at night, when water potentials were above thresholds for cavitation. While it is quite possible that under conditions of low soil water potential reduced hydraulic conductance may cause stomatal closure at high D, the results of these experiments make it unlikely that this is commonly the cause of stomatal closure at high D for herbaceous plants in wet soil.

Despite the mechanical advantage of epidermal cells over guard cells (DeMichele & Sharpe 1973; Franks, Cowan & Farquhar 1998), it is possible that reductions in bulk leaf water potential could directly cause stomatal closure. This is because guard cells could have a larger passive reduction in turgor pressure than the surrounding cells for the same reduction in total water potential (Klein *et al*. 1996). However, recognizing that the mechanical advantage of epidermal cells over guard cells might prevent low bulk leaf water potential at high D from directly causing stomatal closure (and might even cause opening), Buckley, Mott & Farquhar (2003) developed a hydromechanical and biochemical model of g in which g responds to D by a reduction in leaf water potential, decreasing the effectiveness of ATP in generating an osmotic gradient across guard cell membranes. Our results indicate that whatever the cause of reduction guard cell turgor compared to that of surrounding epidermal cells at low water potential, that effect can be overcome by the effect of low $[CO_2]$ in increasing the turgor difference. In the Buckley *et al*. model, the response of g to $[CO_2]$ is based on $[CO_2]$ effects on [ATP]. However, at high light and D, the model as currently parameterized has virtually no stomatal response to $[CO_2]$ (Buckley *et al*. 2003; fig. 4). This aspect of the model does not agree with our experimental observations. The importance of the observed increase in stomatal sensitivity to C_i with increasing D for stomatal closure at high D was evidenced by the much smaller relative reduction in g by high D at lower than at higher external $[CO_2]$. The ability of increased PPFD to increase g at high D is also consistent with responsiveness of g to $[CO_2]$ at high D, because increasing PPFD would have lowered C_i unless g increased. However, I did not attempt to separate the response to PPFD into direct effects of light on g from effects of C_i.

One concept that would be consistent with the data presented here is if the concentration of abscisic acid (ABA) at the guard cells increased with D and caused stomatal closure. It would be expected that stomatal closure induced by increased ABA would be reversible by lowering the $[CO_2]$, as stomatal sensitivity to ABA is strongly $[CO_2]$-dependent (Raschke 1975; Dubbe, Farquhar & Raschke 1978; Leymarie, Lasceve & Vavasseur 1999). Modification of the model of g to have [ABA] mediate the response to leaf water potential seems logical, because ABA certainly does what the model has low leaf water potential doing, which is to decrease the efficiency of the development of osmotic gradient across the guard cell membrane. In the data presented in this paper, a hypothetical increase in [ABA] at the guard cells at high D could have been caused by a reduction in leaf water potential, because there was no evidence of feedforward stomatal response to D. Whether the accumulation of apoplastic ABA at guard cells with increasing D (Zhang & Outlaw 2001) could result in a feedforward response is unclear, but as discussed earlier, so is the existence of truly feedforward stomatal behaviour. While there is considerable circumstantial evidence that ABA may be involved in stomatal responses to D (Franks *et al*. 1997; Bunce 1998; Grantz & Schwartz 1998, Macfarlane, White & Adams 2004), g of mutants of *Arabidopsis* with reduced sensitivity to ABA was still sensitive to D (Assmann, Snyder & Lee 2000), and also to $[CO_2]$ (Leymarie, Vavasseur & Lasceve 1998).

For whatever reason it occurred, an increase in the relative sensitivity of g to C_i at high D accounted for most of the reduction in g at high D in these species. It would be of interest to compare the responses of these species to those of other species reputed to have stomata insensitive to $[CO_2]$. In any event, knowledge of the mechanism by which $[CO_2]$ affects g will be necessary to understand the hydraulic control of g by D, at least in the species examined in this study.

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