

Does transpiration control stomatal responses to water vapour pressure deficit?

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ABSTRACT

Three types of observations were used to test the hypothesis that the response of stomatal conductance to a change in vapour pressure deficit is controlled by whole-leaf transpiration rate or by feedback from leaf water potential. Varying the leaf water potential of a measured leaf by controlling the transpiration rate of other leaves on the plant did not affect the response of stomatal conductance to vapour pressure deficit in *Glycine max*. In three species, stomatal sensitivity to vapour pressure deficit was eliminated when measurements were made at near-zero carbon dioxide concentrations, despite the much higher transpiration rates of leaves at low carbon dioxide. In *Abutilon theophrasti*, increasing vapour pressure deficit sometimes resulted in both decreased stomatal conductance and a lower transpiration rate even though the response of assimilation rate to the calculated substomatal carbon dioxide concentration indicated that there was no 'patchy' stomatal closure at high vapour pressure deficit in this case. These results are not consistent with stomatal closure at high vapour pressure deficit caused by increased whole-leaf transpiration rate or by lower leaf water potential. The lack of response of conductance to vapour pressure deficit in carbon dioxide-free air suggests that abscisic acid may mediate the response.

Key-words: *Abutilon theophrasti*; *Chenopodium album*; *Glycine max*; stomatal conductance; transpiration; vapour pressure deficit.

INTRODUCTION

Monteith (1995) presented a reinterpretation of data on stomatal responses to vapour pressure deficit (VPD) in which he concluded that most data are consistent with the hypothesis that stomatal closure as the VPD between a leaf and the surrounding air increases is caused by an increase in the rate of transpiration from the whole leaf. It was recognized that responses in which transpiration and stomatal conductance both decrease as VPD increases, i.e. the tradi-

tional evidence of a 'feedforward' response (Farquhar 1978), are not consistent with this hypothesis. Such data were excluded from consideration on the basis that stomatal closure may have been 'patchy' (Mott & Parkhurst 1991). It is not surprising that, if one excludes all data indicating a 'feedforward' stomatal response, the remaining data are consistent with a 'feedback' response. The question is whether it is justifiable to exclude all data in which transpiration decreases as VPD increases on the basis that this only occurs during 'patchy' stomatal closure. This paper presents data indicating that this exclusion is not justifiable.

Monteith (1995) did not propose a mechanism by which high transpiration would cause stomatal closure, but it could do so by increasing the water potential gradient between the guard cells and other epidermal cells or by lowering leaf water potential (Stewart & Dwyer 1983), either of which could directly decrease the turgor pressure of guard cells relative to other epidermal cells or affect hormonal distribution. A dependence of the stomatal conductance response to VPD on leaf water potential was examined in the present paper by comparing stomatal responses to VPD in the same leaves held at different water potentials by manipulations of the transpiration rate of other leaves on the shoot. In particular, it was expected that, if leaf water potential was involved in the feedback system, it would be possible to eliminate the stomatal response to VPD by holding leaves above the threshold water potential for stomatal closure (Stewart & Dwyer 1983).

The reduced sensitivity of stomatal conductance to VPD in leaves measured at an elevated carbon dioxide concentration (e.g. Morison & Gifford 1983; Bunce 1993) is consistent with control of sensitivity by transpiration rate, since elevated carbon dioxide partly closed stomata and lowered transpiration rate. In this paper we have tested whether carbon dioxide concentrations near zero increase the sensitivity of stomatal conductance to VPD as they increase transpiration rate.

MATERIALS AND METHODS

Glycine max (L.) Merr. cv. Clark, and *Abutilon theophrasti* (L.) and *Chenopodium album* (L.) from local populations were grown in controlled-environment chambers. Plants were grown one per pot in 20-cm-diameter plastic pots,

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with vermiculite as the rooting medium. Pots were flushed daily with a complete nutrient solution. Plants were grown at 25 ± 0.3 °C air temperature, 18 ± 2 °C dew-point temperature, 370 ± 30 cm³ m⁻³ carbon dioxide, with 14 h d⁻¹ of light from a mixture of metal halide and high-pressure sodium lamps at a photosynthetic photon flux density (PPFD) of 1.0 mmol photons m⁻² s⁻¹. Leaf gas exchange measurements were made on the third mainstem trifoliolate leaf in *G. max*, the third leaf in *A. theophrasti*, and the sixth leaf in *C. album*, shortly after those leaves had become fully expanded in area. The three species were grown at different times.

During leaf gas exchange measurements, plants were in a controlled-environment chamber in the daytime growth conditions, with the exception that in *G. max* some of the measurements were made with the chamber lights off, so that all leaves except the leaf in which gas exchange was measured were in darkness.

Leaf gas exchange was measured using either of two independent systems, a CIRAS-1 portable gas exchange system with a broad-leaf chamber, lamp, infrared temperature sensor and automated gas blending system (PP Systems, Haverhill, MA), and an open gas exchange system enclosing whole leaves or leaflets, with control of light, temperature, carbon dioxide concentration and VPD (Bunce 1993). There was no active control of leaf temperature in the CIRAS system, but leaf temperature varied by less than 1 °C during VPD response curves. Several of the experiments were repeated using both systems (see later).

Experiment I: effect of leaf water potential

G. max plants were taken from the growth chamber during the dark and placed in darkness in the measurement chamber. The whole terminal leaflet of the third trifoliolate leaf was enclosed in the gas exchange cuvette, and illuminated at a PPFD of 1.5 mmol m⁻² s⁻¹. The remainder of the plant was loosely covered with aluminium foil to exclude stray light. The leaf was maintained at 25 °C and 370 cm³ m⁻³ carbon dioxide concentration, and the VPD was initially adjusted to 10–15 mPa Pa⁻¹. After carbon dioxide and water vapour exchange rates had stabilized (at least 1 h was required), VPD was increased in 3–4 gradual steps up to 25–30 mPa Pa⁻¹, and steady-state gas exchange rates were determined at each step in VPD. Leaf water potential was determined on discs excised from darkened side leaflets of the measured leaf taken at the end of the VPD response curve. Water potential was measured with an insulated Wescor C-52 chamber chamber and HR-33 dew-point hygrometer. Upon completion of the VPD curve, the measured leaf was darkened for about 15 min and returned to low VPD. The chamber lights were then turned on, and the measured leaflet illuminated as before for a 2 h equilibration period. The VPD response was again determined, using approximately the same steps in VPD, and the leaf water potential of illuminated side leaflets was determined at the end of the VPD response curve. This experiment was repeated using the CIRAS-1 system, except that leaf water

potentials were determined on discs taken from the terminal leaflet on tissue outside the cuvette, and the PPFD on the measured section of the leaf was 1.1 mmol m⁻² s⁻¹.

Experiment II: effect of carbon dioxide concentration

The effect of low carbon concentration on the stomatal response to VPD was determined in all three species with the CIRAS system. Leaves were placed in the gas exchange system at a VPD of 7–11 mPa Pa⁻¹, a PPFD of 1.1 mmol m⁻² s⁻¹ and a carbon dioxide concentration of 0–50 cm³ m⁻³. When stomatal conductance was constant, the water content of inlet air was decreased to 0 in three steps, and steady-state conductances determined. After these measurements had been completed, similar measurements were made on a different section of the same leaf, but at a carbon dioxide concentration of 350 cm³ m⁻³. Because of the differences in conductance between carbon dioxide treatments, the minimum VPD achieved at the higher carbon dioxide concentration was 10–14 mPa Pa⁻¹. Measurements of the response of stomatal conductance to VPD at low carbon dioxide were also made on *G. max* leaflets in the laboratory gas exchange system at a PPFD of 1.5 mmol m⁻² s⁻¹.

Experiment III: feedforward and patchiness

These experiments were conducted with *A. theophrasti* using the CIRAS system at a PPFD of 2.0 mmol m⁻² s⁻¹. Two sets of measurements were made on the same section of a leaf: a VPD response at a constant external carbon dioxide concentration of 370 cm³ m⁻³, and a carbon dioxide response at constant low VPD. The sequence of these measurements was varied between measured plants. Leaves were kept at each step in VPD or carbon dioxide for 15 min, with data recorded at 1 min intervals for the last 5 min. These data were used to determine whether high VPD altered the response of assimilation rate to internal carbon dioxide concentration. Assimilation and internal carbon dioxide concentration were calculated by the CIRAS system.

RESULTS

The response of stomatal conductance to VPD in *G. max* leaves did not vary depending on the illumination of the rest of the shoot (Fig. 1). Neither slopes nor intercepts of linear regressions differed at $P = 0.05$. Mean water potentials of other leaflets of the measured leaf were -0.61 MPa in the dark and -1.06 MPa in the light, which were significantly different at $P = 0.05$, using a paired *t*-test. The same results, i.e. different water potentials but equivalent responses of conductance to VPD, were obtained with the CIRAS system (not shown).

Low carbon dioxide concentration during the measurement eliminated the response of stomatal conductance to VPD which was present at the growth carbon dioxide con-

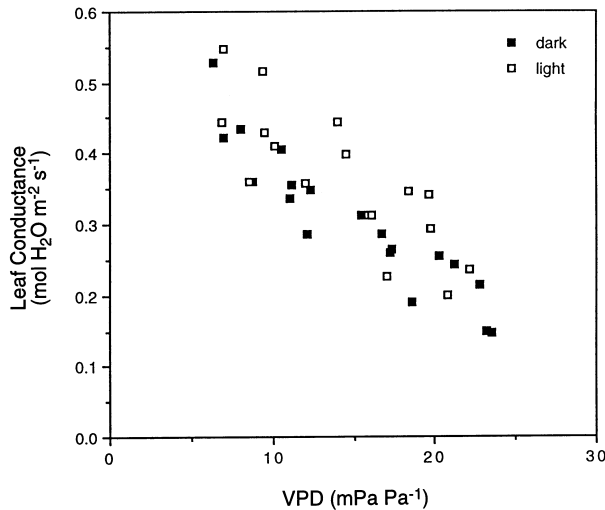


Figure 1. Relationships between leaf conductance to water vapour and the vapour pressure deficit from inside the leaf to outside the leaf boundary layer for leaves of *Glycine max* with all other leaves on the plant either in the light or in the dark. Each of four leaves was measured first in the dark and then in the light. Each data point represents a single measurement.

centration (Table 1). The same response was found in all three species examined. Because the low carbon dioxide concentration caused stomatal opening, transpiration rates were higher at the low carbon dioxide concentration, especially at the higher VPDs (Table 1). Lack of response of conductance to VPD at low carbon dioxide concentration was also found in *G. max* using the laboratory gas exchange system (not shown).

In *A. theophrasti*, the transpiration rate decreased with increasing VPD at the highest VPDs in two of the leaves measured, and remained constant in the third leaf (Fig. 2). Plotting conductance versus transpiration rate (Fig. 3) clarifies that there was no unique value of conductance for a given transpiration rate, and that not all the data for any leaf fit a negative linear relationship as proposed by Monteith (1995). The response of assimilation to internal carbon dioxide concentration obtained from the VPD

response measurements did not differ from that obtained by varying external carbon dioxide concentration at low VPD in any leaves measured (Fig. 4).

DISCUSSION

The ability accurately to predict stomatal conductance as a function of environment would be useful in many endeavors, such as climate modelling, irrigation management, and predicting plant responses to global change. It would be desirable to have mechanistic models of stomatal responses, because they may be more reliably extrapolated to new environmental combinations. VPD is recognized as one of the most important sources of variation in stomatal conductance, but the mechanism of the response is unknown. A simple and appealing mechanism would be one in which stomata responded to VPD by means of a feedback system based on the effect of whole-leaf transpiration rate on leaf water status, or the gradient in water potential between guard cells and other epidermal cells. A feedback mechanism had long ago been rejected as inconsistent with numerous observations of stomatal closure at high VPD accompanied by lower transpiration rate (cf. Farquhar 1978) or increased leaf water potential (Saliendra *et al.* 1995). Recently, Monteith (1995) suggested that most data were consistent with a feedback response to whole-leaf transpiration rate, and that data showing feedforward differed qualitatively because stomatal closure was patchy, based on observations by Mott & Parkhurst (1991).

Complete closure of stomata in patches can occur at high VPD (e.g. Beyschlag *et al.* 1992). Patchy stomatal closure results in a reduction in assimilation rate and stomatal conductance with no reduction in calculated internal carbon dioxide concentration (Bunce 1988), i.e. it causes a reduction in assimilation rate at a given calculated internal carbon dioxide concentration. Because *A. theophrasti* had no change in the rate of assimilation at a given calculated internal carbon dioxide concentration at high VPD, there was unlikely to be patchy stomatal closure. The feedforward response of stomatal conductance to VPD in this species can therefore probably not be attributed to patchy stomatal closure.

Species	CO ₂ 0 to 50 cm ³ m ⁻³			CO ₂ 350 cm ³ m ⁻³		
	VPD (mPa Pa ⁻¹)	G (mmol m ⁻² s ⁻¹)	E (mmol m ⁻² s ⁻¹)	VPD (mPa Pa ⁻¹)	G (mmol m ⁻² s ⁻¹)	E (mmol m ⁻² s ⁻¹)
Glycine max	10.8	758	8.2	13.0	427*	5.6
Chenopodium album	15.7	732	11.5	17.1	361	6.2
Abutilon	7.8	1248	9.7	9.7	765*	7.3
theophrasti	12.8	1270	16.2	14.8	539	8.0
	7.1	1060	7.5	9.0	750*	6.8
	12.4	1024	12.7	12.4	605	7.5

Table 1. Responses of stomatal conductance (*G*) and transpiration (*E*) to VPD at the leaf surface in three species measured either at 0 to 50 or at 350 cm³ m⁻³ external carbon dioxide concentrations. Each value is the mean for three leaves

*indicates a significant effect of VPD on *G* at *P* = 0.05 for that species and carbon dioxide concentration.

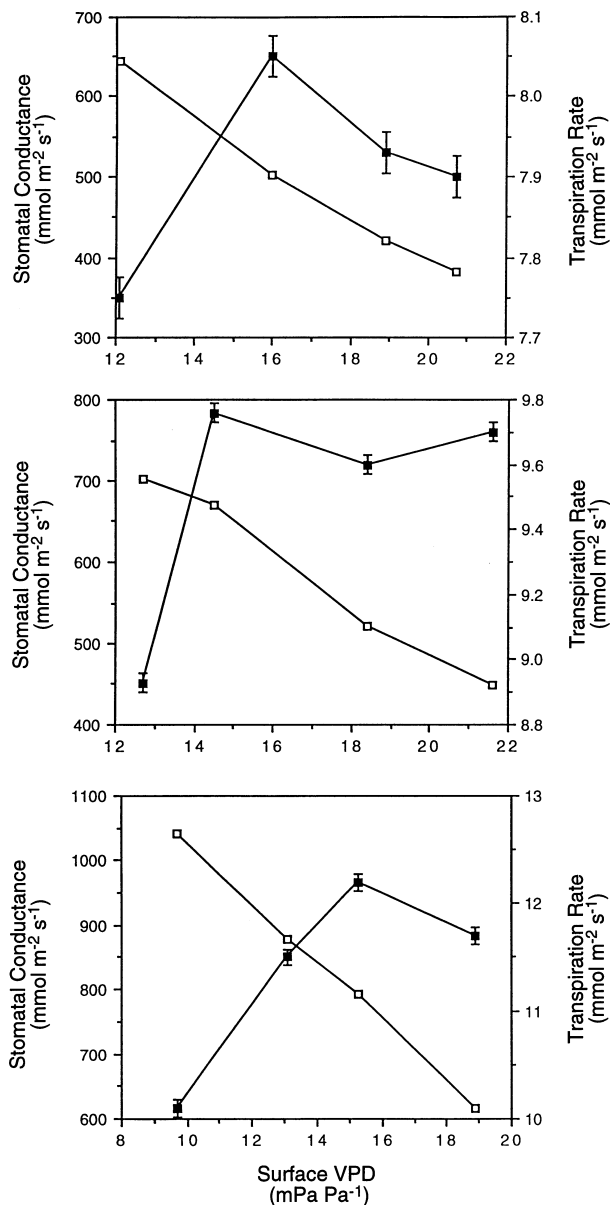


Figure 2. Stomatal conductance (open symbols) and transpiration rate (closed symbols) in three leaves of *Abutilon theophrasti* measured at four steps in vapour pressure deficit at the leaf surface. Vertical bars indicate the standard error of five successive measurements on the same leaf. Standard errors are smaller than the symbols for conductance and for VPD.

Mott & Parkhurst (1991) clearly demonstrated that stomatal conductance did not respond directly to VPD, but to VPD corrected for the diffusion coefficient of water vapour in the medium around the leaf. While this indicates that stomata responded to transpiration rather than to VPD, the experiments did not differentiate between control by whole-leaf transpiration rate and control by peristomal transpiration. The data for *Vicia faba* (Mott & Parkhurst 1991; Figs 3 & 4), which did not show patchy stomatal closure, clearly show that stomatal conductance can be reduced by increasing VPD without any increase in whole-

leaf steady-state transpiration rate. This response suggests, as do the data presented here for *A. theophrasti*, that stomatal responses to VPD are controlled by peristomal transpiration rate. As many others have pointed out, lack of increase in steady-state transpiration as VPD increases does not necessarily mean that there was no transient increase in transpiration as the increase in VPD was imposed.

The failure of the substantial change in leaf water potential between light and dark to affect the stomatal response to VPD in *G. max* is further evidence that change in guard cell water potential does not directly cause the stomatal response to VPD. It is more likely that stomatal conductance is influenced by the difference in water potential between guard cells and other epidermal cells, as this could be affected by peristomal transpiration somewhat independently of bulk leaf water potential. Whether a gradient in water potential within the epidermis suffices as an explanation for stomatal responses to VPD depends on actual sites of evaporation.

The elimination of stomatal responses to VPD at very low carbon dioxide concentrations argues against a direct control by epidermal water relations, whether that is transpiration rate, water potential or peristomal transpiration. Carbon dioxide is apparently needed to mediate the response. This suggests that abscisic acid may be involved in the stomatal response to VPD, because it is known that carbon dioxide is required for stomatal responses to abscisic acid (Raschke 1975). One could envision that changes in peristomal transpiration caused by changes in vapour pressure deficit could alter the rate of delivery of abscisic acid to the guard cells in the transpiration stream.

In summary, the data presented here do not support the hypothesis that stomatal responses to VPD are controlled by whole-leaf transpiration rate or by leaf water potential.

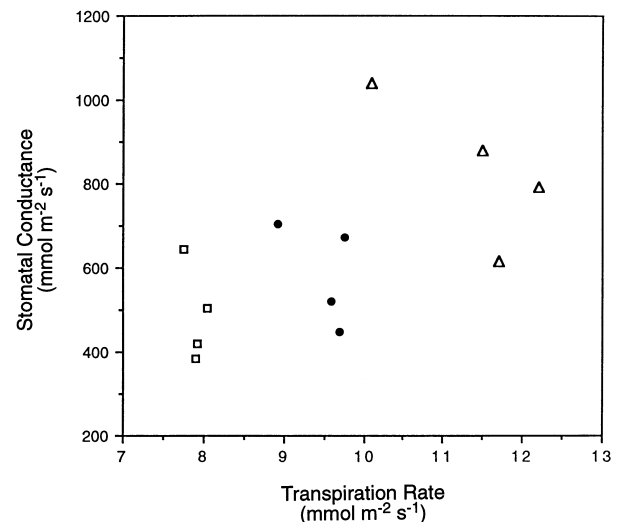


Figure 3. Stomatal conductance as a function of transpiration rate in three leaves of *Abutilon theophrasti* (each type of symbol represents a different leaf from Fig. 2) measured at four steps in vapour pressure deficit at the leaf surface.

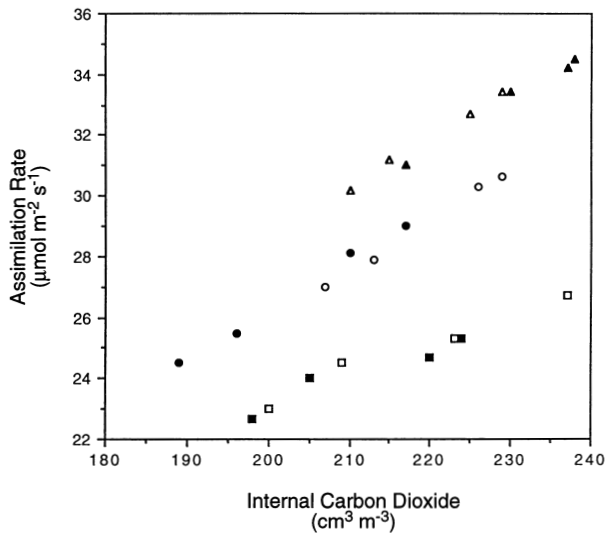


Figure 4. Rate of assimilation of carbon dioxide as a function of calculated internal carbon dioxide concentration in three leaves of *Abutilon theophrasti*, when internal carbon dioxide concentration was altered by changing external carbon dioxide concentration (open symbols) and by changing vapour pressure deficit (closed symbols) in each leaf. Each type of symbol represents a different leaf (same leaves as in Figs 2 & 3). Open symbols represent changing carbon dioxide; closed symbols represent changing VPD.

Rather, the results suggest the importance of peristomatal transpiration. The lack of response of conductance to vapour pressure deficit in carbon dioxide-free air indicates that even changes in peristomatal transpiration alone are

insufficient to cause closure, and suggest the possible involvement of abscisic acid.

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