A hydromechanical and biochemical model of stomatal conductance

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ABSTRACT

A mathematical model of stomatal conductance is presented. It is based on whole-plant and epidermal hydromechanics, and on two hypotheses: (1) the osmotic gradient across guard cell membranes is proportional to the concentration of ATP in the guard cells; and (2) the osmotic gradient that can be sustained per unit of ATP is proportional to the turgor pressure of adjacent epidermal cells. In the present study, guard cell [ATP] is calculated using a previously published biochemical model of C₃ mesophyll photosynthesis. The conductance model for Vicia faba L. is parameterized and tested as with most other stomatal models, the present model correctly predicts the stomatal responses to variations in transpiration rate, irradiance and intercellular CO₂. Unlike most other models, however, this model can predict the transient stomatal opening often observed before conductance declines in response to decreases in humidity, soil water potential, or xylem conductance. The model also explicitly accommodates the mechanical advantage of the epidermis and correctly predicts that stomata are relatively insensitive to the ambient partial pressure of oxygen, as a result of the assumed dependence on ATP concentration.

Key-words: gas exchange; guard cell; photosynthesis; stomata; transpiration.

INTRODUCTION

A model of stomatal conductance (gₛ or simply g; see Table 1 for a list of symbols) is required to predict plant gas exchange accurately. Most models of leaf and canopy gas exchange use a phenomenological model for g (e.g. Jarvis 1976; Ball, Woodrow & Berry 1987; the latter modified by Leuning 1995; and more recently by Tuzet, Perrier & Leuning 2003). These models have been successful because they are mathematically simple, and because they agree with direct measurements of g under many conditions. However, it is difficult to interpret their mathematical structures in terms of the regulatory mechanisms that they presumably mimic. This limits their usefulness as tools for probing stomatal and leaf functioning and constrains the confidence with which their predictions can be extended to future climates. To address these limitations, several authors have attempted recently to model g in a more mechanistically explicit fashion (e.g. Dewar 2002; Gao et al. 2002). However, those models were based on assumptions about epidermal water relations and stomatal hydromechanics that are inconsistent with recent experiments and they calculated guard cell osmotic pressure (πₛ) from irradiance or photosynthetic variables in a phenomenological fashion, much like the Jarvis and Ball–Berry models (Jarvis 1976; Ball et al. 1987) discussed above. Our goal was to develop and present a model for g that overcomes some of these limitations.

Many stomatal responses are driven by changes in πₛ, which is determined partly by solute influx in response to a proton-motive force created by plasma membrane H⁺-ATPases (e.g. Tominaga, Kinoshita & Shimazaki 2001). Although this is a well-established paradigm in stomatal physiology, it is rarely incorporated explicitly into models of stomatal conductance. One of the few attempts to do so (Farquhar & Wong 1984) assumed that g itself, rather than πₛ, is proportional to the concentration of ATP in photosynthetic cells, which could be calculated from the biochemical photosynthesis model developed by Farquhar, Caemmerer & Berry (1980). That conductance model predicted observed responses to irradiance, temperature, CO₂ partial pressure, O₂ partial pressure and leaf chlorophyll content. However, it could not predict any response to hydraulic factors such as humidity or water supply to the leaf, because it did not explicitly include the hydromechanical context that links guard cell osmotic pressure to stomatal conductance. A single value of πₛ can produce a wide range of stomatal apertures and conductances, depending on the relationships between guard cell turgor pressure and volume, between guard and epidermal cell water potentials and between stomatal aperture and guard and epidermal cell turgor pressures.

Intensive study of these hydromechanical factors reveals a paradox. When the rate of water loss from the leaf is experimentally increased (for example, by decreasing ambient humidity), leaf turgor and stomatal aperture both decline in the steady state (Shackel & Brinkmann 1985; Monteith 1995; Mott & Franks 2001). However, pressure...
Table 1. Mathematical terms used in this paper

<table>
<thead>
<tr>
<th>Name</th>
<th>Symbol</th>
<th>Value</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terms in the model (Eqn 6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stomatal conductance to water vapour</td>
<td>$g_{sv}$</td>
<td>–</td>
<td>mol air m$^{-2}$ s$^{-1}$</td>
</tr>
<tr>
<td>ATP concentration</td>
<td>$\tau$</td>
<td>–</td>
<td>mmol ATP m$^{-2}$</td>
</tr>
<tr>
<td>Hydromechanical/biochemical response parameter</td>
<td>$\beta$</td>
<td>1.17 ± 0.27$^a$</td>
<td>[mmol ATP m$^{-2}$]$^{-1}$</td>
</tr>
<tr>
<td>Residual epidermal mechanical advantage</td>
<td>$M$</td>
<td>0.98$^b$</td>
<td>unitless</td>
</tr>
<tr>
<td>Guard cell resistive advantage</td>
<td>$\rho$</td>
<td>0.3$^c$</td>
<td>unitless</td>
</tr>
<tr>
<td>Effective hydraulic resistance to the epidermis</td>
<td>$R$</td>
<td>0.0456$^b$</td>
<td>MPa [mmol H$_2$O m$^{-2}$s$^{-1}$]$^{-1}$</td>
</tr>
<tr>
<td>Epidermal osmotic pressure</td>
<td>$\pi_e$</td>
<td>0.525$^o$</td>
<td>MPa</td>
</tr>
<tr>
<td>Apoplastic osmotic pressure</td>
<td>$\pi_a$</td>
<td>0$^o$</td>
<td>MPa</td>
</tr>
<tr>
<td>Leaf-to boundary layer H$_2$O mole fraction gradient</td>
<td>$D_i$</td>
<td>10 [5–30]</td>
<td>mmol H$_2$O mol$^{-2}$air</td>
</tr>
<tr>
<td>Source water potential</td>
<td>$\psi_i$</td>
<td>0 $^*$</td>
<td>MPa</td>
</tr>
<tr>
<td>Turgor-to-conductance scaling factor</td>
<td>$\chi$</td>
<td>0.105$^p$</td>
<td>mol air m$^{-2}$ s$^{-1}$ MPa$^{-1}$</td>
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<tr>
<td>Terms in simplified form of the model (Eqn 7)</td>
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<td></td>
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<tr>
<td>Guard cell advantage</td>
<td>$\alpha$</td>
<td>–</td>
<td>unitless</td>
</tr>
<tr>
<td>ATP-saturated stomatal conductance</td>
<td>$g_m$</td>
<td>–</td>
<td>mol air m$^{-2}$ s$^{-1}$</td>
</tr>
<tr>
<td>‘Michaelis constant’ for $\alpha$</td>
<td>$K_m$</td>
<td>–</td>
<td>unitless</td>
</tr>
<tr>
<td>Hydroactive compensation point</td>
<td>$\gamma$</td>
<td>–</td>
<td>unitless</td>
</tr>
<tr>
<td>Other terms in the model derivation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epidermal mechanical advantage [fitted value]</td>
<td>$m$ $[\mu]$</td>
<td>1.98$^n$</td>
<td>unitless</td>
</tr>
<tr>
<td>Guard cell osmotic pressure</td>
<td>$\pi_t$</td>
<td>–</td>
<td>MPa</td>
</tr>
<tr>
<td>Water potential of $\zeta$</td>
<td>$\psi_t$</td>
<td>–</td>
<td>MPa</td>
</tr>
<tr>
<td>Turgor pressure of $\zeta$</td>
<td>$P_t$</td>
<td>–</td>
<td>MPa</td>
</tr>
<tr>
<td>Resistance from y to $\zeta$</td>
<td>$r_{yt}$</td>
<td>–</td>
<td>MPa [mmol H$_2$O m$^{-2}$s$^{-1}$]$^{-1}$</td>
</tr>
<tr>
<td>Effective hydraulic resistance to the guard cells</td>
<td>$r_{gt}$</td>
<td>–</td>
<td>MPa [mmol H$_2$O m$^{-2}$s$^{-1}$]$^{-1}$</td>
</tr>
<tr>
<td>Fraction of transpiration that occurs from $\zeta$</td>
<td>$f_{gt}$</td>
<td>–</td>
<td>unitless</td>
</tr>
<tr>
<td>Leaf transpiration rate</td>
<td>$E$</td>
<td>–</td>
<td>mmol H$_2$O m$^{-2}$ s$^{-1}$</td>
</tr>
<tr>
<td>Boundary layer resistance to water vapour</td>
<td>$r_{bw}$</td>
<td>–</td>
<td>[mol air m$^{-2}$ s$^{-1}$]$^{-1}$</td>
</tr>
<tr>
<td>Terms in the ATP submodel</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaf net CO$_2$ assimilation rate</td>
<td>$A$</td>
<td>–</td>
<td>µmol CO$_2$ m$^{-2}$ s$^{-1}$</td>
</tr>
<tr>
<td>Rate of respiration that continues in the dark</td>
<td>$R_a$</td>
<td>–</td>
<td>µmol CO$_2$ m$^{-2}$ s$^{-1}$</td>
</tr>
<tr>
<td>Photosynthetic CO$_2$ compensation point</td>
<td>$g_a$</td>
<td>–</td>
<td>Pa</td>
</tr>
<tr>
<td>Intercellular CO$_2$ partial pressure</td>
<td>$P_i$</td>
<td>–</td>
<td>Pa</td>
</tr>
<tr>
<td>Michaelis constant for RuBP carboxylation</td>
<td>$K_c$</td>
<td>40.4$^l$</td>
<td>Pa</td>
</tr>
<tr>
<td>Michaelis constant for RuBP oxidation</td>
<td>$K_o$</td>
<td>2.48 × 10$^{10}$</td>
<td>Pa</td>
</tr>
<tr>
<td>Light-limited potential electron transport rate</td>
<td>$J$</td>
<td>–</td>
<td>µmol e$^{-}$ m$^{-2}$ s$^{-1}$</td>
</tr>
<tr>
<td>Light-saturated potential electron transport rate</td>
<td>$J_m$</td>
<td>(2.02 ± 0.48) × $V_o$$^h$</td>
<td>µmol e$^{-}$ m$^{-2}$ s$^{-1}$</td>
</tr>
<tr>
<td>Curvature parameter for $J(I/J_o)$</td>
<td>$\theta_i$</td>
<td>0.908 + 0.036$^d$</td>
<td>unitless</td>
</tr>
<tr>
<td>Incident photosynthetically active irradiance</td>
<td>$I$</td>
<td>1100 [50–1600]</td>
<td>µmol photons m$^{-2}$ s$^{-1}$</td>
</tr>
<tr>
<td>Product of absorbance and effective quantum yield</td>
<td>$k$</td>
<td>0.195 ± 0.023$^e$</td>
<td>electrons photon$^{-1}$</td>
</tr>
<tr>
<td>Ambient O$_2$ partial pressure</td>
<td>$P_{O_2}$</td>
<td>(2.10 [0.2–4]) × 10$^3$</td>
<td>Pa</td>
</tr>
<tr>
<td>Ambient CO$_2$ concentration</td>
<td>$c_a$</td>
<td>365 [50–1000]</td>
<td>p.p.m.</td>
</tr>
<tr>
<td>Atmospheric pressure</td>
<td>$p$</td>
<td>10$^3$</td>
<td>Pa</td>
</tr>
<tr>
<td>ATP concentration</td>
<td>$\tau$</td>
<td>–</td>
<td>mmol ATP m$^{-2}$</td>
</tr>
<tr>
<td>ATP concentration when $W_i &gt; W_j$</td>
<td>$\tau_i$</td>
<td>–</td>
<td>mmol ATP m$^{-2}$</td>
</tr>
<tr>
<td>ATP concentration when $W_i &gt; W_j$</td>
<td>$\tau_j$</td>
<td>–</td>
<td>mmol ATP m$^{-2}$</td>
</tr>
<tr>
<td>Basal ATP level provided by other processes</td>
<td>$\tau_b$</td>
<td>1.6$^d$</td>
<td>mmol ATP m$^{-2}$</td>
</tr>
<tr>
<td>Total concentration of adenylates (τ + [ADP])</td>
<td>$a$</td>
<td>12.6×$V_o$$^{ad}$</td>
<td>mmol A-P m$^{-2}$</td>
</tr>
<tr>
<td>Concentration of photophosphorylation sites</td>
<td>$P$</td>
<td>2.5×$V_o$$^{ad}$</td>
<td>mmol sites m$^{-2}$</td>
</tr>
<tr>
<td>Potential RuBP pool size</td>
<td>$B_p$</td>
<td>–</td>
<td>µmol RuBP m$^{-2}$</td>
</tr>
<tr>
<td>Total concentration of Rubisco active sites</td>
<td>$E_i$</td>
<td>–</td>
<td>µmol sites m$^{-2}$</td>
</tr>
<tr>
<td>Rubisco turnover number</td>
<td>$k_i$</td>
<td>–</td>
<td>CO$_2$ site$^{-1}$ s$^{-1}$</td>
</tr>
<tr>
<td>Carboxylation rates</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Limited by CO$_2$ and Rubisco, but not by RuBP</td>
<td>$W_i$</td>
<td>–</td>
<td>µmol CO$_2$ m$^{-2}$ s$^{-1}$</td>
</tr>
<tr>
<td>Limited by RuBP and CO$_2$, but not by Rubisco</td>
<td>$W_j$</td>
<td>–</td>
<td>µmol CO$_2$ m$^{-2}$ s$^{-1}$</td>
</tr>
<tr>
<td>Limited by Rubisco only</td>
<td>$V_o$</td>
<td>(8.86 ± 0.215) × 10$^a$</td>
<td>µmol CO$_2$ m$^{-2}$ s$^{-1}$</td>
</tr>
<tr>
<td>Limited by potential RuBP pool size only</td>
<td>$V_i$</td>
<td>2.27×$V_o$$^{b}$</td>
<td>µmol CO$_2$ m$^{-2}$ s$^{-1}$</td>
</tr>
</tbody>
</table>

Values are given where appropriate; where ranges are given in brackets, standard values are given in italics, and for parameters estimated by gas exchange, standard deviations are given, preceded by the ± symbol. Sources: *assumption; **Appendix 4; *Caemmerer et al. (1994); *Farquhar & Wong (1984). The notation $[V_o]$ means the numerical value of $V_o$, i.e. $V_o$/[µmol CO$_2$ m$^{-2}$ s$^{-1}$]. The subscripts $i$, $o$, or $m$, referring to epidermal cells, guard cells, xylem, and mesophyll cells, respectively. Where experimental precision was known, non-significant digits are subscripted but retained for accuracy.
probe experiments suggest that equal reductions in guard cell and epidermal turgor should cause stomatal aperture to increase; this is because aperture responds negatively, and more strongly, to the ‘backpressure’ of epidermal cells than to the opening force provided by guard cell turgor (Franks, Cowan & Farquhar 1998). Therefore, guard and epidermal cell turgors must be decoupled from one another during the steady-state response of \( g \) to changes in hydraulic supply and demand (Buckley & Mott 2002a). Two principal hypotheses have been advanced to explain this decoupling. The first, which we call the ‘metabolic regulation hypothesis’, suggests that \( \pi \) is actively regulated in proportion to the water potential or turgor pressure of cells near the evaporating site (Haefner, Buckley & Mott 1997). The second, which we call the ‘drawdown hypothesis’, suggests that steady-state stomatal responses to hydraulic perturbations are caused by a water potential gradient from epidermal to guard cells (Dewar 1995, 2002).

Each of these hypotheses can explain the steady-state humidity response. However, to explain both the transient and steady-state phases of the humidity response, the drawdown hypothesis requires the hydraulic conductivity from epidermal to guard cells to vary with VPD in complex fashion (Buckley & Mott 2002a), but there is neither any established role in stomatal behaviour for cell-to-cell conductivity regulation, nor any proven mechanism to effect such regulation. In contrast, the metabolic regulation hypothesis is based on a simple, monotonic relationship between \( \pi \) and \( P \), both in the steady-state and transient phases of the humidity response, and it predicts a monotonic steady-state relationship between \( \pi \) and VPD (Buckley & Mott 2002a). For these reasons, and because it explains short-term hydraulic responses in terms of the same mechanism – osmotic regulation – that drives most other stomatal responses, the metabolic regulation hypothesis seems most parsimonious.

In this study, we derive a closed-form model of \( g \) based on two hypotheses: (1) the osmotic gradient across guard cell membranes, \( \delta \pi \), is limited by guard cell ATP concentration, \( \tau \); and (2) the osmotic gradient that can be sustained per unit of ATP is proportional to epidermal turgor pressure, \( P \), (the metabolic regulation hypothesis). We simulate \( \tau \) in the present study using the model of Farquhar \& Wong (1984) for [ATP] in C\(_3\) mesophyll cells, which is based on the model of Farquhar et al. (1980) for C\(_3\) mesophyll photosynthesis. The use of that ATP submodel entails the implicit assumption that similar biochemical processes control [ATP] in guard cells and in mesophyll cells; however, the model’s validity does not rest on this assumption, and requires merely that [ATP] respond to environmental factors as required to produce observed conductance responses. We assume that stomatal aperture is determined by guard and epidermal cell turgor pressures in the manner shown by Franks et al. (1995, 1998). We parameterize and test the stomatal model for *Vicia faba* L., interpret its behaviour with the help of some algebraic simplifications, and discuss its structure and behaviour in relation to other stomatal models.

SYNOPSIS OF THE MODELLING APPROACH

Our model, like several other recent efforts (Dewar 1995, 2002; Haefner et al. 1997; Gao et al. 2002), is based on five assertions that form a mathematical ‘closed loop.’ These are: (1) stomatal conductance is proportional to stomatal aperture \( (g = a) \); (2) aperture is controlled by guard cell turgor pressure \( (a = P) \); (3) turgor is the sum of water potential and osmotic pressure \( (P = \psi + \pi) \); (4) water potential is ‘drawn down’ to guard cells from a source and through a resistance, by transpiration \( (\psi = \psi_e - E R_g) \) (Fig. 1 shows a resistance diagram); and (5) transpiration rate is the product of conductance and evaporative gradient \( (E = g D) \). Combining the first two assertions as \( g = \chi P \) (with \( \chi \) a constant), the solution of these equations (derived as Eqn A7 in Appendix 1) is

\[
g = \chi \frac{\psi_s + \pi_s}{1 + R_y D_1}
\]

[The symbol \( \psi \), which also appears on Eqn 4 below, indicates that this expression is not part of our model – it is presented only for heuristic purposes.] Equation 1 is consistent with the observation that stomata open more in well-watered
plants (high \(\psi_s\)), less in dry air (high \(D_g\)) and less under high hydraulic resistance (\(R_g\)), and that stomatal opening in the light is accompanied by an increase in guard cell osmotic pressure. This expression is the hydromechanical framework of the model of Gao et al. (2002). It contains a single negative hydraulic feedback loop (loop no. 1 in Fig. 2a), formed by the assertions underlying Eqn 1: an increase in \(g\) decreases \(\psi\), which lowers \(P_e\) reducing stomatal aperture and thus \(g\). This feedback loop is what causes the steady-state responses to \(D_g\), \(\psi_s\), and \(R_g\) in Eqn 1 and in the Gao model.

However, Eqn 1 does not account for the effect of epidermal turgor pressure, \(P_e\), on aperture. Theoretical analysis and pressure probe experiments (DeMichele & Sharpe 1973; Edwards, Meidner & Sheriff 1976; Sharpe, Wu & Spence 1987; Franks et al. 1998) show that stomatal aperture responds positively to guard cell turgor pressure (\(P_e\)), but negatively, and more strongly, to epidermal cell turgor (\(P_e\)). Thus, the assertion that \(g = \chi P_e\) is replaced by

\[
g = \chi(P_e - \hat{m}P_s), \quad \hat{m} > 1 \tag{2}
\]

The parameter \(\hat{m}\) is often termed the ‘mechanical advantage’ of the epidermis and \(\hat{m} = \hat{m} - 1\) is the ‘residual’ mechanical advantage. The observation that \(\hat{m} > 1 (\hat{m} > 0)\) creates some complications: (1) guard cells are ‘downstream’ from epidermal cells in the transpiration stream, so they may have a lower water potential than epidermal cells (\(\psi_s < \psi_e\)) and support a different fraction of transpiration (\(f_g\)); (2) the hydraulic resistance for water flow to guard cells (\(R_g\)) may be higher than that for the epidermis (\(R\)), so \(R_g = R + f_e R_g\) (see resistance diagram in Fig. 1); and (3) guard and epidermal cells may also have different osmotic pressures (\(\pi_s > \pi_e\) generally). When these features are added to the assertions underlying Eqn 1, the solution (derived as Eqn A10 in Appendix 1) is

\[
g = \chi \left(\frac{\psi_s - \pi_e + \pi_s - \pi_e}{1 - \hat{m}D_e(M - f_e R_g/R)} \right) (M = \hat{m} - 1) \tag{3}
\]

Despite being more complicated than Eqn 1, this expression seems incorrect at first glance, because the response to source water potential is now negative, and if \(M > f_e R_g/R\), the responses to hydraulic resistance and humidity are also in the wrong direction. This occurs because the positive feedback that operates via \(P_e\) (loop no. 2 in Fig. 2a) is stronger than the negative feedback via \(P_s\) (loop no. 1 in Fig. 2a) because \(m > 1\).

Dewar (2002) suggested a resolution to this problem. He noted that \(M\) could be considered zero if one interprets \(P_e\) as the ‘bulk’ epidermal turgor (averaged over all epidermal cells, not only the ‘subsidiary’ cells that immediately adjoin the guard cells), and if \(\pi_e\) is lower in non-subsidary than in subsidiary epidermal cells. If the postulated difference between \(\pi_e\) and \(\pi_{ebulk}\) is large enough to overcome the mechanical advantage of the subsidiary cells and the increase in water potential that should occur with distance from each stomatal pore, then Eqn 2 can be replaced by \(g = \chi(P_e - P_{ebulk})\), implying \(M = 0\). Applied to Eqn 3, this yields a new solution (Eqn A11 in Appendix 1):

\[
g = \chi \left(\frac{\pi_e - \pi_{ebulk}}{1 + f_e R_g D_e} \right) \tag{4}
\]

[The \(^{\ast}\) symbol indicates this is not part of our model, as for Eqn 1.] By nullifying the mechanical advantage, the Dewar resolution weakens the positive hydraulic feedback that occurs via \(P_e\), making its intrinsic strength equal to that of the negative feedback via \(P_s\). The negative feedback is then strengthened by a hydraulic gradient from epidermal to guard cells, equal to \(f_e R_g D_e\). This resolution produces the correct negative steady-state response to \(D_g\), but by focusing direct hydraulic responses in the epidermal-to-guard cell gradient, it eliminates the direct effects of \(\psi_s\), and \(R\) – necessitating an additional model to predict stomatal responses to those factors. Dewar (2002) used a soil–plant hydraulic model to calculate epidermal water potential (\(\psi_e\)) and then postulated an effect of \(\psi_s\) on the sensitivity of guard cell solute leakage to xylem sap ABA. To produce observed responses to intercellular CO2 concentration (\(c_i\)) and irradiance, Dewar assumed \(\pi_e\) proportional to the rate of gross photosynthesis, and inversely proportional to \(c_i\); these effects correspond to feedback loop no. 4 in Fig. 2a.

However, the core assumption underlying Eqn 4 is called into question by pressure probe experiments that found no systematic variation in turgor between subsidiary and non-subsidary epidermal cells (Franks et al. 1995, 1998; Mott & Franks 2001). Additionally, it is often observed that stomata initially respond in the ‘wrong direction’ when \(\psi_s\), \(R\) or \(D\), are varied and then reverse course and slowly converge to the ‘correct’ steady-state response. Equation 4 does not predict these ‘wrong-way’ responses, whereas Eqn 3 does.

A different resolution

We accept at face value the experimental evidence suggesting that \(M > 1 (\hat{m} > 1)\) in Eqn 2), regardless of where in the epidermis \(P_e\) is measured. As a result, the net hydropassive feedback that results from a change in \(D_g\), \(\psi_s\), or \(R\) is positive, because the mechanical advantage renders the hydropassive feedback through \(P_e\) (loop no. 2 in Fig. 2a) stronger than that via \(P_s\) (loop no. 1 in Fig. 2a). Buckley & Mott (2002a, b) proposed a resolution that avoids the need to assume a spatial gradient in \(\pi_s\) or a large value of \(f_e R_g\), and that predicts both the steady-state and temporary ‘wrong-way’ responses to \(D_g\), \(\psi_s\) and \(R\) with a single mechanism. Below, we formalize that resolution and use it to derive a new steady-state model of stomatal conductance.

Specifically, we hypothesize that the steady-state osmotic gradient across guard cell membranes (\(\delta \pi_g\)) is proportional to guard cell ATP concentration (\(\tau\)), and that the sensitivity of \(\delta \pi_g\) to \(\tau\) scales with epidermal turgor pressure, \(P_e\). These hypotheses create another feedback loop that operates via \(P_e\) (loop no. 3 in Fig. 2a), but which has negative gain. This negative, hydropassive feedback gradually overrides the initial positive hydropassive feedback caused by the mechanical advantage, so that at steady state.
Figure 2. Diagrams showing influences among key variables in three recent hydromechanical models of stomatal conductance (Gao et al. 2002; Dewar 2002; and the model presented in this study). (a) Generic diagram with four important feedback loops highlighted and numbered from 1 to 4. (b) Diagrams of each model, modified from the generic diagram, and showing where each of six stomatal effectors ($D_s$, $y_s$, $R$, $c_i$, PFD and $pO_2$) influences the system directly. (c) Feedbacks in each model that are purely physical or hydraulic feedbacks (hydropassive). In the Dewar model, the assumption that $M = 0$ collapses loops no. 1 and no. 2 into one term, $\Delta P(P_e - P_i)$, which then uniquely determines aperture. The resulting combined feedback loop has negative gain because any resistance from epidermal to guard cells ($f_{rg}$) causes $P_g$ to decrease more than $P_e$ when $E$ increases. In our model, the mechanical advantage makes the loop no. 2 stronger than loop no. 1, so the net hydropassive feedback is positive. (d) Feedbacks with a biochemical component. The Dewar model uses loop no. 3 to produce responses to $y_s$ and $R$; our model uses loop no. 3 to override the positive hydropassive feedback shown in (c). Both models also include negative feedback from the photosynthetic apparatus (loop no. 4). (e) Major stomatal responses (listed on the left) and the feedback loops, numbered as in (a), that are responsible for initiating those responses.

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\[ \delta \pi_x = \beta t P_c \]  

where \( \beta \) is a sensitivity parameter, assumed constant. When applied to the general solution (Eqn 3), this leads to yet another solution:

\[ g = \frac{\chi (\beta t - M)(\psi_s + \pi_c) - \pi_a + \pi_s}{1 + \chi R D_s (\beta t - M + \rho)} \]  

where a new term, the guard cell resistive advantage, \( \rho = f_r \pi_c R = (R_\text{g}/R - 1) \), has been introduced for clarity, and \( \pi_s \) is the osmotic pressure in the apoplasm near the stomatal complex. (Eqs 5 & 6 are derived as Eqns A12 & A15 in Appendix 1).

THE MODEL

Equation 6 can be simplified into a compact and useful form that is algebraically similar to the Michaelis–Menten expression for the rate of an enzyme-mediated reaction:

\[ g = \frac{g_m (\alpha - \gamma)}{\alpha + K_b} \]  

In Eqn 7, \( g_m \) is the maximum conductance in the absence of feedback limitation, \( \alpha \) is the guard cell advantage, \( K_b \) is the ‘Michaelis constant’ for \( \alpha \), and \( \gamma \) is the hydroactive compensation point. These new terms are defined by Eqns 8–11 and described below:

\[ g_m = \frac{\psi_s + \pi_c}{R D_s} \]  

\[ \alpha = \beta t - M + \rho \]  

\[ K_b = \frac{1}{\chi R D_s} \]  

\[ \gamma = \frac{\pi_s - \pi_a}{\pi_s + \psi_s + \rho} \]  

The maximum conductance, \( g_m \), is the conductance required for transpiration to match the maximum possible flow rate through the plant, which occurs when the gradient that drives water flow to the leaf, \( \psi_s - \psi_c \), reaches its most negative possible value, \( \psi_s + \pi_c \). Then \( g = E/D_s = [(\psi_s + \pi_c)/R]/D_s = g_m \). As \( g \) approaches \( g_m \), hydroactive and hydropassive feedback cease to constrain transpiration, so \( g_m \) represents the conductance in the absence of feedback limitation.

The guard cell advantage, \( \alpha \), is central to the interpretation of our model. It is the balance of three different effects of leaf water status on stomatal conductance. The first influence, \( \beta t \), is a positive, hydroactive effect that we call the guard cell metabolic advantage. The second influence, \( M \), is a negative, hydromechanical effect caused by the epidermal mechanical advantage. The third influence, \( \rho \), is a positive hydraulic effect that we call the guard cell resistive advantage, caused by any water potential drawdown that may occur from epidermal cells to guard cells.

The ‘Michaelis constant’ for \( \alpha \), \( K_b \), is a measure of the sensitivity of stomatal conductance to ATP; if \( K_b \) is small, \( g \) saturates at low \( \alpha \) and therefore at lower irradiance. \( K_b \) also represents a measure of the intrinsic balance between the hydraulic supply and demand: the transport capacity (hydraulic conductance) of the xylem equals \( 1/R \), and the evaporative demand of the atmosphere equals \( D_s \). The ‘hydroactive compensation point’, \( \gamma \), the value of \( \alpha \) required to overcome epidermal turgor to induce stomatal opening. The period of time during which \( \pi_a \) increases in response to light after a period of darkness, but before \( \alpha \) reaches \( \gamma \), is commonly referred to as the Spannungsphase (Stählefeld 1929). The resistive advantage (\( \rho \)) appears in \( \gamma \) because, if stomata are closed, there is no transpirational flux to create a standing gradient from epidermal to guard cells, so \( \rho \) has no effect; in other words, when \( \alpha < \gamma \), only \( \beta t \) is available to overcome \( M \). Our model includes a basal level of ATP (\( \tau_c \)) that does not depend directly on irradiance, and which makes \( \alpha \) positive in the dark, reducing the photon flux density (PFD) required to open stomata. In this context, stomatal opening in darkness would imply \( \tau_c > (\gamma + M - \rho)/\beta \).

Most biologists are familiar with the archetypal topology of Michaelis–Menten curves, so Eqn 7 may help to visualize the model’s behaviour, although the analogy with enzyme kinetics is limited, because \( g_m \) and \( K_b \) co-vary through \( R \) and \( D_s \). For example, an increase in soil water potential raises \( g_m \), permitting higher stomatal conductance and thus greater water use rates. An increase in \( D_s \) has two effects: it decreases \( K_b \) (the ‘Michaelis constant’ for \( \alpha \)), which steepens the response of \( g \) to \( \alpha \), making stomata more sensitive to changes in light or photosynthetic capacity, and it decreases \( g_m \), lowering the conductance achieved for a given irradiance and water supply (see Fig. 5a, discussed below).

MODEL BEHAVIOUR

To evaluate the behaviour of the model, we parameterized it using gas exchange and pressure probe experiments on *Vicia faba* L. (Appendix 4). We then performed additional gas exchange experiments to document stomatal responses to changes in environmental variables (Appendix 5) and simulated those experiments, as well as other ‘thought experiments’, in the model (Appendix 3).

Figure 3 compares measured and modelled responses of stomatal conductance to variations in ambient CO₂ concentration (\( c_\text{a} \)), incident irradiance (\( I \)), leaf-to-air water vapour mole fraction gradient (\( D \)) and ambient O₂ concentration (\( P_{\text{O}_2} \)). Conductance declines with increasing \( D \) and \( c_\text{a} \) and rises with incident irradiance (\( I \)) (Fig. 3a–c). However, the relative decline with \( c_\text{a} \) is steeper at low irradiance (Fig. 3a), because ATP concentration responds more steeply to increasing CO₂ supply when photosynthesis is limited by RuBP regeneration (see Fig. 4a, discussed below). Similarly, the light response saturates more quickly at low \( c_\text{a} \) than at high \( c_\text{a} \) (Fig. 3b), because photosynthesis is saturated at lower irradiances when \( c_\text{a} \) is low. Figure 3a also shows another response of \( g \) to \( c_\text{a} \) at high PFD, using a
larger value of \( V_m \) (RuBP carboxylation capacity), chosen to make the modelled and observed responses match and to show that the value of \( c_a \) at which the response slope changes is strongly dependent on \( V_m \). The value of \( V_m \) could not be measured for the leaves whose responses are shown in Fig. 3, so the simulations used a ‘standard’ value of \( V_m \), calculated as an average from five leaves (see Appendix 4 and Table 1); those five estimates varied by nearly 200%, so it is likely that the measured leaves shown in Fig. 3 each had a different \( V_m \), which may have differed substantially from the ‘standard’ model value.

The model predicts that stomata can either open or close slightly in response to variations in ambient oxygen concentration, \( \text{pO}_2 \) and observations showed negligible responses (Fig. 3d). Although the match between our model and the data was less convincing for oxygen than for the \( \text{CO}_2 \), light and humidity responses, other stomatal models generally perform worse and they do not predict that the response can be either positive or negative (see Fig. 7, discussed below).

The biochemical substructure of the model, which controls the responses to \( \text{CO}_2 \), irradiance, and oxygen, is deeply...
embedded in \( \tau \) (Eqn 6) or \( \alpha \) (Eqn 7). In turn, \( \tau \) and \( \alpha \) respond to those environmental factors indirectly, via their effects on photosynthesis as described by the model of Farquhar \textit{et al}. (1980) (Appendix 2). Because we used the \( \tau \) model of Farquhar \& Wong (1984), our model responds to photosynthetic effectors in similar fashion to theirs. However, our model also explicitly includes hydraulic feedback, which warps the responses of \( g \) relative to the purely biochemical responses of \( \tau \) specified by the Farquhar and Wong model. Figure 4 illustrates the linked biochemical and hydraulic control of stomatal conductance by showing how \( \tau \), \( \alpha \) and \( g \) vary with \( c_i \), at a series of irradiances. \( \tau \), \( \alpha \) and \( g \) respond to \( c_i \); with very similar shapes, although the shape of \( g \) versus \( c_i \) is slightly different for different values of \( D \) (Fig. 4b). These features can be understood in terms of the Michaelis–Menten analogy (Eqn 6): near-linearity between \( g \) and \( \alpha \) implies that \( K_a \) is large relative to \( \alpha \). However, \( K_i \) and \( g_m \) both depend on \( D \) (Eqn 8). Figure 5a shows that as \( D \) increases, \( g \) saturates more quickly and at a lower value of \( \alpha \), because both \( K_a \) and \( g_m \) decrease. (Fig. 5c shows how \( D \) affects the response of \( g \) to irradiance itself, rather than \( \alpha \).)

The Michaelis–Menten analogy also provides a way to interpret the effects of declining soil water potential and osmoregulation. If \( \psi_s \) declines but epidermal osmotic pressure is 'osmoregulated' to match the decline in \( \psi_s \), then \( g_m \) will not change, but the hydroactive compensation point, \( \gamma \), will increase. As a result, the shape of \( g \) versus \( \alpha \) will be unaffected, but the curve will shift to lower \( g \) (Fig. 5b). If, on the other hand, epidermal osmoregulation only matches part of the decline in \( \psi_s \), then \( g_m \) will decline and \( \gamma \) will increase further still, changing both the shape and vertical position of the curve. Figure 5d shows how these hypothetical variations in \( \psi_s \) and \( I_a \) affect the light-response curve itself; note that a higher irradiance is required to open stomata at low \( \psi_s \), because of the larger hydroactive compensation point.

**Parameter sensitivity and spatial averaging**

Figure 6 shows how parameter variation affects modelled responses to humidity, \( \mathrm{CO}_2 \) and light. Halving or doubling the residual mechanical advantage (\( M \)) has a fairly small effect on the shape and position of most of these responses (Fig. 6a–c); most significantly, the irradiance required to open stomata is higher when \( M \) is larger (Fig. 6c), because \( M \) decreases the guard cell advantage, requiring higher \( \tau \) to overcome epidermal turgor and drive \( \alpha \) over \( \gamma \). The insensitivity to \( M \) seems paradoxical in light of the importance of the epidermal mechanical advantage to stomatal hydraulic, but the reason is simply that \( \beta \) over \( \tau \), which was introduced for the explicit purpose of overcoming \( M \), is much larger than \( M \). Because \( R \) and \( D \) are algebraically interchangeable in our model (see Eqns 6–10), varying \( R \) merely compresses the x-axis for the response to \( D \), and has the same effect as variation in \( D \) on the light-response curve, discussed above (cf. Figs 6f & 5c). Increasing \( \beta \) steepens and magnifies the stomatal responses to each of \( D \), \( c_i \), and \( I \) (Fig. 6g–i), highlighting the dual roles of \( \beta \) as an independent control on stomatal sensitivity and as a link between hydraulic and biochemical factors.

To provide a broader perspective on the model’s behaviour under different parameter regimes, we performed a Monte Carlo analysis, in which many parameters are simultaneously and randomly varied (Fig. 6j–o; see Appendix 3 for details). In Fig. 6j–l, and ten \( g \) response curves are shown for \( D, c_i \), and \( I \); each curve represents a different leaf (or patch of leaf) with a different set of parameters. Figure 6m–o show the mean and standard deviations among 150 response curves from a set of Monte Carlo simulations. One possible interpretation of the mean curves (solid lines in Fig. 6m–o) is that they represent the behaviour of the model averaged over many ‘leaves’ with different parameter values but identical environmental conditions; however, that interpretation bears the caveat that the averaged ‘leaves’ are functionally independent. The averaging tends to smooth out the kinks caused by transition from Rubisco to light limitation.

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DISCUSSION

Several stomatal models already exist that can predict most commonly observed variations in stomatal conductance (Jarvis 1976; Ball et al. 1987; Leuning 1995; Jarvis & Davies 1998; Dewar 2002; Gao et al. 2002; Gutschick & Simonneau 2002; Tuzet et al. 2003). However, we are unaware of any other single model that is consistent, in both structure and behaviour, with all of the following empirical constraints: (1) $g$ can vary with $E$ despite constant $D_s$ and with $c_i$ despite constant $c_s$; (2) under most conditions, stomata are fairly unresponsive to oxygen; (3) increases in $D_s$ and $R$ cause conductance to increase transiently, and then decline in the steady state; (4) aperture is more sensitive to epidermal turgor than guard cell turgor, implying that a uniform decrease in guard cell turgor should cause stomata to open, rather...
Figure 6. Effects of parameter variation on stomatal responses to $D_s$, $c_a$ and $I$ predicted by the model. In each of plots (a–i), only one parameter differed from the standard values in Table 1 ($M$ varied in a, b and c; $R$ in d, e and f; and $\beta$ in g, h and i). Dashed and dotted lines are simulations at non-standard values of $M$, $R$ or $\beta$ and solid lines are simulations at standard values (as described in the legends at right). Plots (j–o) show Monte Carlo simulations (detailed in Appendix 3), in which all parameters were randomly varied. (j) (k) (l): ten individual ‘leaves’ with randomly varied parameters. (m) (n) (o): mean ± standard deviation (SD) among 150 Monte Carlo simulations in which only $\beta$, $V_m$, $J_m/V_m$, $\theta$ and $F$ were varied, using measured values of SD given in Table 1 (*, dashed lines), or in which $R$, $M$, $\chi$ and $\pi$ were also varied, with SD = 0.175 mean (**, dotted lines).
than close; and (5) increases in $\psi$, by root de-pressurization cause immediate, reversible responses that are similar to the responses to $D_s$ and $R$, suggesting root signals are probably not involved. Our model satisfies each of these constraints:

1. **Responses to $E$ and $c_c$.** It is clear from experimental evidence that the stomatal response to $D_s$ is actually a response to $E$ (Mott & Parkhurst 1991), and that the stomata respond to $c_c$ independently of $E$ (Mott 1988). These mechanistic aspects of stomatal behaviour were missing from most early models of stomatal conductance, but several recent models accommodate them (Dewar 2002; Gao et al. 2002; Tuzet et al. 2003).

2. **Oxygen response.** Stomata are generally unresponsive to experimental variation in ambient oxygen partial pressure, $pO_2$ (Gauhl 1976; Nobel, Longstreth & Hartsock 1978; Farquhar & Wong 1984; Fig. 3d). In our model, stomata respond to changes in $pO_2$ via changes in guard cell ATP concentration, $\tau$, which we simulated using the model of Farquhar & Wong (1984). That model predicts very small responses to $pO_2$ (which our hydromechanical framework dampens slightly by its hyperbolic dependence on $\tau$), and negligible responses were measured by gas exchange (Fig. 3d). In contrast, most of the models listed above either do not respond to oxygen at all (Gao et al. 2002), or they always respond strongly and negatively (Fig. 7), either by a direct response to net CO2 assimilation rate ($A$) (BBL, Gutschick & Simonneau 2002), the ratio of $A$ to $c_c - \Gamma$ (Tuzet et al. 2003), or the ratio of gross photosynthetic rate to $c_c$, $(A + R_g)/c_c$ (Dewar 2002). An exception is the model of Jarvis & Davies (1998), which captures responses to light and CO2 via the quantity $A_m - A$ (where $A_m$ is photosynthetic capacity). Because $A$ responds negatively to oxygen and $A_m$ does not respond at all, that model (discussed below) responds positively to $pO_2$.

3. **Transient wrong-way responses and the epidermal mechanical advantage.** The steady-state responses to short-term variations in $D_s$, $R$, and $\psi$ are typically preceded by a transient change in $g$ in the opposite direction to the steady-state response (Darwin 1898; Raschke 1970; Farquhar & Cowan 1974; Kappen, Andresen & Losch 1987; Comstock & Mencuccini 1998). Hydropassive responses to increases in $D_s$ are controlled by two feedback loops – negative feedback occurs via $P_r$, positive feedback occurs via $P_g$ (these are labelled as 1 and 2, respectively, in Fig. 2a), and the positive feedback is stronger because of the epidermal mechanical advantage. However, the total feedback must be negative for $g$ to decrease in the steady state as observed. The Gao model achieves this by excluding the $P_g$ loop entirely; the Dewar model achieves it by assuming that $M = 0$, and that a large drawdown in $\psi$ occurs from epidermal to guard cells (Fig. 2b).

Evidence suggests $M > 0$ (DeMichele & Sharpe 1973; Franks et al. 1998), and our model takes this evidence at face value. As a result, its net hydropassive feedback is positive (Fig. 2c) and the hydropassive responses to $D_s$, $R$, and $\psi$ are in the ‘wrong’ direction. However, our core hypothesis – that $P_r$ affects the sensitivity of $\delta_g$ to $\tau$ (Eqn 5) – creates another negative feedback loop (loop no. 3 in Fig. 2a). This negative *hydroactive* feedback overcomes the hydropassive effects to produce steady-state behaviour consistent with observations. Because changes in $\delta_g$ follow causally from changes in $P_r$, the latter must be the first to change, so hydropassive ‘wrong-way’ responses must precede hydroactive steady-state responses. The duration of the wrong-way response is determined by the ratio of the time constants.

**Figure 7.** Responses of stomatal conductance to ambient oxygen concentration measured by gas exchange (symbols) and simulated (lines) using five different stomatal models, including the model described in this paper, for three different sets of conditions (a) (b) and (c). $g$ is expressed relative to its value at 21% oxygen in all cases; in (c), the $y$-axis is broken at $g = 1.5 \text{ mol m}^{-2} \text{s}^{-1}$ and condensed at higher values to accommodate the large negative responses of some models. Simulations were performed using measured values of $A$, $c_c$, $I$ and $pO_2$ and the photosynthetic compensation point, $\Gamma$, was calculated from the biochemical model of photosynthesis (Appendix 2) (accounting for the dependence of $\Gamma$ on $pO_2$). The models of Jarvis & Davies (1998) and Tuzet et al. (2003) contain unknown hydraulic parameters, so we only simulated their biochemical components ($A_m - A$ and $A/(c_c - \Gamma)$, respectively; $A_m = J/4$ at the current irradiance), which do not account for hydraulic feedback. (The data and the simulations from our model are the same as in Fig. 3d).
short-term stomatal responses to variations in root potential:

\[ \frac{\partial Y}{\partial t} = \frac{1}{1 + sD_s(A_m - A)} \]

where \( A \) is the net CO₂ assimilation rate, \( A_m \) is the value of \( A \) at saturating \( c_i \) and \( s \) and \( G \) are empirical parameters. Jarvis and Davies obtained Eqn 12 by positing abstractly that \( g \) is controlled by two linked feedback loops. First, \( g \) is proportional to the ‘residual photosynthetic capacity’, \( A_m - A \); that is \( g = G^*(A_m - A) \). In the hydromechanical context, this is feedback loop no. 4 in Fig. 2a. Second, the proportionality factor \( G^* \) declines from a maximum value, \( G \), with increasing transpiration rate: \( G^* = G - sE \). This corresponds to feedback loop no. 3 in Fig. 2a. Comparison of Eqn 12 with Eqns 6–11 suggests \( s \propto R \), \( G \propto (\psi + \psi_i) \) and \( (A_m - A) \propto \alpha \). The relations are not precise because two other independent parameters (\( \gamma \) and \( \beta \)) link the relevant features dimensionally in our model, and also because, in describing explicitly the hydraulic feedback loop posited by JD, our model introduces hydromechanical terms such as \( M_p \) and \( R \).

Despite these distinctions, both models produce the three photosynthetically related features of stomatal behaviour (the responses to CO₂ and irradiance, and the correlation with photosynthetic capacity) by supposing that stomata respond positively to some measure of how much faster CO₂ could be fixed if stomata did not limit its supply (\( \tau \) in our model, \( A_m - A \) in JD). In contrast, other models predict positive responses to \( I \) and \( A_m \) by including a direct response to \( A \) itself; therefore, to predict the negative response to \( c_i \), they must also include an explicitly negative response to some surrogate for CO₂ supply (e.g. \( c_i, c_E, c_m - \Gamma \), or \( c_i - \Gamma \)). The fact that JD predicts a positive response to oxygen in all conditions, whereas the observed response is negative in some conditions and positive in others (see Figs 3d & 7), suggests that if stomata do respond to residual photosynthetic capacity, that response is mediated by a less direct surrogate than \( A_m - A \). Guard cell ATP concentration is one obvious candidate for that surrogate.

### Co-variation of conductance and photosynthesis

The rationale for modelling \( g \) in proportion to \( A \) (as most other models do), rather than \( A_m - A \) or \( \tau \), is based on the observation that \( g \) and \( A \) co-vary linearly as irradiance varies for a single leaf, or as photosynthetic capacity varies among leaves (Wong, Cowan & Farquhar 1979). Figure 8 illustrates how this feature emerges in our model on a short time scale, as irradiance varies. For any given value of \( c_i \), there are two independent constraints on \( g \) that must be satisfied simultaneously: the biochemical and hydromechanical model (Eqn 6 or 7) and the expression for CO₂ diffusion (Eqn A25). The actual state of the leaf corre-
sponds to the intersection of these constraints. Figure 8a shows how these two constraints vary with \( \alpha \) (the latter determined by the biochemical model of photosynthesis, and driven by independent variation of \( c_i \)) at five different irradiances. The intersection points at different irradiances are almost linearly related. Furthermore, if the same constraints are plotted against \( c_i \) rather than \( \alpha \), the intersections occur at similar values of \( c_i \) except at low PFD (Fig. 8b) – showing how our model produces the well-known conservation of \( c_i \), or the ratio of \( c_i/c_a \) (Fig. 8c).

The conservation of \( c_i \) can also be interpreted mathematically; specifically, the ratio of \( A/g \) must be constant. Comparing Eqsns A18–A20 at constant \( c_i \) with Eqn 7, this implies

\[
\frac{\alpha(I)-\gamma}{\alpha(I)+K_g} = \begin{cases} 
J(I) & \text{if } W_c > W_i \\
V_m & \text{else} 
\end{cases}
\]  

where the guard cell advantage, \( \alpha \), and potential electron transport rate, \( J \), are expressed as functions of irradiance, \( I \). The two conditions on the right apply when electron transport or Rubisco, respectively, limit photosynthesis. The electron transport-limited condition – that \( J \) should increase in similar hyperbolic fashion as \( \alpha \) with irradiance – seems reasonable at first glance, because \( J \) is calculated from a hyperbolic function of \( I \) (Eqn A21). The Rubisco-limited condition, that the hyperbolic function of \( \alpha \) on the left should be constant as irradiance increases, implies either that \( \alpha \) is insensitive to irradiance or that \( \alpha \) is large relative to \( K_g \); the former reason is stronger here because Fig. 4a verifies that \( \alpha \) is relatively insensitive to \( I \) under Rubisco-limited conditions (low \( c_i \) and high \( I \)), whereas \( K_g \) is between 6 and 21 and \( \alpha \) is between 10 and 14. In summary, our model conserves \( c_i \) because (a) when electron transport is limiting, \( \alpha \) increases roughly linearly with \( I \), whereas \( g \) and \( J \) respond hyperbolically to \( \alpha \) and \( I \), respectively; and (b) when Rubisco is limiting, \( V_m \) is insensitive to irradiance, and \( \alpha \) and \( g \) are nearly so.

On a longer time scale, our model would produce a correlation between conductance and photosynthetic capacity if all elements of the latter were assumed to scale together, at least in guard cell chloroplasts (this includes carboxylation, electron transport, and photophosphorylation capacities and the potential RuBP and ATP pools – \( V_m, J_m, R_p \), and \( a_s \), respectively). If that were the case, then guard cell ATP concentration would be simply proportional to \( V_m \) for a given irradiance (see Eqns A22 & A23). Conservation of the ratio \( V_m/J_m \) (Wullschleger 1993; Gonzales-Real & Baille 2000; Meir et al. 2002) provides some evidence that different elements of photosynthetic capacity scale together, but it does not prove that \( a_s, R_p \), and \( p \) all co-vary with \( V_m \) and \( J_m \) in a similar fashion. Furthermore, the hypothesized correlation between \( g \) and \( V_m \) via \( \tau \) is mediated by the parameter \( \beta \) (Eqn 6), which may be regulated independently of \( V_m \). Nevertheless, by having \( g \) depend explicitly on the concentration of specific components of the photosynthetic apparatus, our model provides a testable, mechanistic hypothesis to explain the observed correlation between conductance and photosynthetic capacity, and thus to study how leaves coordinate the constraints on carbon gain caused by multiple limiting resources – water, nitrogen and light.

**Interpretation of the hypothesis that \( \delta x = \beta t \).**

The core hypothesis of our model, Eqn 5 (Eqn A12 in Appendix 1) actually consists of two complementary hypotheses. First, the guard cell osmotic gradient must increase with the turgor pressure of adjacent epidermal cells. We suggested an interpretation of this putative response in Appendix 1, following the reasoning of Dewar.

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**Figure 8.** Diagram showing the two independent constraints that link stomatal conductance to net assimilation rate (\( A \)) and intercellular CO₂ concentration (\( c_i \)). The ‘biochemical constraint’ on \( g \) is Eqn 6 or Eqn 7, in which \( g_w \) depends indirectly on \( c_i \) via ATP concentration. The ‘diffusion’ constraint is simply \( 1.6 A(c_i)/(c_s - c_i) \), where \( A(c_i) \) is the biochemical model of photosynthesis given in Appendix 2. Panel (a) shows these constraints as relationships between \( g \) and \( A \) and panel (b) shows them as relationships between \( g \) and \( c_i \) itself. At any given irradiance, the actual state of the leaf corresponds to the intersection of the two constraints. In the solution (as opposed to in either constraint alone), \( g \) versus \( A \) is roughly linear and \( c_i \) is nearly constant. Panel (c) shows how the solution, expressed in terms of the ratio of \( c_i/c_a \), varies with irradiance for different values of \( D_1 \) and \( c_o \).

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(2002), wherein the resistance to passive osmotic leakage out of guard cells is proportional to epidermal turgor, but our model does not rest on this interpretation. Second, the guard cell osmotic gradient must be proportional to the cytosolic ATP concentration in guard cells, \( \tau \). This hypothesis is supported by recent data of Tominaga et al. (2001) showing that guard cell chloroplasts in Commelina benghalensis supply the ATP necessary to drive proton pumping, and that the pumping rate is limited by ATP supply.

Observed stomatal responses (e.g. Fig. 3) place empirical constraints on the behaviour of \( \tau \): it must increase with light, decrease with \( \text{CO}_2 \) and be fairly insensitive to oxygen. The model of Farquhar & Wong (1984) behaves in this manner, suggesting that it is an empirically adequate submodel for \( \tau \). However, for this submodel to be interpreted as a mechanistic component of our model, a third core hypothesis must be satisfied: that [ATP] is controlled by similar biochemical processes in guard cells and mesophyll cells. Some evidence is inconsistent with this hypothesis – for example, biochemical assays (Outlaw et al. 1979; Outlaw 1989) have reported no evidence for substantial Calvin cycle activity in guard cells – but other evidence supports it. Cardon & Berry (1992) found that guard cell fluorescence in discs from white areas of variegated Tradescantia albiflora leaves responded to \( \text{CO}_2 \) as would be expected if Rubisco-limited \( \text{CO}_2 \) fixation were the major sink for photosynthetic ATP, and that oxygen produced a response, but only at low \( \text{CO}_2 \) – also consistent with a role for Rubisco. Those results were recently confirmed by Lawson et al. (2002), whose apparatus allowed concurrent measurements of guard and mesophyll cell chloroplast fluorescence in green regions of \( T. \) albiflora leaves; they also extended the results to a second species (\textit{Commelina communis}).

It is worth noting that our model for stomatal conductance does not rest on the validity of any particular hypothesis about the biochemical pathways responsible for controlling guard cell [ATP]; mathematically, the model rests only on the assertion that guard cells contain some quantity, \( \tau \), that responds to changes in environmental conditions in the manner required to produce observed conductance responses. If future experiments suggest \( \tau \) is not [ATP], then the mechanism underlying the biochemical component of our model must be re-interpreted, but the model’s hydromechanical framework – arguably its main novel feature – would be unaffected by such evidence. Our model shows how observed stomatal responses to non-hydraulic environmental factors (such as irradiance and \( \text{CO}_2 \)) can be integrated with both ‘wrong-way’ and steady-state responses to hydraulic factors (such as humidity, xylem resistance and source water potential) under the auspices of a single mechanism of guard cell osmotic regulation.

**CONCLUSION**

This study presents a mathematical model that predicts stomatal conductance from the balance of opposing hydromechanical and biochemical influences in and around guard cells. These influences interact directly in the control of the guard cell osmotic gradient, which we hypothesize is proportional to the concentration of ATP in guard cells (a sensor of the balance between \( \text{CO}_2 \) supply and demand in photosynthesis) and to the turgor pressure of adjacent epidermal cells (a sensor of the balance between \( \text{H}_2\text{O} \) supply and demand in transpiration). We used a previously published model based on \( \text{C}_5 \) mesophyll photosynthesis (Farquhar & Wong 1984) to simulate \( \tau \), parameterized and tested the stomatal model directly for \textit{Vicia faba} L. and found that it reproduces the well-known short-term stomatal responses to environmental variables. Unlike other models, ours also predicts that stomata should be relatively insensitive to the ambient oxygen concentration, and it accounts for the epidermal mechanical advantage, which controls critically the direction of passive stomatal responses to hydraulic perturbations.

Our model represents a step towards producing a stomatal model based entirely on reduced processes at the cellular level. As such, it allows properties of gas exchange in intact leaves to be interpreted directly in terms of processes at the cellular level, and it suggests a direct mechanistic nexus between hydraulic and photosynthetic capacities. Finally, our model provides a mathematical framework to help understand how plants coordinate the economic tradeoffs of multiple limiting resources in intact leaves.

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### APPENDIX 1: DERIVATION OF HYDROMECHANICAL MODEL

#### General hydromechanical model

The hydromechanical core of our model consists of five relationships. First, stomatal conductance ($g$) is proportional to stomatal aperture ($a$):

$$ g = \frac{c}{\tau} a \tag{A1} $$

where $\chi$ and $c$ are proportionality constants. Second, stomatal aperture is a linear combination of guard cell and epidermal turgor pressures ($P_g$ and $P_e$, respectively):

$$ a = c(P_g - \hat{m}P_e) \tag{A2} $$

Formally, $P_g$ represents the turgor pressure of ‘subsidiary’ epidermal cells, that is, the cells that immediately adjoin the guard cells, but as pressure probe experiments (Franks et al. 1995, 1998; Mott & Franks 2001) have shown no systematic variation in turgor between subsidiary and non-subsidiary epidermal cells, $P_g$ can also be interpreted as the turgor of the ‘bulk’ epidermis. The parameter $\hat{m}$ in Eqn A2 is sometimes called the ‘mechanical advantage of the epidermis’ and labelled as ‘$m$’. (We use a different symbol in Eqn A2 because $m$ is actually defined as $-\frac{\partial \Phi}{\partial \phi}$, and because Franks et al. (1995, 1998) reported a non-linear relationship between $a$, $P_g$, and $P_e$, so $\hat{m} \neq m$ formally. We fitted Eqn A2 to the Franks data (Fig. 9a & b) and found $\hat{m} = 1.98$.) Third, $P_g$ and $P_e$ are sums of water potential ($\psi$) and osmotic pressure ($\pi$) terms:

$$ P_g = \psi_g + \pi_g \quad P_e = \psi_e + \pi_e \tag{A3} $$

(where $\pi_g$ and $\pi_e$ are positive by convention.) Fourth, each of these water potentials forms one end of a gradient that drives a liquid flow in proportion to the transpiration rate ($E$). To describe these flows, we consider the leaf diagrammed in Fig. 1, in which transpiration occurs from three sites (mesophyll, epidermal and guard cells) in the proportions $f_m$, $f_e$ and $f_g$, respectively (note $f_m + f_e + f_g = 1$) and which is fed water by a single conduit with zero capacitance and resistance given by $R_e$, connected to the soil at water potential $\psi_s$. Then

$$ E = \frac{\psi_s - \psi_f}{R_e} = \frac{\psi_s - \psi_f}{(f_m + f_e + f_g)R_e} = \frac{\psi_s - \psi_g}{f_g R_e} \tag{A4} $$

where $r_e$ and $r_g$ are resistances from the xylem to epidermal cells and from epidermal to guard cells, respectively.

Equation (A4) can be rewritten in terms of $\psi_g$, $\psi_e$, and $\psi_s$: $\psi_e = \psi_s - R_e E$

$$ \psi_g = \psi_s - R_e E \tag{A5} $$

where $R = r_e + f_g R_{e,x}$ and $R_{e,x} = R + f_e r_{e,x}$. Fifth, transpiration rate is the product of stomatal conductance and the evaporative gradient ($D_e$, the difference in water vapour mole fraction between the leaf’s intercellular spaces and the boundary layer):

$$ E = gD_e \tag{A6} $$

To derive Eqn 1 in the main text, we set $\hat{m} = 0$ in Eqn A2 so that $g = \chi P_e$, apply this to Eqn A3 to give $g = \chi[(\psi_g - R_e E) + \pi_g]$ and then apply Eqn A6 and rearrange to solve for $g$:

$$ g = \chi \frac{\psi_g + \pi_g}{R_e D_e} \tag{A7} $$

This expression is marked with a symbol to indicate that it is not part of our model; it is a special case derived for heuristic purposes. To derive Eqn 3 in the main text, we combine Eqs A1–A3 and A5 directly to yield

$$ g = \chi[(\psi_s - R_e E) + \pi_g] - \hat{m}[(\psi_g - R_e E) + \pi_g] \tag{A8} $$

Pooling similar terms and defining the residual mechanical advantage of the epidermis as $M = (\hat{m} - 1)$, we have:

![Figure 9](https://example.com/figure9.png)

Figure 9. The dependence of stomatal aperture, $a$, on guard cell turgor pressure, $P_g$, and epidermal cell turgor pressure, $P_e$. (a) Experimental data of Franks et al. (1998), using the parameters for low $P_g$ calculated by Buckley & Mott (2002a). (b) Equation A2 $a = \max(c(P_g - \hat{m}P_e), 0)$ fitted to the Franks data.
Finally, using Eqn (A6) to replace $E$ with $gD_s$ and solving for $g$ yields:

$$g = \chi\left[-M(\psi_s + \pi_s) + \pi_g - \pi_e + (MR - f_q \gamma_q)E\right] \quad (A9)$$

Equation 4 in the main text is found simply by setting $M = 0$ in Eqn A10:

$$g = \chi\left(\frac{\pi_g - \pi_e}{1 + \chi RD_s}\right) \quad (A11)$$

Again, Eqn A11 is marked with a $^\circ$ symbol to indicate that it is not part of our model.

**Steady-state model with metabolic regulation**

To derive our steady-state model, we constrain Eqn A10 with an expression for the steady-state guard cell osmotic gradient ($\delta\pi_t = \pi_t - \pi_e$, where $\pi_t$ is the osmotic pressure of the apoplastic region near the stomatal complex, assumed uniform). We propose two hypotheses. First, $\delta\pi_t$ is proportional to the concentration of ATP in guard cells, represented by the symbol $\tau$. Second, the sensitivity of $\delta\pi_t$ to $\tau$ is proportional to epidermal turgor pressure, $P_e$. These hypotheses imply:

$$\delta\pi_t = \beta \tau P_e \quad (A12)$$

where $\beta$ is an empirical coefficient. Applying Eqn A12 to Eqn A9, we have

$$g = \chi\left[-M(\psi_s + \pi_s) + (\beta \tau P_e + \pi_t - \pi_e) + (MR - f_q \gamma_q)E\right] \quad (A13)$$

We use Eqns A3 and A5 to express $P_e$ in terms of $\psi_s$, $\pi_t$, $R$ and $E$ and rearrange to pool similar terms:

$$g = \chi\left((\beta \tau - M)(\psi_s + \pi_t) - (\pi_t - \pi_e) + (MR - f_q \gamma_q)E\right) \quad (A14)$$

Finally, we apply Eqn A6 and solve for $g$:

$$g = \chi\left[\frac{(\beta \tau - M)(\psi_s + \pi_t) - \pi_e + \pi_s}{1 + \chi RD_s(\beta \tau - M + p)}\right] \quad (A15)$$

This is Eqn 6 in the main text. A new unitless term, $p$, defined as $f_q\gamma_q/R$, has been introduced in Eqn A15. (Note that $p$ also equals $(R_e - R)/R$ or $R_e/R - 1$). The hydroactive effect represented by $\beta \tau$ overcomes the hydropassive effect caused by $-M$ and the occurrence of a transient hydropassive response to perturbations in either $D_s, R,$ or $\psi_s$ is easily explained by a finite time constant for adjustment of $\delta\pi_t$ in response to changes in $P_e$.

The model form given in Eqn A15 is not strictly a closed-form solution because $D_s$ is the evaporative gradient from the intercellular spaces to the leaf surface and it can not be measured directly. It is inferred from the leaf-to-atmosphere gradient, $D$, given the ratio of stomatal conductance, $g$, and boundary layer resistance, $r_{bw}$; thus, $D_s$ is an implicit function of $g$. The correct closed form solution is the greater root of a quadratic expression: $g = [-q_1 + (q_1^2 - 4q_2q_0)^{0.5}]/2q_2$, where the quadratic coefficients $q_n$ are given by Eqn A16:

$$q_2 = n_{bw}$$

$$q_1 = 1 + \chi[RD\alpha - n_{bw}(\psi_s + \pi_t)(\alpha - \gamma)] \quad (A16)$$

$$q_0 = -\chi(\psi_s + \pi_t)(\alpha - \gamma)$$

The terms $\alpha$ and $\gamma$ are defined by Eqns 9 and 11 in the main text. Equation A15 is easily obtained by substituting $n_{bw} = 0$ and $D_s = D$ into the above.

**The meaning of $\beta$**

Following Dewar (2002), one possible interpretation of the metabolic response parameter, $\beta$ (Eqn A12) is that the rate of active solute uptake by guard cells ($\pi_t$) is proportional to $\tau$ ($\pi_t = \xi \tau$) and that the resistance to outward diffusion is proportional to $P_e$, so that the rate of passive efflux is $\pi_t = \xi \delta\pi_t/P_e$. The net rate of change of $\pi_t$, $\pi_t - \pi_e$, is zero at steady state, so that

$$\xi \tau = \xi \delta\pi_t/P_e \Rightarrow \delta\pi_t = \frac{\xi}{\xi + \tau} P_e \quad (A17)$$

In this interpretation, $\xi$ is the passive efflux rate at a reference $P_e$ of 1 MPa, and $\xi \tau$ is the pumping rate per mmol m$^{-2}$ of ATP. This interpretation would appear to be challenged by experimental data of Fischer & Hsiao (1968) showing that stomata in epidermal peels with punctured epidermal cells remain open after being first illuminated, then placed in darkness. However, the possibility remains that the conductance of guard cell membranes to outward solute diffusion is normally near zero, and that intact epidermal cells are required to generate a signal that causes them to leak (in the absence of closing signals arising in distant tissues, such as ABA from drying roots).

**APPENDIX 2: DESCRIPTION OF THE MODEL OF [ATP]**

Farquhar & Wong (1984) derived expressions for the concentration of ATP in mesophyll chloroplasts of leaves of C$_3$ species, from the mathematical model of photosynthesis presented by Farquhar et al. (1980). The latter model is

$$A = \left[1 - \frac{F_0}{P_i}\right] \min\{W_e, W_j\} - R_d \quad (A18)$$

where $A$ is the leaf net CO$_2$ assimilation rate, $F_0$ is the photorespiratory compensation point, $P_i$ is the partial pressure of CO$_2$ in the intercellular spaces, $W_e$ is the RuBP-saturated rate of RuBP carboxylation, $W_i$ is the rate of carboxylation that can be sustained by the current rate of electron transport, and $R_d$ is the rate of leaf respiration that continues in the dark. $W_e$ and $W_i$ are given by

$$W_e = \frac{V_mP_i}{p_i + K_c(1 + pO_2/K_a)} \quad (A19)$$

where $K_c$ and $K_e$ are the Rubisco Michaelis–Menten constants for RuBP carboxylation and oxygenation, respectively, $V_{	ext{m}}$ is the maximum velocity of RuBP carboxylation, $J$ is the potential electron transport rate and $pO_2$ is the partial pressure of oxygen in the intercellular spaces. $J_T$ depends on $pO_2$ by the empirical relation: $J_T = k_cK_c pO_2/(2k_cK_e)$, where $K_c$ and $K_e$ are the turnover numbers for RuBP oxidation and carboxylation, respectively, by Rubisco; we assumed that $k_c/2k_e = 0.105$, as found at 25°C by Badger & Andrews (1974). Following Farquhar & Wong (1984), $J$ is modelled as the hyperbolic minimum of the light-saturated potential electron transport rate ($J_w$) and the product of incident irradiance ($I$) with the parameter $F$ ($F$ is the product of leaf absorptivity to PAR and the effective quantum yield), so that

$$J = \min[J_w, FI, \theta I]$$

(A21)

where $\min[x, y, \theta I]$ is the root $Z$ of a quadratic expression given by $6Z^2 - (x + y)Z + xy = 0$. The concentration of ATP provided by photophosphorylation is modelled as one of two different values: $\tau_c$, which applies when $W_c < W_p$, and $\tau_g$ which applies when $W_l < W_j$.

$$\tau_c = a_i - \frac{W_c}{W_j}$$

(A22)

$$\tau_g = (a_i - p)\left(\frac{V_r}{V_m - 1}\right)\left(\frac{W_c}{W_j} - 1\right)$$

(A23)

$$\tau = \begin{cases} \tau_c & \text{if } W_c < W_j \\ \tau_g & \text{else} \end{cases}$$

(A24)

In Eqn A24, $\tau_c$ is the basal level of ATP provided by other processes, such as ongoing mitochondrial respiration, $a_i$ is the total concentration of adenylates ($\tau + [ADP]$), $p$ is the concentration of phosphorylation sites and $V_r$ is the CO$_2$- and Rubisco-saturated potential rate of carboxylation (i.e. limited only by the availability of CO$_2$ acceptors). $V_r$ and $V_m$ are given by $k_cR_p$ and $k_cE_c$, respectively, where $k_c$ is the Rubisco turnover number for RuBP carboxylation, $R_p$ is the potential RuBP pool size and $E_c$ is the concentration of Rubisco active sites (proportional to $V_m$). The simulations presented here assumed that $a_i, p, J_m$ and $R_p$ are proportional to $E_c$ and therefore to $V_m$, on the premise that all components of the photosynthetic apparatus should scale with one another to maintain a functional balance. Therefore, in practice, $V_r$, $a_i$ and $p$ were each calculated as fixed proportions of $V_m$, given in Table I. The numerical value of $\tau_c$ was chosen arbitrarily, to satisfy the empirical constraints that $\tau_c$ increases as irradiance approaches zero (e.g. Ball & Critchley 1982) (which requires that $A$ decline to zero at a higher irradiance than g, that is, $\alpha > \gamma$ at the photosynthetic light compensation point), and that stomata close in the dark (i.e. $\alpha > \gamma$ at zero irradiance). Note that $\tau$ does not numerically represent guard cell ATP concentration per se; rather, we assume the latter is proportional to $\tau$, and use parameter values that are based on mesophyll pools and expressed on a leaf area basis.

### APPENDIX 3: NUMERICAL PROCEDURES

A value for $\tau$ is needed to solve the expression for stomatal conductance (Eqn A15), but this in turn requires a value for $c_i$, which depends on stomatal conductance according to the standard expression for CO$_2$ diffusion through stomata (which is an Ohm’s Law adaptation of Fick’s First Law of Diffusion). It is easily shown that

$$g = \frac{A}{(c_d - p_i/p_t)}(0.23 + 1.37\omega)$$

(A25)

where $\omega$ is the ratio of total and stomatal conductances to water vapour [$\omega = (1 + g_{\text{stomal}})^{-1}$]. Equations A25 and A15 represent independent constraints on $g$, and must be solved numerically. We solved the system by varying $c_i$ upwards (starting at 1.1 p.p.m. above $I_T$) until the estimate of $g$ from Eqn A15 was smaller than that from Eqn A25; at that point, the stepsize was halved and the direction of change in $c_i$ was reversed. This procedure was repeated until the relative difference between the two estimates of $g$ was less than 10$^{-3}$. When irradiance was below the light compensation point for photosynthesis [i.e. the value of $I$ such that $J(I) < 4R_d(c_i + 2I_T)/(c_i - I_T)$] but above the irradiance causing stomatal opening ($I$ such that $\alpha(I) > \gamma$), the sense of the algorithm must be reversed: $c_i$ is varied upwards from $c_i + 1$ p.p.m., and reversed when $g$ from Eqn A25 becomes smaller than that from Eqn A15. Finally, when $I$ is sufficiently low that $\alpha(I) > \gamma$ we set $g = A = 0$ and $c_i = I(1 + 8R_d)/(1 - 4R_d)$, a user-friendly interface that solves the model is available as a downloadable executable file from the authors at <http://bioweb.usu.edu/kmott/ >.

For the Monte Carlo simulations (Fig. 6j–o), response curves were obtained for each of a number of different simulated ‘leaves’ (six for Fig. 6j–l and 150 for Fig. 6m–o), in which several parameters were randomly varied using normal distributions (normal deviates were calculated as described by Press et al. 1992; pp. 289–290, using the random number generator described on p. 279 of the same text). Experimental estimates of the mean and SD were available for the parameters $R, M, \chi$ and $\pi$, we took the standard values (Table 1) to be the means of the parameter distributions, and we assumed coefficients of variation (CV = SD/mean) of either zero or 0.175; the latter value was the average CV among the five parameters in Table 1. Figure 6m–o present SD lines using both CV estimates for $R, M, \chi$ and $\pi$.

### APPENDIX 4: PARAMETER ESTIMATION

Values for several parameters were estimated from previously published gas exchange and pressure probe measurements on *Vicia faba*. First, we fitted a floored plane
(a = max[c(P_e – \dot{m}P_e), 0]) by least-squares regression to the relationship between a, P_e and \dot{m} given by Franks et al. (1998) for V. faba (using parameters for low P_e calculated by Buckley & Mott (2002a)), which yielded c = 3.70 μM MPa⁻¹ and \dot{m} = 1.98. This plane and the observed relationship to which it was fitted are shown in Fig. 9 and discussed in Appendix 1. Second, the aperture plane was combined with Eqns A1–A6 to yield a direct relationship between P_e and the product of aperture and D, (P_e = -aD[\chi(c) + \psi_e + \pi_e]). Buckley & Mott (2002a) measured P_e, a and D, concurrently by pressure probe and gas exchange, and reported a linear regression between P_e and \alphaD, with slope -0.0013; MPa μmol⁻¹ [mmol H₂O mol⁻¹ air]⁻¹; this slope was used to calculate R\chi and the product R\chi/t (0.0048 mol air mmol⁻¹ H₂O) was estimated by applying the value of c from the aperture plane. Third, R was estimated independently by Mott from measurements of transpiration rate and epidermal turgor pressure (submitted for publication) to be 0.0456 MPa [mmol H₂O mol⁻¹ air]⁻¹. Buckley & Mott (2002a) measured R\chi versus \psi_e. Fourth, \psi_e was estimated as the intercept (0.52; MPa) of the P_e versus \alphaD regression data collected with a standard single-pass gas exchange system that has been described previously (e.g. Buckley & Mott 2000). N₂, O₂ and CO₂ were mixed from pure compressed sources using mass flow controllers, and water vapour was added to the mixture by bubbling a portion the dry gas stream through degassed distilled water. The absolute concentration of O₂ in the mixture was measured with an O₂ electrode (Rank Brothers, Cambridge, UK); the absolute concentration of CO₂ was measured with an infrared gas analyser (ADC Mark III set in absolute mode; ADC, Hoddesdon, UK); and the absolute concentration of water vapour was calculated from the dewpoint of the mixture, which was measured with a chilled-mirror dewpoint hygrometer (Dew 10; General Eastern, Watertown, MA, USA). A portion of the gas flow was diverted for the reference cell of the differential infrared gas analyser (see below) and the rest was delivered to the leaf chamber. Flow rate to the chamber was measured with a mass flow meter. Gas returning from the chamber was picked up at ambient pressure and pumped through the analysis cell of a CO₂ and H₂O infrared gas analyser (LiCor 6262; LiCor Instruments, Lincoln, NE, USA). The gas in the leaf chamber was circulated by small rotary fans, and boundary layer conductance was 3.3 mol m⁻² s⁻¹. Leaf temperature was measured with a fine-wire chromel–constantan thermocouple. Light was provided by a Xenon source and delivered to the leaf via a liquid light guide. Stomata were assumed to be in steady state when conductance did not change more than instrumental noise for 10 min. This often required an hour or more following a step change in environmental conditions.

APPENDIX 5: MATERIALS AND METHODS FOR GAS EXCHANGE MEASUREMENTS

Vicia faba L. plants were grown in 1 L pots containing equal parts peat moss, perlite and vermiculite. Plants were grown in a controlled environment greenhouse with day and night temperatures of 30 and 20 °C, respectively, and day-length was extended to 16 h when necessary with high-pressure sodium lamps that provided a PFD of approximately 500 μE m⁻² s⁻¹ at the top of the plant. Pots were drip watered to excess once a day with a nutrient solution containing 9.1 mM nitrogen, 1.8 mM phosphorus, 2.7 mM potassium and 11 μM chelated iron (Peter’s 20–10220; Grace Sierra Horticultural Products, Milpitas, CA, USA).

Leaves were selected for uniformity of age and appearance. Gas exchange data were collected with a standard single-pass gas exchange system that has been described previously (e.g. Buckley & Mott 2000). N₂, O₂ and CO₂ were mixed from pure compressed sources using mass flow controllers, and water vapour was added to the mixture by bubbling a portion the dry gas stream through degassed distilled water. The absolute concentration of O₂ in the mixture was measured with an O₂ electrode (Rank Brothers, Cambridge, UK); the absolute concentration of CO₂ was measured with an infrared gas analyser (ADC Mark III set in absolute mode; ADC, Hoddesdon, UK); and the absolute concentration of water vapour was calculated from the dewpoint of the mixture, which was measured with a chilled-mirror dewpoint hygrometer (Dew 10; General Eastern, Watertown, MA, USA). A portion of the gas flow was diverted for the reference cell of the differential infrared gas analyser (see below) and the rest was delivered to the leaf chamber. Flow rate to the chamber was measured with a mass flow meter. Gas returning from the chamber was picked up at ambient pressure and pumped through the analysis cell of a CO₂ and H₂O infrared gas analyser (LiCor 6262; LiCor Instruments, Lincoln, NE, USA). The gas in the leaf chamber was circulated by small rotary fans, and boundary layer conductance was 3.3 mol m⁻² s⁻¹. Leaf temperature was measured with a fine-wire chromel–constantan thermocouple. Light was provided by a Xenon source and delivered to the leaf via a liquid light guide. Stomata were assumed to be in steady state when conductance did not change more than instrumental noise for 10 min. This often required an hour or more following a step change in environmental conditions.

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