Title
How do stomata respond to water status?

Author
Buckley, Thomas N

Publication Date
2019-05-09

DOI
10.1111/nph.15899

Peer reviewed

New Phytologist

Review

Tansley review

The control of stomata by water balance

Author for correspondence:
Tom Buckley
Tel: +1 435 797 3567
Fax: +1 435 797 1575
Email: tom_buckley@alumni.jmu.edu

Received: *6 June 2005*
Accepted: *7 July 2005*

Thomas N. Buckley

Environmental Biology Group & Cooperative Research Centre for Greenhouse Accounting, Research School of Biological Sciences, The Australian National University, GPO Box 475, Canberra City, ACT 2601, Australia; Present address: Biology Department, Utah State University, Logan, UT 84322–5305, USA

Contents

Key words: cavitation, feedback, feedforward, stomatal conductance, transpiration, water potential.

Summary

It is clear that stomata play a critical role in regulating water loss from terrestrial vegetation. What is not clear is how this regulation is achieved. Stomata appear to respond to perturbations of many aspects of the soil–plant–atmosphere hydraulic continuum, but there is little agreement regarding the mechanism (or mechanisms) by which stomata sense such perturbations. This review discusses feedback and feedforward mechanisms by which hydraulic perturbations are putatively transduced into stomatal movements, in relation to generic empirical features of those responses. It is argued that a metabolically mediated feedback response of stomatal guard cells to the water status in their immediate vicinity ('hydro-active local feedback') remains the best explanation for many well-known features of hydraulically related stomatal behaviour, such as transient 'wrong-way' responses and the equivalence of hydraulic supply and demand as stomatal effectors. Furthermore, many curious phenomena that appear inconsistent with feedback, such as 'apparent feedforward' humidity responses and 'isohydric' behaviour (water potential homeostasis), are in fact expected to emerge from the juxtaposition of hydro-active local feedback and the well-known hysteretic and threshold-like effect of water potential on xylem hydraulic resistance.

New Phytologist (2005) **168**: 275–292

 doi: 10.1111/j.1469-8137.2005.01543.x

I. Introduction

Stomata regulate leaf diffusive conductance, and thereby influence water loss and carbon gain. Most stomatal responses counteract, at least partially, imposed changes in the balance between water supply and evaporative demand. For example, reducing atmospheric humidity shifts hydraulic balance towards demand, which reduces leaf water status; however, stomata respond by reducing their apertures, which restricts water loss and mitigates the potential decline in water status. The same tendency to reverse shifts in supply and demand is evident in the stomatal responses to changes in other hydraulically related variables, including xylem hydraulic resistance and water status elsewhere in the soil–plant–atmosphere continuum. These generic tendencies, as well as a great deal of concrete empirical evidence, suggest that stomatal guard cells respond by negative feedback to a local measure of leaf water potential, ψ_l.

Consensus remains elusive, however, regarding the mechanism by which stomatal conductance (g_s) and water balance are coordinated: is it passive feedback, active feedback, feedforward, or some combination of these? Is the core effector actually ψ_l, or a close proxy thereof, or do guard cells directly sense other properties such as plant resistance or the threshold water potential inducing xylem cavitation? My aims in this review are (1) to evaluate the major alternative hypotheses that have been used to explain short-term stomatal responses to hydraulic perturbations, by detailing explicitly what is required for them to explain common features of hydraulically related stomatal behaviour, and (2) to show how the 'hydro-active negative feedback' hypothesis – that guard cell osmotic pressure is actively regulated in response to the water status of the epidermal evaporating site – may easily be reconciled with several phenomena that appear inconsistent with feedback, by considering the amplifying effect of other known processes.

II. Background: stomatal hydromechanics

In 1898, Charles Darwin's son Francis, an early pioneer in stomatal research, commented that 'the problem of the stoma is still in the mechanical rather than the physiological stage of development' (Darwin, 1898). A century later, it is still possible to write with greater confidence about the mechanical and hydraulic context that translates guard cell osmotic pressure into stomatal conductance than about the physiological control of guard cell osmotic pressure itself. This section reviews the hydro-mechanical basis for stomatal movements, in order to provide a generic model to assist discussion in later sections.

1. Aperture, turgor and the mechanical advantage

Stomatal aperture (a_s) is positively related to the turgor pressure of the guard cells that form the pore (P_g), but negatively related to the pressure of adjacent subsidiary or epidermal cells (P_e) (Figs 1a, 2a). Experiments in which these two opposing pressures were measured and/or manipulated directly with a cell pressure probe (Meidner & Edwards, 1975; Edwards *et al.*, 1976; Franks *et al.*, 1995, 1998) have shown conclusively that, at least in those species that have been studied, the backpressure of epidermal cells is more effective in regulating aperture. This observation is consistent with theoretical analyses (DeMichele & Sharpe, 1973; Cooke *et al.*, 1976; Cowan, 1977), which termed the effect a 'mechanical advantage of the epidermis.' Franks *et al.* (1998) performed a thorough experimental study of aperture vs pressure relationships, and found that a_s responded to P_g in saturating fashion at low P_e, but in sigmoidal fashion at high P_e (Fig. 2a). A useful approximation is:

$$a_s \propto (P_g - P_e) - MP_e \qquad \text{Eqn 1}$$

where M is a parameter, the *residual* or *net* mechanical advantage, which is positive. (The simple 'mechanical advantage', m, is $M + 1$.) Equation (1) is illustrated in Fig. 2(b).

Both P_g and P_e are uniquely related to water potential and osmotic pressure (π), by the standard expression of plant–water relations: $P_g = \psi_g + \pi_g$, and $P_e = \psi_e + \pi_e$ (taking the convention that osmotic pressure is positive). In turn, these water potentials are determined by factors that influence liquid-phase water supply and evaporative demand (discussed in the next subsection). Epidermal osmotic pressure (π_e) may be fairly constant on the timescale of typical diurnal stomatal responses (Frensch & Schulze, 1988; Nonami *et al.*, 1990).

The simplest and most direct way for the plant to control stomatal aperture, however, is through actively mediated changes in guard cell osmotic pressure (π_g). By definition, $\pi_g = n_g RT/V_g$, where n_g and V_g are guard cell osmotic content (mol) and volume, respectively. (R and T are the gas constant and absolute temperature, respectively.) A complex web of signal transduction pathways controls n_g by modulating the activity of electrogenic proton pumps, which drive active ion uptake; by regulating ion channels and pores in the plasmalemma and tonoplast, which regulate the cell's permeability to osmolytes; and by intracellular production of osmolytes such as malate and sucrose. Those processes are beyond the scope of this article; the reader is directed to numerous recent reviews on the topic (Assmann, 1999; McAinsh *et al.*, 2000; Assmann & Wang, 2001; Hetherington, 2001; Schroeder *et al.*, 2001; Zeiger *et al.*, 2002; Dodd, 2003; Hetherington & Woodward, 2003; Vavasseur & Raghavendra, 2005). These variations in n_g change π_g and hence ψ_g, causing water to move into or out of guard cells. The resulting volume changes are translated by the guard cell walls' elastic properties into variations in turgor pressure. Water flow stops when P_g has changed enough to bring ψ_g ($= P_g - \pi_g$) back into hydraulic steady state between the guard cells and their surroundings.

2. Generic model for steady-state stomatal hydraulics

It is possible to form an expression for g_s in terms of reduced water relations parameters by combining the effect of water

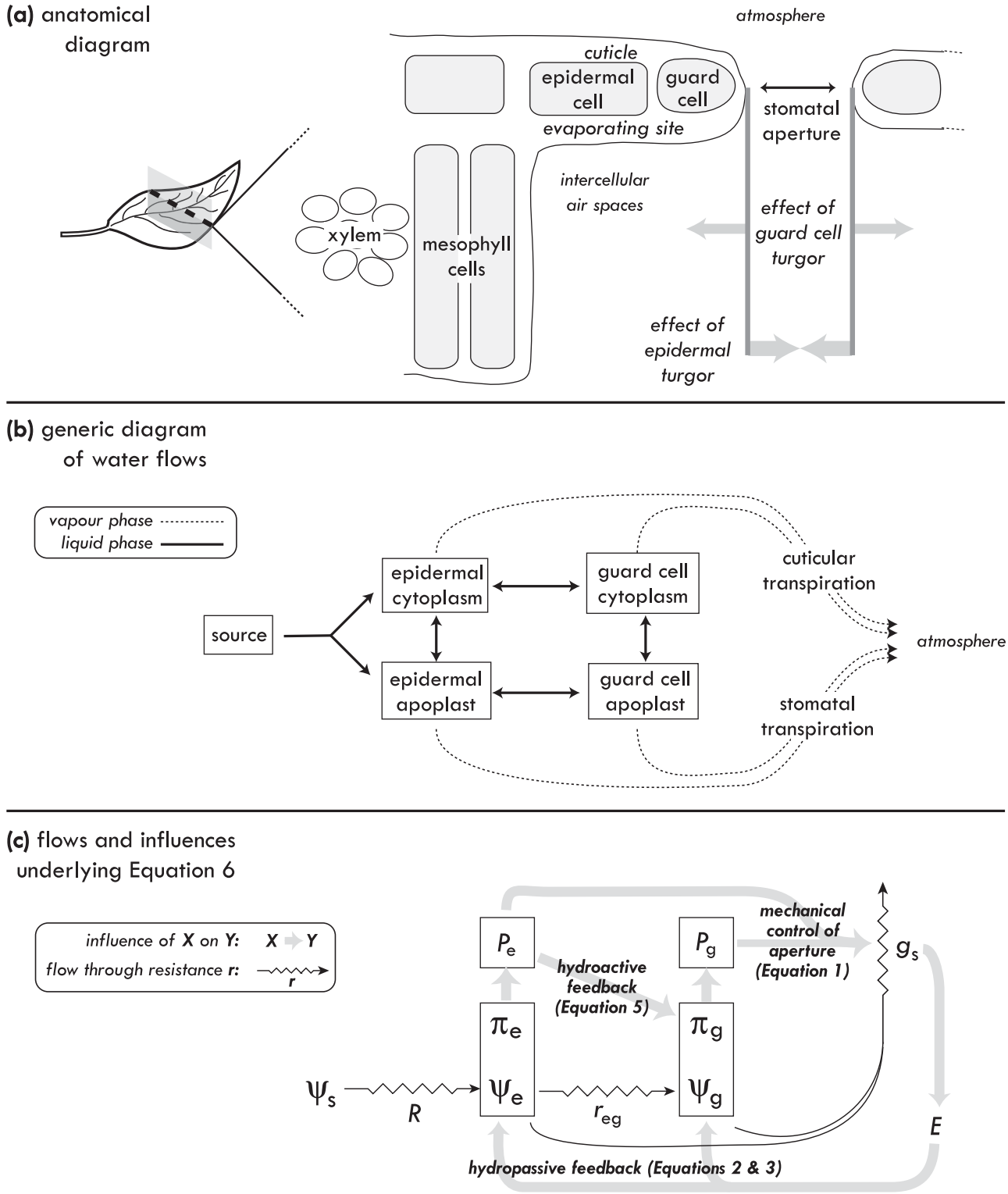

Fig. 1 Diagram of structures, flows and influences associated with stomatal hydraulics, to assist the reader in following the discussion in the text. (a) Anatomical situation of structures referred to in the text, and indicating the opposing effects of guard and epidermal cell turgor pressures on stomatal aperture. (b) Water-exchanging compartments associated with the stomatal complex, and possible flows among them. (c) Primary water flows (solid lines) and physiological or physical influences (thick shaded lines, referenced to equations in the text) that are mathematically embedded in Eqn 6, which is based on the hydro-active local feedback hypothesis. ψ_s, ψ_e, ψ_g: source, epidermis, and guard cell water potentials, respectively; π_e, π_g: epidermal and guard cell osmotic presures; P_e, P_g: epidermal and guard cell turgor pressures; R, effective resistance from source to epidermis; r_{eg}, resistance from epidermis to guard cell; g_s, stomatal conductance; E, transpiration rate.

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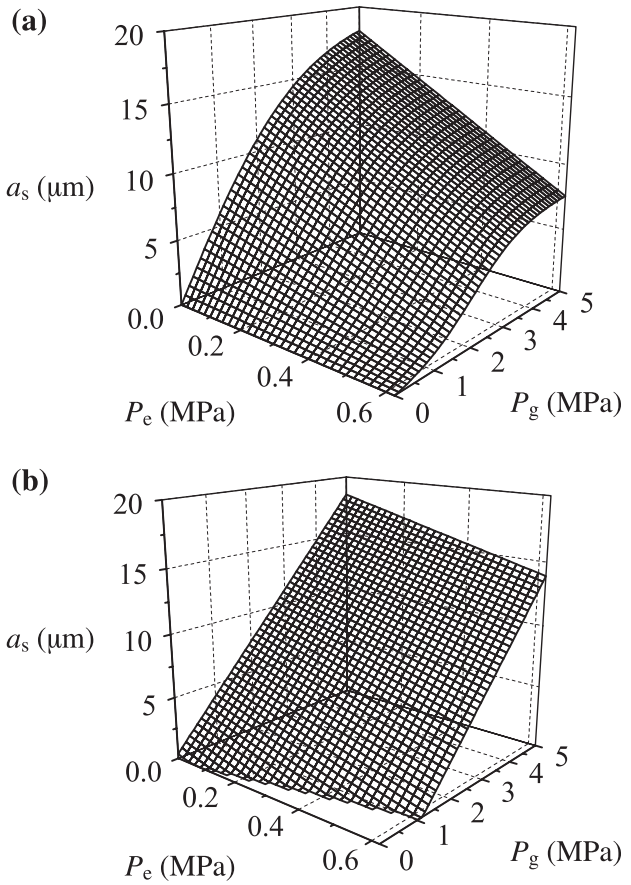

Fig. 2 Diagram showing the effects of epidermal turgor pressure (P_e) and guard cell turgor pressure (P_g) on stomatal aperture (a_s). (a) Empirical model developed by Franks *et al*. (1998) from pressure-probe data, with parameters estimated for *Vicia faba* as described by Buckley & Mott (2002a). (b) A floored plane (a_s = max{0, $c(P_g - (M + 1)P_e)$}, where c is an empirical proportionality factor) fitted to the surface in (a), with $c = 3.7$ and $M = 1.0$. (Reproduced from Buckley *et al*., 2003, copyright Blackwell Publishing.)

balance on stomatal aperture (Eqn 1) with the effect of aperture on water balance via transpiration rate (E). However, this requires that we relate a_s to g_s, and E to water balance. The first presents a dilemma because g_s is defined only in relation to boundary layer conductance (g_b), and vice versa. Total conductance to water vapour, g_{tw}, is well defined as the ratio of transpiration rate (E) to H_2O mole fraction difference (D) between the leaf interior and ambient air, and g_s and g_b are defined as parallel complements in g_w: $g_w = (g_s^{-1} + g_b^{-1})^{-1}$, so $g_s = E/(D - (E/g_b))$. As a result, the dependence of g_s and g_b on reduced quantities such as windspeed or stomatal aperture is difficult to work out in theory (see Nobel, 1991; Jones, 1992; Lushnikov *et al*., 1994). For simplicity, I will use $E = g_s D_s$, where D_s is the leaf boundary layer evaporative gradient, and I will assume that g_s is linearly and homogeneously proportional to a_s.

The effect of E on water balance at steady state can be modeled with a gradient/resistance approach. If epidermal and guard cells sustain fractions, f_e and f_g, respectively, of noncuticular transpiration rate E, and fractions f_{ec} and f_{gc} of cuticular transpiration rate E_c, then epidermal and guard cell water potentials (ψ_e and ψ_g, respectively) may be written as:

$$\psi_e = \psi_s - f_e r_{se} E - f_{ec} r_{se} E_c \qquad \text{Eqn 2}$$

$$\psi_g = \psi_e - f_g r_{eg} E - f_{gc} r_{eg} E_c \qquad \text{Eqn 3}$$

where ψ_s is the water potential of the soil or other source, and r_{se} and r_{eg} are resistances from source to epidermis, and epidermis to guard cells, respectively. Figure 1(b) illustrates these compartments and flows diagrammatically. Then, assuming $g_s \propto a_s$, it is easily shown that Eqns (1–3), together with the definition of water potential and the diffusion constraint, $E = g_s D_s$, imply that

$$g_s = \chi \frac{\pi_g - m\pi_e - M\psi_s + (f_{ec} r_{se} - f_{gc} r_{eg}) E_c}{1 - \chi(MR - f_g r_{eg}) D_s} \qquad \text{Eqn 4}$$

where $R = f_e r_{se}$ and χ is a proportionality constant. Equation (4) assumes nothing about the metabolic control of π_g – it is merely an expression of the (simplified) physical constraints relating g_s to parameters of water relations and stomatal mechanics. It is also not a dynamical model, because Eqns (2) and (3) assume hydraulic steady state. With the exception of π_g, most terms in Eqn (4) have not traditionally been thought to be actively regulated on short timescales, although they may vary passively. For example, the data of Franks (1998) suggest that M is not constant, but that it varies somewhat with both P_g and P_e. Some evidence suggests that epidermal osmotic pressure, π_e, is fairly conservative during short-term variations in stomatal conductance (Frensch & Schulze, 1988; Nonami *et al*., 1990), although π_e may shift in parallel with long-term regulation of bulk leaf osmotic pressure (Morgan, 1984). Plant hydraulic resistance, R, can also vary passively on short timescales as a result of xylem embolism, but recent evidence also shows that it can be endogenously regulated (McCully *et al*., 1998; Zwieniecki & Holbrook, 1998; Tyree *et al*., 1999; Zwieniecki *et al*., 2001).

III. The parsimony of hydro-active local feedback

The simplest explanation for hydraulic feedback control of stomatal conductance would be that guard cell water status, and hence turgor pressure, responds directly to variations in hydraulic supply and demand. Unfortunately, this hydro-passive effect can not explain most aspects of hydraulically related stomatal responses. The crux of the problem is the mechanical advantage of the epidermis. In this section, I review the phenomenology of stomatal responses to short-term (minutes to hours) perturbations of the soil–plant–atmosphere hydraulic continuum, highlighting the apparent fundamental need for an *active* (i.e. biochemically mediated) feedback response of π_g to changes in water status in or near the epidermis.

1. Short-term responses are fundamentally similar

The most easily observed (and perhaps the most familiar) stomatal response to perturbation of leaf water balance is the response to humidity. When the humidity around a leaf is reduced, g_s typically increases for 5–15 min and then declines for another 20–75 min, ultimately approaching a steady state g_s that is lower than the initial value (Cowan & Farquhar, 1977; Kappen *et al.*, 1987; Grantz, 1990; Mott & Parkhurst, 1991; Monteith, 1995; Oren *et al.*, 1999). However, other perturbations of the hydraulic continuum induce the same archetypal two-phase response. Comstock & Mencuccini (1998) imposed stepwise changes in atmospheric pressure around the roots of a desert shrub, *Hymenoclea salsola*, and found wrong-way responses 5–10 min in duration followed by steady-state responses around 30–60 min long (Fig. 3). The effects were reversible, consistent with feedback. Raschke (1970) found similar responses to pressure changes in the water supply to detached maize leaves. Likewise, Rufelt (1963) reduced the water potential of the solution bathing roots of a wheat plant by adding sodium chloride, and observed a transient opening and subsequent closing response. Fuchs & Livingston (1996) reported similar results for seedlings of Douglas-fir and alder, although without any significant transients.

Leaf excision also induces a similar response pattern: stomata first open, then close, except that in the case of leaf excision, they usually close completely. The excision response was first reported by Darwin (1898) but is often called the 'Iwanoff effect', after a paper by Iwanoff (1928), who attributed the response to the release of xylem tension and a reduction in xylem resistance caused by air influx. In an elegant study involving manipulation of ψ_s and distinct components of R in potted walnut trees (*Juglans regia* × *nigra*) by soil drought, soil chilling and shoot embolism, Cochard *et al.* (2002) concluded that stomata did not respond directly to these perturbations, but to some measure of local water status (either ψ_l or xylem tension in the leaf rachis).

Changes in transpiration rate in other parts of the plant can also affect g_s in this manner. Numerous studies have shown that epidermal turgor declines in response to increased vapour pressure difference (VPD) (Shackel & Brinkmann, 1985; Frensch & Schulze, 1988; Nonami *et al.*, 1990), and Mott *et al.* (1997) and Mott & Franks (2001) demonstrated that these changes can be propagated to neighbouring stomata, causing them to respond despite no change in local VPD. Similar responses, propagated over a greater distance, were observed when transpiration rate was adjusted by changing irradiance over only one half of a wheat leaf (Buckley & Mott, 2000): stomata in the unperturbed region responded by closing, then opening, when g_s and E fell to zero in the darkened region. The reverse was observed upon re-illumination of the latter region, and transient wrong-way responses were also evident in most cases. Comparable effects were observed at an even larger scale by Whitehead *et al.* (1996), who found rapid and reversible stomatal responses in one part of a *Pinus radiata* canopy when another part of the same canopy was shaded or re-illuminated.

In summary, all of these results suggest that stomata respond similarly to any perturbation in the hydraulic continuum. This implies that the core effector is affected by both supply and demand in similar fashion, an obvious candidate being water potential somewhere in the transpiration stream. The fact that stomata respond similarly to local variations in epidermal turgor that are too small to change bulk leaf water potential suggests that the sensor is close to guard cells, perhaps in the epidermis.

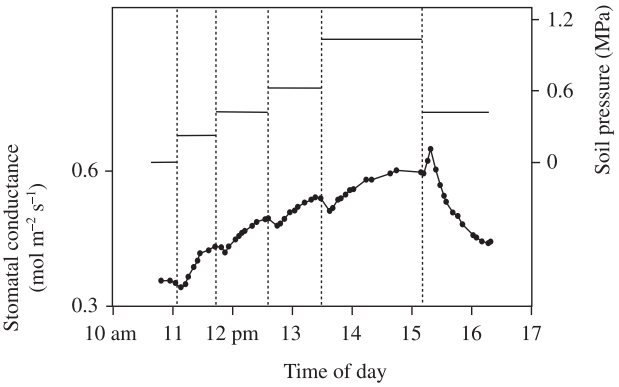

Fig. 3 Response of stomatal conductance (symbols, lower half of figure) to step changes in soil water status by soil pressurisation (horizontal line segments, upper half of figure), showing transient 'wrong-way' and steady-state 'right-way' responses to both increases and decreases in soil water status. (Reproduced from Comstock & Mencuccini, 1998, copyright Blackwell Publishing.)

2. Three criteria: decoupling, transients, and supply–demand symmetry

To explain the archetypal stomatal responses to D_s, ψ_s and R described above, any mechanism must satisfy three criteria: (a) it must decouple guard and epidermal turgor in the steady state; (b) it must produce transient wrong-way, then steady-state 'right-way' responses; and (c) it must satisfy these criteria when either hydraulic supply or demand is perturbed. The second and third criteria are self-evident from the phenomenology of short-term responses. The first criterion is demanded by the mechanical advantage of the epidermis (Eqn 1), which ensures that g_s will increase if guard and epidermal turgors decline by equal amounts. There are two generic hypotheses to explain decoupling. According to one hypothesis, reduced water status causes turgor to decline in both cells by similar amounts, so aperture increases; subsequently, active adjustment of guard cell osmotic pressure reduces guard cell turgor enough to produce the right-way response. Another hypothesis holds that guard cells are separated from epidermal cells by a large water potential gradient – caused either by a large hydraulic resistance or by the accumulation of osmolytes in the guard cell apoplast – so an increase in evaporation rate reduces guard

cell turgor more than epidermal turgor, and aperture declines. These hypotheses are discussed in the following subsections.

3. Mechanism 1: metabolic response to local water status

It is difficult to identify the primary source for this hypothesis; Darwin (1898) clearly had the idea, and it has re-appeared many times since then (Darwin & Pertz, 1911; Stalfelt, 1929; Meidner, 1986). There is little direct evidence either for or against the idea, but much circumstantial evidence in support of it. One point is that guard cell osmoregulatory responses (e.g. to light) typically follow saturation kinetics preceded by a lag period. For example, Grantz & Zeiger (1986) reported similar kinetics for the stomatal responses to VPD and light, the latter being known to involve active guard cell osmoregulation. Buckley & Mott (2002a) used a model to infer π_g from stomatal aperture during a humidity response, concluding that guard cell osmoregulation in response to VPD was monotonic and exponential in time – similar to the kinetics of ion flux observed for guard cell responses to light (Grantz, 1990). Numerous dynamic models of g_s based on this idea exhibit the archetypal two-phase response (Cowan, 1972; Delwiche & Cooke, 1977; Haefner *et al.*, 1997). These features, when combined with the fact that epidermal turgor responds almost immediately to hydraulic perturbations (Mott & Franks, 2001), fulfil criterion (b). Criteria (a) and (c) are satisfied directly by the hypothesis statement: decoupling is produced by metabolic adjustment of π_g, and symmetry is conferred by choosing the evaporating site as the sensor, and hence situating the sensor in the transpiration stream.

Buckley *et al.* (2003) derived a closed-form model for g_s based on the osmoregulation hypothesis. Specifically, they combined two hypotheses: that π_g is regulated in direct proportion to epidermal turgor pressure (P_e), and that π_g is proportional to the concentration of adenosine triphosphate (ATP) in guard cells (τ), which was assumed to vary with CO_2 and light in the manner predicted for mesophyll [ATP] by the model of Farquhar & Wong (1984). The formal expression of these hypotheses is: $\pi_g = \beta\tau P_e$, where β is an empirical constant. This may be generalised to:

$$\pi_g = BP_e \qquad \text{Eqn 5}$$

where B is a proportionality factor that incorporates the effects of light and CO_2, but does not necessarily depend on the ATP hypothesis. When this expression is applied to Eqn (4) and when cuticular water loss is assumed to be negligible, the following equation results after some rearrangement:

$$g_s = \chi \frac{\alpha(\psi_s + \pi_e) - \pi_e}{1 + \chi(\alpha R + f_g r_{eg})D_s} \qquad \text{Eqn 6}$$

Figure 1(c) illustrates the flows and influences that are incorporated into this expression. Equation (6) contains a new term, α, the guard cell advantage, which is defined as $B - M$. The guard cell advantage is determined by the balance of two opposing effects: B captures the positive effect of hydro-active control of π_g, and M captures the negative effect of epidermal backpressure and its mechanical advantage. $\alpha > 0$ when light levels are adequate to promote stomatal opening. Equation (6) predicts similar steady-state responses to ψ_s, R and D_s, consistent with the observations outlined above.

Grantz & Schwartz (1988) found no evidence of guard cell osmoregulation in response to changes in mannitol concentration in the solution bathing epidermal peels of *Commelina communis* L., which seems to contradict the osmoregulation hypothesis. It is possible that guard cells normally sense variations in ion or hormone concentrations in the apoplastic evaporating site, and hence respond not to water potential *per se*, but to local apoplastic water content, the two being decoupled by immersion. Additionally, the authors found an immediate decline in stomatal aperture following mannitol addition, without any wrong-way response. This may indicate an absence of epidermal backpressure in the peels under study (epidermal peeling usually ruptures most epidermal cells in *Vicia faba*, regardless of peeling contact angle; Joe Shope, Utah State University, pers. comm.).

4. Mechanism 2: water potential drawdown to guard cells

Alternatively, guard and epidermal turgor pressures could be decoupled by a water potential difference, if guard cells support enough direct evaporation. This gradient could be produced either by flow through a large resistance (Farquhar, 1978; Maier-Maercker, 1983; Dewar, 1995, 2002) or by accumulation of an osmolyte such as sucrose in the guard cell apoplast (Outlaw & De Vlieghere-He, 2001). One interpretation of these hypotheses attributes humidity sensing to cuticular water loss from guard cells; another, to water loss from the inner (substomatal) surface of guard cells. Confusingly, both are labelled 'peristomatal transpiration' by some authors.

To produce the transient wrong-way humidity response, epidermal turgor must respond more quickly than guard cell turgor to a change in humidity. This directly contradicts the hypothesis that humidity is sensed primarily and proximally via cuticular transpiration from guard cells (other difficulties face the cuticular drawdown hypothesis as well; see Section IV.1), so that hypothesis does not satisfy criterion (b). Humidity sensing via water loss from the *inner* surfaces of guard cells could produce a two-phase response, subject to the following two additional assumptions. (i) The epidermis must be strongly hydraulically coupled to the evaporating site – either via direct water loss from an evaporating site that is hydraulically separated from the guard cell evaporating site, or via dependence on a shared apoplastic evaporating pool whose water status is quasi-static with respect to VPD. The latter alternative is incompatible with a drawdown in apoplastic water potential,

whether caused by osmotic accumulation or by hydraulic resistance; it is compatible with a symplastic water potential gradient, but such a gradient could occur only transiently if there were a lower-resistance, quasi-steady apoplastic pathway for water delivery to guard cells. (ii) The halftime for relaxation of water potential gradients across guard cell membranes must be of the same order as the wrong way response, i.e. over 30 min in some cases (Mott & Franks, 2001; Buckley & Mott, 2002a). This is much slower than halftimes commonly observed for plant cell membranes, which tend to be well under one minute (Steudle, 1994). Recent experiments on epidermal peels of *Vicia faba* (K. Mott & J. Shope, unpublished) found that guard cell volume responded to step changes in water potential of the surrounding medium with halftimes on the order of 30–60 s. However, exogenous membrane trafficking inhibitors increased the halftime dramatically, suggesting that guard cells can down-regulate membrane hydraulic permeability to slow down water flow when cell membrane surface area is unable to 'keep up' for some reason. These and other data (Huang *et al.*, 2002) suggest that guard cells possess regulatable aquaporins. The possibility remains, therefore, that the hydraulic resistance from epidermal to guard cells (r_{eg}) is dynamically and actively regulated in such a way as to produce a wrong-way and subsequent steady-state response (Buckley & Mott, 2002b). However, it is difficult to see the adaptive benefit of such a kinetically complex response, particularly when its only effect is to delay establishment of the new target state.

Regardless, the drawdown hypothesis predicts the wrong steady-state responses to R and ψ_s, as seen by inspection of Eqn (4), and hence violates criterion (c). The reason is simply that the epidermis–guard cell water potential gradient, however large or small it may be, is insensitive to any properties of the flow continuum proximal to the epidermis. This is the case regardless of whether the relevant water loss occurs through stomata or through the cuticle. In a recent model (Dewar, 2002), the resistance-based version of the drawdown hypothesis is used to predict the steady-state response to humidity, but the R and ψ_s responses are captured by calculating epidermal water potential, ψ_e, with an external model, and then positing an interaction between ψ_e and absciscic acid (ABA) in the metabolic control of guard cell osmotic pressure. However, a ψ_e-sensitive ABA response is also an ABA-sensitive ψ_e response, phenomenologically – in other words, Dewar's model is also based, in part, on the hypothesis that guard cells respond metabolically to variations in epidermal water status.

5. Summary and extension

Several lines of argument suggest that stomata respond to short-term perturbations of humidity, xylem resistance, soil water potential, and anything that directly influences leaf water status, by a mechanism involving active guard cell osmoregulation in response to the water status of cells near the evaporating site (hydro-active local feedback). The next section discusses several features of stomatal control that appear difficult, at first glance, to explain solely by hydro-active local feedback.

However, there are three other important features of stomatal control that have not been discussed yet, but which are predicted, at least qualitatively, by a feedback response to local water status. The first involves the effect of osmoregulatory responses to soil drought, and is discussed in Section IV.3. The second feature is the tendency, evidenced by a growing body of data, for g_s to 'track' plant hydraulic conductance, analogous to the tracking of photosynthetic capacity by stomata (Meinzer & Grantz, 1990; Meinzer *et al.*, 1995; Saliendra *et al.*, 1995; Hubbard *et al.*, 2001; Franks, 2004; for a review, see Meinzer, 2002). The direct effect of whole-plant hydraulic resistance on stomatal conductance under hydro-active local feedback (Eqn 6) predicts this correlation without recourse to any additional regulatory mechanism. It does not, however, specify the slope of the correlation, which is probably influenced by the converse effect (i.e. the effect of g_s on R by way of xylem cavitation). This is discussed in detail by Sperry (2000); I will touch on it later in the context of 'apparent feedforward' humidity responses (Section IV.1) and stomatal optimisation of water use (Section IV.4).

The third feature is the height-related increase in the relative stomatal limitation to photosynthetic carbon gain. It is well known that stomatal conductance tends to track photosynthetic capacity (A_m) among leaves, such that the prevailing ratio of intercellular to ambient CO_2 mole fraction (c_i/c_a) is highly conserved (e.g. Wong *et al.*, 1979, 1985). It is also often observed that leaf-specific hydraulic conductance decreases, and hence R increases, with height (Saliendra *et al.*, 1995; Mencuccini & Grace, 1996; McDowell *et al.*, 2002; Barnard & Ryan, 2003; Mokany *et al.*, 2003; Delzon *et al.*, 2004). On the other hand, c_i/c_a is often found to be lower in leaves of taller trees, or in leaves at greater elevation within an individual tree (Yoder *et al.*, 1994; McDowell *et al.*, 2002; Barnard & Ryan, 2003; Delzon *et al.*, 2004; Koch *et al.*, 2004), which suggests the coordination of stomatal conductance and photosynthetic capacity is sensitive to height, perhaps via R. One explanation for this trend is that it is not g_s *per se*, but rather the hydraulic maximum stomatal conductance, g_m – the limiting value as transpiration rate and hydraulic resistance approach zero, and hence as leaf water potential approaches soil water potential – that tracks photosynthetic capacity. Monteith (1995) used the symbol g_m to represent the value approached by g_s in the absence of hydraulic demand ($E \to 0$). Extending the definition of g_m to include negligible supply constraints ($R \to 0$), g_m is the limit of Eqn (4) as R and D_s approach zero: $g_m = \alpha\chi(\psi_s + \pi_e) - \chi\pi_e$. (Note that this '$g_m$' differs from the use of the same symbol by Buckley *et al.* (2003), but is analogous to Monteith's use of the symbol.) Comparing this with Eqn (4) and dividing by A_m, we see that:

$$\frac{g_s}{A_m} = \left(\frac{g_m}{A_m}\right)\frac{1}{1 + \chi(\alpha R + f_g r_{eg})D_s} \qquad \text{Eqn 7}$$

Thus, if g_m/A_m is conserved, then Eqn (7) predicts the observed height-related decline in g_s/A_m as a necessary and passive consequence of the observed height-related increase in R.

IV. Feedforward, or feedback plus? Emergent properties of marginally stable feedback control

Section III argued that the best explanation of archetypal hydraulic responses is an active feedback response of guard cells to local water status. However, hydro-active local feedback may appear to contradict several other curious aspects of hydraulic regulation. These include the occasional report of transpiration rate declining as leaf-to-air evaporative gradient increases, often called 'direct' or 'feedforward' humidity responses (discussed in Section IV.1); the more common finding that bulk leaf water potential (ψ_l) often remains nearly constant despite large changes in hydraulic driving variables (Section IV.2); the frequent observation that stomatal closure in drought precedes any decline in ψ_l (Section IV.3); and the prediction that optimal stomatal control sometimes requires feedforward phenomenology (Section IV.4).

Do these phenomena truly contradict hydro-active local feedback, or can they be accommodated by modifying or complementing local feedback? I will argue that these features are not only consistent with, but are in fact expected to emerge from, the combination of hydro-active negative feedback with three other well known processes: the hydro-passive effect of ψ_l on stomatal aperture, captured by Eqn (1); the hysteretic, threshold behaviour of plant hydraulic resistance in response to ψ_l; and the effect of exogenous ABA on g_s. The section concludes by briefly discussing stomatal oscillations and patchiness, two other phenomena that appear to emerge from the spatiotemporal instability caused by this fusion of processes (Section IV.5).

1. Apparent feedforward responses to humidity

A feedback response must involve a monotonic relationship between two variables. In other words, if the two variables are plotted against one another, the slope of the relationship can never be zero or infinite, because that would either permit one variable to change independently of the other, or create an ambiguity in the predicted effect of a change in one variable. The stomatal response to variations in E caused by changing D_s usually satisfies these criteria: as D_s increases, E rises but g_s falls (Monteith, 1995). Occasionally, however, a further increase in D_s results in a decline in both E and g_s in the steady state (Schulze *et al.*, 1972; Franks *et al.*, 1997). Because this can not be explained solely by negative feedback between g_s and E, it has been termed 'feedforward' (e.g. Farquhar, 1978).

Various mechanisms have been proposed to explain this phenomenon. Many involve the direct loss of water through the outer surface of guard cells (Farquhar, 1978; Maier-Maercker, 1983; Dewar, 1995, 2002). The plausibility of this idea rests upon the assumption that cuticular water loss makes guard cell turgor more sensitive than epidermal turgor to humidity, even when stomata are wide open (otherwise, reduced humidity would still passively open stomata). However, this seems unlikely, given that epidermal turgor is quite sensitive to stomatal transpiration (Frensch & Schulze, 1988; Nonami *et al.*, 1990; Mott *et al.*, 1997; Mott & Franks, 2001; see Section III.1), which, in turn, is usually many times larger than cuticular transpiration under typical mid-day conditions (Boyer *et al.*, 1997). Cowan (1994) also pointed out that a mechanism requiring perpetual, uncontrolled water loss is a strange way to effect water conservation. As discussed in Section III.4, it is also difficult to reconcile humidity sensing by cuticular water loss from guard cells with the transient 'wrong-way response' to humidity. Furthermore, Mott & Parkhurst (1991) demonstrated that stomata are insensitive to ambient humidity *per se*.

There is another difficulty with the hypothesis that stomata sense humidity by any feedforward mechanism: although the variables linked by feedforward need not be monotonically related, they must still be uniquely related – that is, only one value of the dependent variable (g_s, in this case) can correspond to any given value of the core independent variable (humidity or D_s). In other words, the current humidity should be the only information needed to predict g_s, if other stomatal effectors are controlled. In contrast, Franks *et al.* (1997) found that in the few cases where E declined at high D_s, the effect was hysteretic (i.e. irreversible in the short term), and hence would more accurately be termed 'apparent feedforward.' These authors noted that if the effect of ABA on stomata is also hysteretic, apparent feedforward might be explained by increased production or redistribution of ABA within the leaf at high E. More generally, hysteresis can result when the dependent variable in question (e.g. g_s) is influenced not only by the independent variable being measured (e.g. D_s), but also by any other factor that happens to be covarying with the independent variable. For example, Meinzer *et al.* (1997) found diurnal hysteresis in g_s vs E coincident with diurnal variation in irradiance, temperature and other variables that typically vary *in situ*, and they attributed the effect to feedforward via cuticular transpiration. Similarly, diurnal hysteresis in g_s vs D_s concurrent with observed nonstomatal depression of photosynthesis was described as apparent feedforward by Macfarlane *et al.* (2004).

The hysteretic nature of the apparent feedforward response to humidity is reminiscent of another feature of plant–water relations: the effect of xylem water status on hydraulic conductivity (Tyree & Sperry, 1989). Large negative pressures induce the formation of embolisms in xylem conductive elements; however, because highly tensile water is metastable before cavitating, the phase change associated with embolism formation – and hence the response of xylem hydraulic resistance to tension – is hysteretic. This suggests a mechanism for apparent feedforward humidity responses: hydro-active local

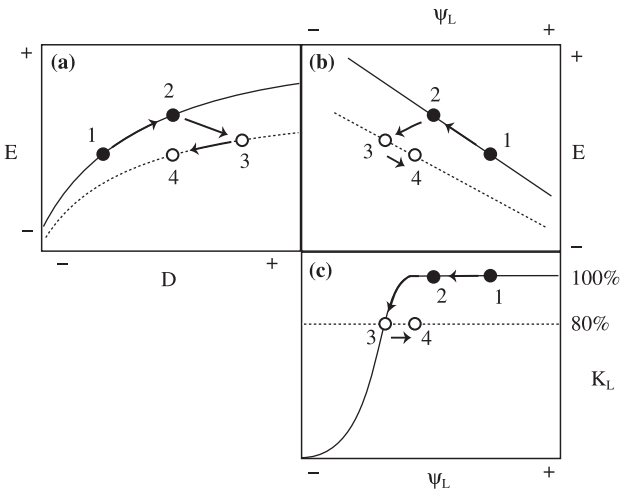

Fig. 4 Diagram illustrating hypothetical sequence of steady states (numbered symbols connected by arrows) giving rise to a hysteretic 'apparent feedforward' relationship between transpiration rate (*E*) and evaporative demand (*D*). Solid and dashed lines in (a) and (b) are steady-state curves predicted by the model of Buckley *et al*. (2003) using parameter values therein, except for effective source–epidermis resistance, *R*, which was set at either 0.12 (solid lines) or 0.15 MPa/[mmol m^{-2} s^{-1}] (dashed lines). (c) Heuristic representation of a vulnerability curve in which the threshold leaf water potential (ψ_l) causing significant loss of xylem conductivity is slightly more negative than the ψ_l value corresponding to steady-state point 2. Increasing *D* beyond that point induces cavitation that results in a 20% loss in conductivity (25% increase in *R*), shifting the leaf to a new steady-state curve (dashed lines and open symbols) and creating the appearance of a feedforward relationship between *E* and *D*. Hysteresis results because the embolisms can not be repaired instantaneously, so an immediate reduction in *D* leads to steady-state point 4, not 2.

feedback is hysteretically amplified by increases in xylem resistance when *E*, and hence xylem tension, becomes large enough to induce cavitation (Oren *et al*., 1999; Buckley & Mott, 2002b). Figure 4 illustrates how this mechanism would produce hysteretic apparent feedforward: when VPD is increased beyond a certain point, water potential crosses the cavitation threshold, increasing *R* and hence reducing *E* at any given VPD. If, following a subsequent reduction in VPD, embolism repair lags behind the recovery of water status, then *E* vs D_s would follow a different trajectory for declining VPD. The extent to which the effect is truly hysteretic should depend on the time constant for cavitation repair, a subject of considerable controversy, which arises again in the context of stomatal oscillations (Section IV.5).

2. Homeostatic control of bulk leaf water potential

In some species, g_s appears to regulate hydraulic supply and demand so tightly that ψ_l does not vary significantly (Jones, 1990; Tardieu, 1993; Saliendra *et al*., 1995). At first glance, such 'isohydric' behaviour seems to preclude a feedback response of g_s to ψ_l, which would require both variables to change at least slightly. Isohydric behaviour would in fact demand a feedforward mechanism if ψ_l were truly independent of the putative water status sensor. However, such independence would also require the sensor to be decoupled from the transpiration stream, thus requiring a separate mechanism for the ψ_s and *R* responses. It would also beg the question of how evaporation from an hydraulically isolated humidity sensor might be replenished – lacking a water source, the sensor would have to be in equilibrium with the atmosphere, requiring relative water contents approximately equal to relative humidity and thus often low enough to preclude metabolic functioning altogether. Furthermore, as the guard cells are known to be hydraulically connected to the rest of the plant (Frensch & Schulze, 1988; Nonami *et al*., 1990; Mott *et al*., 1997; Mott & Franks, 2001), yet another unknown transduction mechanism would be required to relay information from the isolated sensor to guard cells.

If the sensor comprised relatively few cells, separated but not isolated from the bulk of leaf water by a large resistance, then a feedback response to this sensor could produce near-homeostasis in water potential (Sperry, 2000) (perhaps better termed 'pseudo-isohydric' behaviour). This would not be a feedforward response to ψ_l, but rather negative feedback amplified by driving part of the transpiration stream through a large resistor and locating the sensor downstream from that resistor. It is unclear where this resistor would have to be. However, two points suggest it is not located between epidermal and guard cells. The first reason, as discussed earlier (Section III.4), is that this would produce opposite transient responses to perturbations of water balance by supply and demand. The second reason, which is more abstract and difficult to explain, is that an active feedback response of guard cell osmotic pressure to guard cell water potential would contradict the unique relationship implied by physical constraints between those two variables. By definition, guard cell water potential (ψ_g) is a function of osmotic content and cell volume (n_g and V_g, respectively): $\psi_g = P_g - \pi_g = P_g(V_g) - n_g R'T/V_g$, where $P_g(V_g)$ represents the guard cell pressure–volume curve, R' is the gas constant and T is the absolute temperature. However, ψ_g also depends on V_g via the latter's effect on g_s via P_g: say, $\psi_g = \psi_e - (f_g r_{eg} D_s) g_s(P_g(V_g))$ (Eqn 3). Thus the system comprising the states of guard cell water relations (ψ_g, π_g and P_g) contains two independent variables (n_g and V_g) and two constraints, and has no internal freedom. Reversible effects of humidity, resistance, or any other driving variable on the constraint *functions* themselves (e.g. D_s influences the dependence of ψ_g on V_g) would not decouple π_g from ψ_g within the domain of that driving variable; the only way to decouple π_g from ψ_g in the steady state is to make one of the constraints nonunique with respect to ψ_g, π_g or P_g. Cowan (1994) elaborated this idea by postulating that the guard cell pressure–volume curve is hysteretic, but Peter Franks and colleagues disproved it by measuring guard cell pressure–aperture and pressure–volume relations directly and finding only very minimal hysteresis (Franks *et al*., 1995, 1998, 2001).

These points suggest that, if the sensor's responsiveness to humidity is amplified by a resistor, the resistor is probably located upstream of epidermal cells, at least those immediately adjacent to the guard cells. However, sequestering sensory cells downstream of any resistor would not enhance responsiveness to hydraulic perturbations upstream of the resistor, so it could promote isohydric behaviour under varying D_s, but not under varying ψ_s or R. In contrast, Hubbard *et al.* (2001) found homeostatic ψ_l control in ponderosa pine seedlings subjected to increases in xylem hydraulic resistance by air injection. This argues against a central role for active regulation of liquid-phase hydraulic resistance within the leaf (by aquaporins, for example) in producing isohydric behaviour.

At any rate, the feedback vs homeostasis paradox may be a red herring, for two reasons. First, simple negative feedback between g_s and ψ_l need not require ψ_l to vary beyond the range of measurement uncertainty. Figure 5 illustrates this by superimposing the data of Hubbard *et al.* (2001), which clearly show near-homeostasis in ψ_l, on predictions from the hydro-active feedback model of Buckley *et al.* (2003) (see Appendix for details). Neither the observed nor the predicted range of ψ_l variation shown in Fig. 5 would be easily distinguishable from true homeostasis by experiment. More generally, it can be shown (see Appendix) that, according to the hydro-active feedback hypothesis, the relative drop in ψ_l (expressed as the relative increase in soil–leaf ψ gradient) induced by a given relative increase in D_s, is:

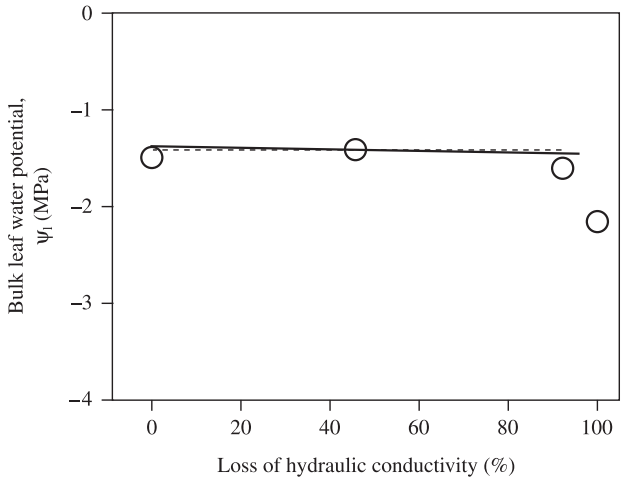

Fig. 5 Circles: observations by Hubbard *et al*. (2001) of bulk-leaf water potential (ψ_l) in ponderosa pine seedlings subjected to a progressive loss in hydraulic conductivity by injecting air into the stem xylem. (Reproduced from Hubbard *et al*., 2001, copyright Blackwell Publishing.) Solid line: the model of Buckley *et al*. (2003), with parameters adjusted where possible to represent the plants studied by Hubbard *et al*. (2001) (see Appendix for parameter estimation details), showing the response of ψ_l predicted by hydro-active local feedback. Dashed line: a perfectly horizontal line, representing perfect homeostasis, shown for reference.

$$\frac{\partial \ln \Delta\psi}{\partial \ln D_s} = \frac{1}{1 + \chi(\alpha R + f_g r_{eg})D_s} \qquad \text{Eqn 8}$$

If the quantity on the right-hand side is very small, then ψ_l is nearly homeostatic under varying D_s. This suggests that quasi-isohydric behaviour is promoted by large χ, R or α. To interpret this more intuitively, first compare Eqns (7) and (8) to see that the quantity on the right-hand side of Eqn (8) equals g_s/g. Comparison with Monteith's (1995) expression, $g_s/g = 1 - E/E_m$, suggests that the degree of water potential homeostasis under varying D_s (one minus Eqn 8, say H_D), is:

$$H_D \equiv 1 - \frac{\partial \ln \Delta\psi}{\partial \ln D_s} \approx \frac{E}{E_m} \qquad \text{Eqn 9}$$

Thus, hydro-active negative feedback regulation of stomatal conductance produces near-homeostasis in ψ_l when the plant's hydraulic system is operating close to capacity ($E \approx E_m$). This occurs when ψ_l is in the vicinity of the cavitation threshold. Cavitation, in turn, can provide the amplification necessary to create true homeostasis. In other words, pseudo-isohydric and truly isohydric behaviour are not only consistent with but are in fact predicted by simple feedback regulation of g_s in response to ψ_l, given the known positive feedback between ψ_l and R and the tendency of many species to operate near the cavitation threshold. It is not necessary to suppose that guard cells sense an amplified proxy of ψ_l.

One final point regarding the interpretation of isohydric behaviour *in situ* relates to the interpretation of apparent feedforward behaviour, as discussed in Section IV.1. Diurnal invariance of ψ_l does not say anything unambiguous about stomatal control unless all other stomatal effectors were held constant. In practice, few published experiments showing isohydric behaviour have satisfied these criteria, so, although the widespread occurrence of the phenomenon is certain, a better understanding of the underlying mechanism awaits experiments designed for that purpose.

3. Pre-emptive responses to soil drought

Changes in soil water status can affect stomata in at least three ways. The initial effect, as described in Section III.1, is the typical two-phase stomatal response, which, in the steady state, reverses part or most of the change in ψ_l that would otherwise result passively. This is consistent with a negative feedback response of g_s to ψ_l. However, when soil water status declines more slowly, over several days or more, g_s often declines without any change in ψ_l (Zhang & Davies, 1990; Gollan *et al.*, 1992). This is known to be initiated by a drought-sensing mechanism located in the roots, which produce ABA and export it to leaves in the transpiration stream. ABA affects guard cells directly by inducing osmotic efflux and hence turgor loss and reduced stomatal aperture (Zhang & Davies, 1990; Assmann & Shimazaki, 1999; Blatt, 2000; Ng *et al.*, 2001). Soil drying

can also lead to up-regulation of leaf osmotic pressure, which permits the maintenance of turgor at lower water potentials. To the extent that 'osmoregulation' promotes greater g_s under moderate soil drought (Morgan, 1984; Jones, 1992), it opposes any ABA effect. This subsection discusses the relationship between these drought responses and the putative hydro-active local feedback control of stomata. Specifically, I ask two questions: where do the effects of root-derived ABA and osmoregulation fit into the mathematical structure outlined above (Eqns 4 and 6), and are these effects feedforward responses or not?

The answer to the first question is straightforward for ABA, in one sense: ABA is directly sensed by guard cells, where it causes a reduction in osmotic content (for reviews, see Assmann & Shimazaki, 1999; Dodd, 2003; Pospíšilová, 2003). The expression of hydro-active local feedback underlying Eqn (6) is that, at steady state, $\pi_g = BP_e$, suggesting that the ABA response is embedded in the parameter B, which would decline as [ABA] increases (Dewar, 2002; Buckley *et al.*, 2003). However, Assmann *et al.* (2000) found normal steady-state humidity responses in ABA-insensitive and ABA-deficient mutants, suggesting that ABA may not be a necessary component of hydro-active local feedback. On the other hand, Zhang & Outlaw (2001) found that guard cell apoplastic [ABA] responded to short-term variations in transpiration rate, apparently due to passive changes in apoplastic water content, as needed to produce the correct steady-state stomatal response. Other evidence also suggests interaction, though not necessarily convergence, between the mechanisms of local water status sensing and ABA responses: the CO_2 and ABA response pathways are closely intertwined (Webb & Hetherington, 1997; Leymarie *et al.*, 1998; Assmann, 1999), and stomatal sensitivity to CO_2 is enhanced by elevated humidity during growth (Talbott *et al.*, 2003). It is not clear how these results may be reconciled with the ABA mutant behaviour, except to speculate that the mutants possess alternative, compensatory mechanisms to sense local water status. Indeed, the tremendous plasticity and redundancy of environmental sensing by guard cells (Zeiger *et al.*, 2002) complicates the interpretation of mutant behaviour.

The effect of bulk leaf osmoregulation on stomatal hydromechanics is entirely a matter of speculation, because the hydromechanical framework (Eqn 4) only accounts directly for epidermal water relations. If epidermal cells osmoregulate in concert with the bulk of leaf tissue, then the positive effect of osmoregulation on g_s is captured by variations in epidermal osmotic pressure, π_e, given the hydro-active feedback hypothesis that $\pi_g = BP_e$. It is worthwhile noting, however, that osmoregulation would have the opposite effect – pre-emptive stomatal closure and drought avoidance, instead of sustained opening and drought tolerance – if the sensor were epidermal water potential (ψ_e) rather than turgor *per se*. (To see this, apply $\pi_g = B\psi_e$ to Eqn 4 instead of $\pi_g = BP_e$, to give $\alpha\psi_s - \pi_e$ in the numerator instead of $\alpha(\psi_s + \pi_e) - \pi_e$; the former responds negatively to π_e.)

The second question (are stomatal responses to ABA and osmoregulation feedforward?) is semantical, but also substantive. First, it is clear that neither response necessarily represents feedback control, because neither necessarily influences the effector, which is soil drying. On the other hand, from the perspective of stomatal physiology, ABA and osmoregulation can be described as independent controls on the gain of the feedback loop between g_s and water status: ABA amplifies the feedback, whereas osmoregulation diminishes it. Furthermore, because the effect of ABA is often hysteretic, it may linger even after soil water status recovers, creating the impression that stomatal conductance is declining while ψ_s is increasing. The distinction is important as a reminder that feedforward phenomenology does not necessarily contradict the hypothesis of an underlying, and ongoing, negative feedback.

4. Optimal stomatal control

This review is concerned primarily with the mechanisms of stomatal control, but there are other ways to interpret and generalise stomatal function. Perhaps the most promising of these is the hypothesis that stomatal behaviour has been shaped by selection such that the underlying control mechanisms achieve, or at least tend to approach, some quantifiable goal. In this subsection, I ask what one would expect from stomatal control mechanisms, in terms of feedback and feedforward phenomenology, if they were, in some sense, optimal.

The first step is to identify the goal of stomatal control. Here we immediately face a dilemma, because there are two obvious but different goals to choose from. One is to maximise the amount of carbon gained per unit of water lost. Because instantaneous water-use efficiency (A/E) is usually greatest at $g_s = 0$, the question is posed on an integrated timescale: what pattern of stomatal behaviour maximises daily total carbon gain ($\int A\,dt = A_t$) for a given daily total water use ($\int E\,dt = E_t$)? The solution is that the diurnal conductance timecourse, $g_s(t)$, maximises A_t for a given E_t if the ratio of the sensitivities of E and A to g_s is constant over time: $(\partial E/\partial g_s)/(\partial A/\partial g_s) = \partial E/\partial A(t) = \lambda$ (where λ is a constant implicitly defined by E_t and other imposed parameters) (Cowan & Farquhar, 1977; Cowan, 1977, 1982). The alternative goal is to prevent runaway xylem cavitation by preventing E and ψ_l from crossing thresholds, say E_{crit} and $\psi_{l,cav}$. These two goals are most easily resolved by recognising that, whereas conductance is a continuously varying quantity, daily maximum E (say E_{max}) is a property of the diurnal course of gas exchange viewed in its entirety. Thus, ensuring that $E_{max} < E_{crit}$ is more akin to ensuring that $\int g_s D_s dt = E_t$, than to ensuring that $\partial E/\partial A(t) = \lambda$; in other words, cavitation avoidance is an aspect of the resource constraint needed to frame the problem, rather than a competing goal.

This point is best illustrated by an example. If the value of λ 'chosen' by the plant is high enough that, for some part of the day, E would exceed E_{crit}, then the cavitation-avoidance

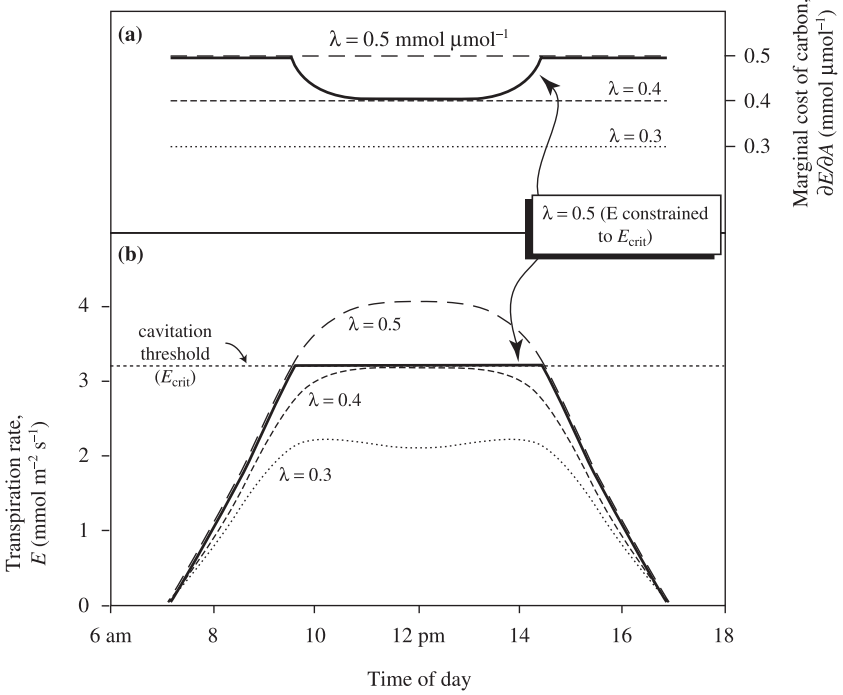

Fig. 6 Diurnal traces of (a) the marginal water cost of carbon gain ($\partial E/\partial A$, upper half of figure) and (b) the transpiration rate (E, lower half of figure). The three dashed trajectories represent optimal stomatal regulation at three different values of λ, as evidenced by the invariance of $\partial E/\partial A$ in (a). The solid lines represent suboptimal regulation demanded by the need to reduce E during mid-day to prevent runaway cavitation when the cavitation threshold is well below the diurnal peak of the theoretical optimum E trace. See the text for further discussion, and the Appendix for a description of how optimal gas exchange traces were calculated.

goal would force E to deviate below the optimal trace – causing $\partial E/\partial A$ to vary over time (solid lines in Fig. 6) and violating the first goal. If instead λ were low enough to ensure $E << E_{crit}$ all day, then gas exchange could remain optimal, but clearly more water could have been used without undue risk. Choosing λ such that E just reaches but does not exceed E_{crit} satisfies both goals while using as much water and gaining as much carbon as is safely possible. The analysis changes if the water supply is known only stochastically, in which case it may be wise to use soil water even more slowly than required to prevent cavitation (Cowan, 1982; Jones & Sutherland, 1991; Bond & Kavanagh, 1999).

Under some conditions, however, optimal gas exchange requires E to decline with increasing D at mid-day, despite increasing irradiance (Fig. 7). In fact, under all three of the scenarios outlined above, the associated relationship between g_s and ψ_l is nonunique and thus feedforward-like (Fig. 7c). Although the effects of VPD and light are confounded in these simulations, they are easily disentangled by holding irradiance constant, and this still yields feedforward-like relationships in some cases (Fig. 7b,d). This can not be produced solely by local hydraulic feedback. However, it can result from the amplification of feedback by a limited degree of xylem cavitation, suggesting that total cavitation avoidance may in fact be suboptimal in some conditions. Recent data showing that embolised vessels can be re-filled quickly and under tension (McCully *et al.*, 1998; Tyree *et al.*, 1999; Melcher *et al.*, 2001; Bucci *et al.*, 2003; Brodribb & Holbrook, 2004) suggest that mid-day cavitation need not irreversibly reduce g_s in the afternoon. The plausibility of this mechanism is supported by data of Bucci *et al.* (2003), who reported mid-day depression of petiole hydraulic conductivity in two tropical savanna tree species, following a mid-morning maximum in E and coincident with a decline in E. Active regulation of liquid-phase hydraulic resistance by aquaporins or other means (McCully *et al.*, 1998; Zwieniecki & Holbrook, 1998; Tyree *et al.*, 1999; Johansson *et al.*, 2000; Zwieniecki *et al.*, 2001; Tyerman *et al.*, 2002; Hill *et al.*, 2004) may provide alternative mechanisms for plants to achieve the required feedforward-like phenomenology, without the risks and hysteresis associated with cavitation. Taken together, these considerations may help to explain further why stomatal behaviour tends to operate so close to the edge of water-supply catastrophe (Tyree & Sperry, 1988; Sperry *et al.*, 2002; Brodribb & Holbrook, 2003).

5. Oscillations and patchiness

The idea that short-term stomatal responses to water balance involve the juxtaposition of positive and negative feedback loops is also useful for explaining two other interesting features of stomatal behaviour. As discussed above (Section III.1), these responses usually include a transient 'wrong-way' response that reinforces the perturbation, followed by an exponential approach to a new steady-state conductance that partially counteracts the perturbation. Sometimes, however, this two-phase pattern repeats itself, producing oscillations that may persist for a while before damping out, or may even persist indefinitely. The simplest explanation for both the two-phase response pattern and oscillations is the existence of

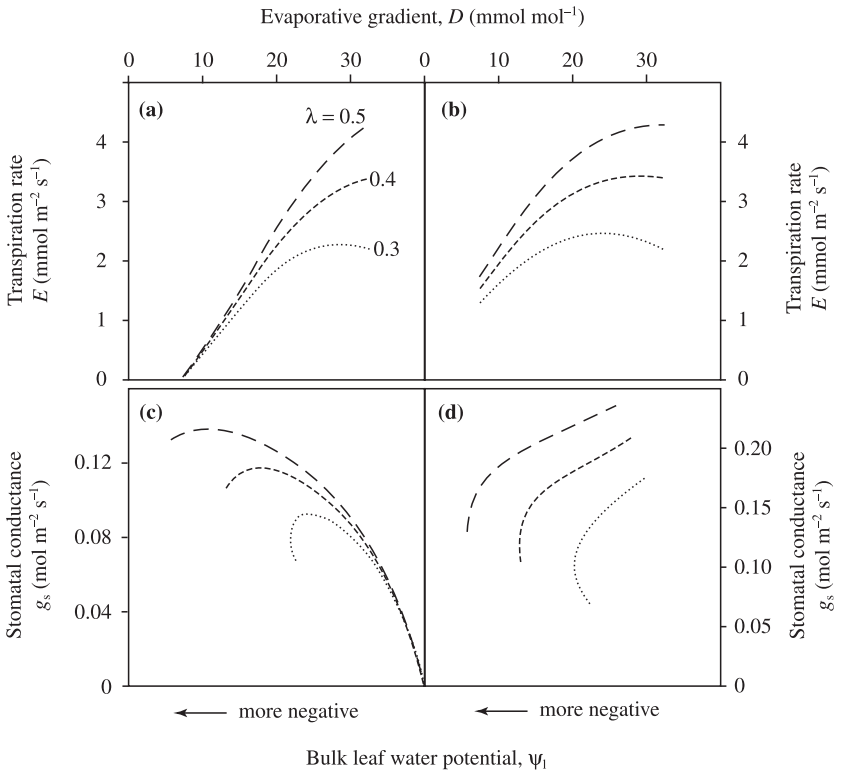

Fig. 7 Relationships between (a,b) evaporative gradient, *D*, and transpiration rate, *E*, and (c,d) between bulk leaf water potential, ψ_l, and stomatal conductance, g_s (ψ_l is calculated as $\psi_s - RE$, and is shown on a nondimensional linear scale to indicate fixed but arbitrary ψ_s and *R*). The traces in (a) and (c) correspond to the simulations shown in Fig. 5; the traces in (b) and (d) are from simulations in which irradiance was fixed at 1000 μE m^{-2} s^{-1} to isolate the effect of *D*, with conditions otherwise the same as for the traces in (a) and (c). When both light and *D* vary, *E* vs *D* is nonunique (i.e. feedforward-like) under the low-λ scenario (dotted line in (a)), whereas g_s vs ψ_l is nonunique in all three cases (c). When only *D* varies, the two lower-λ scenarios require nonunique relationships for both *E* vs *D* (b) and g_s vs ψ_l (d).

two opposed feedback loops with finite natural frequencies of similar order (Cowan, 1972; Jarvis *et al.*, 1999). If this idea is correct, then the tendency for oscillations to occur should be highly sensitive to the gain of either loop – consistent with the observation that high VPD strongly promotes oscillations (Cowan, 1972; Farquhar & Cowan, 1974; Rand *et al.*, 1981; Haefner *et al.*, 1997; Jarvis *et al.*, 1999; Wang *et al.*, 2001).

Another feature that may be explained by the dual-feedback model is 'patchy' stomatal conductance, a phenomenon in which stomatal apertures are coordinated within but not among local regions of a leaf (for reviews, see Terashima, 1992; Pospíšilová & Santrucek, 1994; Weyers & Lawson, 1997; Mott & Buckley, 1998; Mott & Buckley, 2000). Patchiness is most often induced experimentally by a step change in VPD, and is usually dynamic, in that the conductance of each 'patch' changes over time, often oscillating (Cardon *et al.*, 1994; Siebke & Weis, 1995). However, other perturbations can induce patchiness, including large changes in irradiance (Eckstein *et al.*, 1996), and patches are sometimes static, not dynamic. The mechanism of patchiness is unknown. However, a growing body of evidence suggests that it is an emergent property of the spatiotemporal instability caused by two features: the oscillation-promoting combination of positive and negative feedback loops, and hydraulic connectivity, which can coordinate stomatal behaviour locally (Haefner *et al.*, 1997; Mott *et al.*, 1997). The idea is that 'hydraulic coercion' of the sort reported by Mott *et al.* (1997) and simulated by Haefner *et al.* (1997) might also allow stomata in one patch to destabilise stomata in another patch by disturbing the latter's local hydraulic steady state.

One recent discovery is directly relevant to the study of both oscillations and patchiness. Classically, the positive feedback involved in oscillations has been identified solely with the effect of the epidermal mechanical advantage on passive stomatal hydromechanics (Eqn 1). However, positive feedback can also emerge from xylem cavitation in response to increased tension. The transient drop in water status induced by a step increase in VPD, for example, could induce cavitation, perhaps within the leaf or petiole, further reducing water status and amplifying the wrong-way response. Because this effect is often irreversible in the short term, it seems more likely to lead to sustained stomatal closure than oscillations. However, as discussed in Section IV.4, recent evidence suggests that cavitation can be reversed on timescales of similar order as those for guard cell osmotic adjustment, and without significant hysteresis (Brodribb & Holbrook, 2004). Any transient spatial variability in water status within a leaf lamina is also likely to produce spatially heterogeneous patterns of intralaminar cavitation, which could help to entrench and propagate transient stomatal patchiness arising from heterogeneous response kinetics. Whether cavitation plays any role in oscillations or patchiness remains a matter of pure speculation at this point, but it does seem to warrant further investigation.

V. Conclusions

Most aspects of short-term stomatal behaviour in response to changing leaf water balance are consistent with, and are most easily explained by, the hypothesis of 'hydro-active local feedback': a metabolically mediated response of guard cells to local water status. In contrast, two hallmarks of hydraulically related stomatal behaviour – wrong-way responses and equivalence of hydraulic supply and demand as stomatal effectors – are very difficult to explain with the alternative hypothesis, involving water potential gradients between epidermal and guard cells. Furthermore, features of stomatal control that appear inconsistent with hydraulic feedback are in fact easily reconciled with it when other known processes are taken into account, most notably the hysteretic response of xylem resistance to tension and the tendency for leaves to operate near the cavitation threshold.

Many lines of experiment could improve our understanding of this topic, but several strike me as particularly intriguing: (a) testing the hypothesis that hysteretic apparent feedforward responses to humidity involve xylem cavitation; (b) clarifying whether the degree of water potential homeostasis is sensitive to physiological and environmental factors as predicted by Eqns (8–9), or if instead the property is more or less invariant within a taxon; (c) looking for evidence of embolism and its rapid reversal during stomatal oscillations; and, perhaps most significantly, (d) seeking the water status sensor and the mechanism that relays its output to guard cells.

Acknowledgements

I am gratefully indebted to Keith Mott and Graham Farquhar for their generous mentorship and friendship. This work was supported by funding from the Cooperative Research Centre for Greenhouse Accounting at the Research School of Biological Sciences, Australian National University, Canberra.

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Appendix

1. Estimation of parameters for Fig. 5

To simulate the experiment shown in fig. 4 of Hubbard *et al.* (2001), in which near homeostasis in ψ_l was observed during a progressive reduction in hydraulic conductivity due to air injection into the stem xylem of ponderosa pine seedlings, I used the model of Buckley *et al.* (2003) with several parameters modified to mimic the Hubbard experiment ($R = 1/K_L = 1/2.8$ mmol m^{-2} s^{-1} MPa^{-1} = 0.36 MPa $mmol^{-1}$ m^2 s^{-1}; ψ_s (predawn ψ_l) = –0.4 MPa; D_s (air saturation deficit) = 28.5 mmol mol^{-1}), but using other parameter values given by Buckley *et al.* (2003) for *Vicia faba*. I adjusted π_e by trial and error until the g_s value predicted by the model for zero conductivity loss was equal to the observed value (0.1 mol m^{-2} s^{-1}), using the values of ψ_s, R, χ and D_s calculated as described above; this yielded π_e = 1.6 MPa.

2. Derivation of Eqn (8)

The soil–epidermis water potential gradient is simply ER, or $g_s D_s R$; applying Eqn (6) to this yields:

$$\Delta\psi = \frac{R(\chi(\psi_s + \pi_e) - \chi\pi_e)D_s}{1 + \chi(\alpha R + f_g r_{eg})D_s} = \frac{aD_s}{1 + bD_s} \qquad \text{Eqn A1}$$

where a and b are shorthand for $R(\chi(\psi_s + \pi_e) - \chi\pi_e)$ and $\chi(\alpha R + f_g r_{eg})$, respectively. The sensitivity of $\Delta\psi$ to D_s is:

$$\frac{\partial\Delta\psi}{\partial D_s} = \frac{a(1+bD_s) - abD_s}{(1+bD_s)^2} = \frac{a}{(1+bD_s)^2} = \frac{\Delta\psi}{D_s}\frac{1}{1+bD_s} \qquad \text{Eqn A2}$$

Dividing both sides by $\Delta\psi/D_s$ and replacing b with $\chi(\alpha R + f_g r_{eg})$ gives Eqn (8).

3. Calculation of optimal gas exchange traces for Fig. 6

When boundary layer resistances to heat and gas transfer are small, then the optimal value of stomatal conductance (to water vapour) is implicitly defined by the following equation (Buckley *et al.*, 2002):

$$g_s = \frac{\partial A_d}{\partial c_i}\left(\frac{\lambda(c_a - c_i)}{D_s} - 1.6\right) \qquad \text{Eqn A3}$$

where values are specified for λ (the invariant marginal water cost of carbon, $\partial E/\partial A$), c_a (ambient CO_2 concentration) and D_s, and for a biochemical CO_2 demand function $A_d(c_i)$ with specified photosynthetic parameters, including maximum carboxylation velocity ($V_{c,max}$), maximum potential electron transport rate (J_{max}), photosynthetic irradiance (I) and leaf temperature (T_l). The constraint is implicit, not explicit, because c_i depends on g_s, and $\partial A_d/\partial c_i$ in turn depends on c_i. I assumed that c_a = 365 ppm, $V_{c,max}$ = 100 µmol m^{-2} s^{-1}, J_{max} = 210 µmol e^- m^{-2} s^{-1}, convexity = 0.9, leaf PAR absorbance = 80%, I = (1000 µE m^{-2} s^{-1}) $\sin(t_{rel}\pi)$, where t_{rel} is relative time of day (0 at sunrise and 1 at sunset), $T_l = T_a = 15°C + 15\sin(t_{rel}\pi)°C$, where T_a is the air temperature, and $D = e_s(T_l) - e_a$, where e_s is the saturation water vapour mole fraction at T_l and e_a is the ambient vapour mole fraction (= 10 mmol mol^{-1}).

For each of a series of 'candidate' values of g_s, I calculated A_d, and c_i from the intersection of the model of Farquhar *et al.* (1980) (with temperature dependencies given by de Pury & Farquhar, 1997) and a diffusion model ($A = g_s(c_a - c_i)/1.6$), and calculated $\partial A_d/\partial c_i$ using expressions given by Buckley *et al.* (2002). Candidate g_s values were adjusted until a value was found that was within 5×10^{-5} mol m^{-2} s^{-1} of the value given by Eqn (12). This procedure was repeated at each of 200 time points evenly spaced between t_{rel} = 0 and 1.

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