Stomatal Water Relations and the Control of Hydraulic Supply and Demand

By Thomas N. Buckley and Keith A. Mott

1 Introduction

Stomata have fascinated plant biologists for well over 100 years. It is difficult to think of another plant system that responds to so many factors or displays such complexity at so many levels. Indeed, when one considers the number of feedback loops involving stomatal conductance and all of the potential interactions among these feedbacks, it is really quite remarkable that stomata work at all.

There is now general agreement about the environmental factors to which stomata respond, and the advent of relatively low-cost, easy to use gas-exchange systems and porometers has resulted in a surfeit of data describing stomatal conductance responses in natural and laboratory situations. Despite this, the mechanisms by which stomata respond to environmental factors are largely unknown. It has been clear for some time that active regulation of guard cell osmotic pressure plays a large role in most stomatal responses, and there has been some recent progress in elucidating the signal transduction chains and ion transport processes responsible for this aspect of stomatal function. It seems likely that many of these subcellular processes will be worked out in the near future. However, in order to be useful in predicting and interpreting gas exchange, these metabolic processes must be translated into dynamics of stomatal conductance at larger scales. This in turn requires a detailed understanding of the hydraulic factors that link stomatal aperture to guard cell osmotic content and to the water relations of the epidermis. Short-term stomatal responses to perturbations in hydraulic supply or demand are an appropriate context in which to develop this understanding, not only because of the ecological significance of these stomatal responses and their consequent need for explanation, but also because they provide a window on epidermal hydraulics: the system is prodded from different angles, one by one, producing apparently disparate responses that lend themselves to logical synthesis. Our goal in this review is a parsimonious, synthetic, mechanistic explanation of these responses.

Progress in Botany, Vol. 63

2 Hydraulics of Stomatal Responses to Environmental Factors

Although most stomatal responses are driven ultimately by changes in guard cell solute concentration, stomata are fundamentally hydraulic entities, and it is impossible to understand conductance responses to environmental perturbations without taking hydraulics into account. Stomatal dynamics emerge from a highly connected hydraulic medium: water evaporates from a mesh of mesophyll and epidermal cells to which guard cells are hydraulically slaved. Two factors ensure the dynamic and spatial significance of hydraulic interactions among stomata. First, individual stomatal apertures are controlled by both guard cell turgor and epidermal cell turgor, which affect aperture in opposite ways but are hydraulically governed by the same sources and sinks. Second, recent data suggest that in some species the proximity of adjacent stomata causes neighboring guard cell pairs to be, in effect, partially slaved to one another for access to water. It is now becoming apparent that these factors interact in complex and often counterintuitive ways that cannot be understood or predicted without extending the spatial context of stomatal physiology to at least the leaf scale.

An important facet of stomatal hydraulics is that the effect of epidermal turgor on stomatal aperture is greater than that of guard cell turgor by a factor of 1.5 or more (Sharpe et al. 1987; Franks et al. 1998). Thus, for equal increases in guard cell and epidermal cell turgor, stomatal aperture will decrease. In most stomatal movements, this 'mechanical advantage' enjoyed by the epidermal cells is more than offset by the much larger, osmotically induced changes in turgor pressure of the guard cells. Nevertheless, the effect of epidermal turgor on aperture is an important component of most stomatal responses. In light of the importance of both guard and epidermal cell turgor in determining stomatal aperture, four questions are of interest for understanding how aperture responds to environmental perturbations. First, how do guard and epidermal turgor pressures determine aperture? Second, what controls guard cell turgor? Third, what controls epidermal cell turgor? And fourth, how do the factors controlling these two parameters interact? These four questions will be discussed in order, below.

Although some models of stomatal functioning have assumed linear effects of guard and epidermal cell turgor (Cowan 1972; Delwiche and Cooke 1977; Haefner et al. 1997), recent work shows that these effects are, in fact, strongly nonlinear (Franks et al. 1998). In the three species examined by Franks et al. (1998), aperture increased in a saturating fashion with guard cell turgor when epidermal turgor was zero. Nonzero epidermal turgor depressed stomatal apertures for any value of guard cell turgor, and the overall relationship between aperture and guard cell turgor became sigmoidal (Fig. 1, redrawn from Franks et al. 1998, with

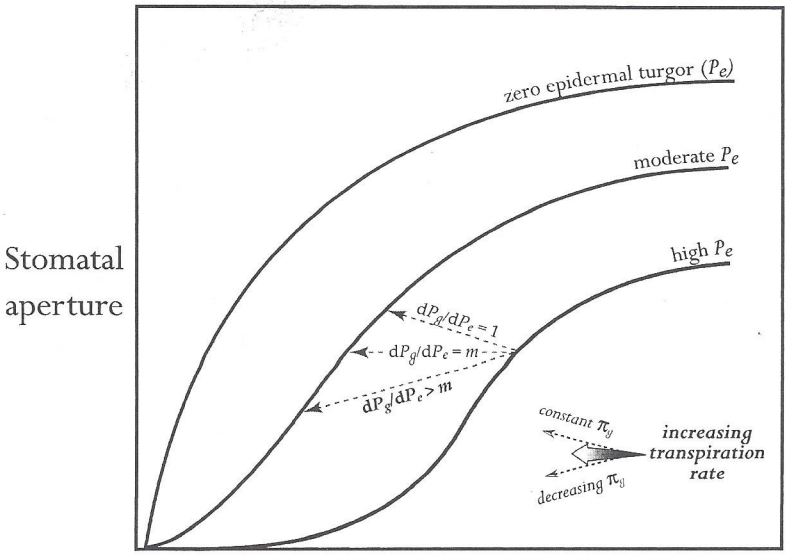

Fig 1. Diagram illustrating the effects of epidermal cell and guard cell turgor pressures (P_e and P_g) on stomatal aperture, based on the data of Franks et al (1998). Superposed on these relationships are three heuristic trajectories for increasing transpiration rate, for a steady-state system governed by the properties of stomatal mechanics implied by these data, and by the assumption that guard cells and epidermal cells share the same water potential at equilibrium. These trajectories show that in such a system, for stomatal aperture to decline as transpiration rate increases, guard cells must actively control osmotic pressure (π_g) in order to overcome the epidermal mechanical advantage, m, defined here as the ratio of the sensitivities of aperture to P_e and P_g. Were π_g allowed to remain constant by passive equilibrium, P_e and P_g would change by equal amounts, causing aperture to increase with transpiration rate (*upper trajectory*); and with some osmotic control, but not enough, aperture would remain constant as P_e and P_g declined (*middle trajectory*). The *lower trajectory*, annotated in *bold*, shows the correct response

annotations). These relationships must be taken into account as the turgor pressures of guard and epidermal cells vary, as discussed below.

Guard cell turgor is a function of both osmotic pressure and water potential. The former is controlled by the cellular processes that affect the osmotic concentration of the guard cells. These processes have been the subject of several recent reviews (Assmann 1999; Assmann and Shimazaki 1999; Blatt 2000) and will not be discussed here. Guard cell water potential is completely controlled by hydraulics, at least in the short term, and is therefore influenced by transpiration rate, soil water potential, and the conductance for water flow from the soil to leaf. All of these have important implications for stomatal responses, and will be discussed in detail in a later section. Transpiration rate, however, is inter-

esting in the immediate discussion because of its interaction with stomatal aperture.

As guard cells accumulate solutes, their turgor pressure and volume increase. This opens the stomatal pore and allows more transpiration, which in turn lowers the water potential and turgor pressure of the surrounding cells (Shackel and Brinckmann 1985; Nonami and Schulze 1989; Nonami et al. 1990; Mott and Franks, in press), which are presumably in hydraulic equilibrium with the guard cells. This decline in guard cell water potential tempers the effect of solute accumulation on turgor, and the magnitude of this decline is controlled by evaporative demand (Δw, the gradient for water vapor between the leaf and air): larger values of Δw will result in larger effects of aperture on transpiration and therefore on guard cell water potential. Therefore, a given increase in guard cell osmotic content will translate into a smaller increase in guard cell turgor at high values of Δw than at low values. The effect of solute accumulation on turgor is also reduced by dilution as the guard cell increases in volume. The dilution of solutes as volume increases occurs in all plant cells, but most plant cells undergo relatively small (~15%) changes in volume as they move from maximum turgor to zero turgor (e.g., Steudle et al. 1977). However, because of the exceptionally large changes in guard cell osmotic pressure and therefore turgor pressure (Franks et al. 1995; Franks et al., in press), volume changes for guard cells will have a substantial effect on osmotic pressure. As turgor pressure increases from 10 to 45 bars in the guard cells of *Vicia faba*, volume increases by approximately 50% (Franks et al, in press). Thus, solute concentration must actually increase by 500% over this range of pressure, instead of by 350%, as would be the case if volume were relatively constant. These considerations make it impossible to define a simple, unique rule that allows one to predict changes in guard turgor from changes in guard osmotic pressure.

The factors controlling epidermal turgor have been studied in much less depth than those controlling guard cell turgor, probably because in most of the species studied, epidermal turgor appears to be a passive function of epidermal water potential. In a notable exception to this generalization, Klein et al. (1996) observed that epidermal cells of *Vicia faba* lost a substantial amount of turgor (estimated by the difference between bulk epidermal water potential and osmotic pressure of detached epidermis) early in the morning before stomata opened, and they concluded that epidermal turgor did not play an important role in controlling stomatal aperture during the day. However, as noted above, pressure probe studies have shown that epidermal turgor and mesophyll turgor respond rapidly to changes in transpiration rate that are caused by perturbations in ambient humidity. Thus, epidermal and mesophyll turgor appear to depend on the prevailing balance between water supply and demand: transpiration removes water from the epidermis, and flow

from the soil to the leaf replaces it. This leads to some interesting and well-documented kinetics. For example, when humidity is suddenly lowered, transpiration increases, causing a rapid decline in epidermal turgor (and presumably guard cell turgor). Because of the mechanical advantage of the epidermal cells, the pore actually opens in response to this decrease in turgor, which further increases transpiration. This sequence of events would constitute a 'runaway' reaction if it were not for the fact that the indirect effect of epidermal turgor on transpiration (via conductance) is much smaller than the direct effect of transpiration on epidermal turgor. The transient opening response persists until active processes in the guard cell cause the osmotic pressure, and hence the turgor pressure, of the guard cell to increase sufficiently to cause a reduction in steady-state aperture. A similar sequence of events, often called the Iwanoff effect (Iwanoff 1928), occurs when leaf turgor is suddenly reduced by cutting off the water supply to the leaf (Raschke 1970), and the opposite sequence of events occurs when leaf water potential is increased by pressurizing the roots (Comstock and Mencuccini 1998). It has also been suggested that the guard cells experience the lion's share of water loss, and the resulting imbalance in water potential drawdown between the epidermal and guard cells is sufficient to overcome the epidermal mechanical advantage; in that case, stomatal closure in low humidity would not require active efflux of osmotica (see Cowan 1994 for a discussion). This hypothesis is discussed in a later section (*Stomata as hydraulic integrators*), but here we note only that it is difficult to explain the transient responses described above with such a hypothesis.

Perhaps more important than these transient effects, but less obvious, is that continuous feedback exists between epidermal turgor pressure and stomatal aperture, even for stomatal movements that are initiated by active changes in guard cell osmotic pressure, rather than by hydraulic perturbations to the epidermis or guard cells. For example, when stomata are induced to open by light, guard cells initiate the response by the active uptake of ions. This results in an increase in guard cell turgor, which overcomes the backpressure of the epidermis and begins to open the pore. As transpiration increases, both epidermal and guard cell turgor decline, with the result (because of the mechanical advantage of the epidermis) that the pore opens even more. Thus, the effect of guard cell turgor on aperture is amplified by the effect of transpiration on epidermal and guard cell turgor. Since the effect of aperture on transpiration is larger at lower atmospheric humidity, this amplification effect is also larger at low atmospheric humidity. Therefore, for a given pumping rate, stomata respond more rapidly to environmental perturbations in dry air, because hydraulic interactions with epidermal cells reinforce the opening or closing responses. This effect has been noted several times in the literature (e.g., Assmann and Grantz 1990; Mott et al. 1999), and may have adaptive value because rapid responses to environmental pertur-

bations will be more important for carbon water balance when humidity is low and water loss rates are high.

It is interesting to note that rapid changes in epidermal osmotic pressure have been observed for some monocot species and could contribute to stomatal responses in these species (Raschke and Fellows 1971). Little information is available concerning the mechanisms for these changes in osmotic pressure or their effects on stomatal aperture, and this area seems worthy of further research.

The interactions between all of these factors are complicated further by the fact that they occur in a hydraulically connected spatial context. The epidermal cells within an areole do not have independent water supply pathways. This is understood most easily by considering the epidermal cells in the center of an areole, which are distal to all other cells in the water supply pathway and thus necessarily share the same water supply as many, if not all, other cells in the areole. Additionally, localized changes in evaporative water loss from a group of epidermal cells will lower the water potential in those cells, drawing water from neighboring cells despite constant evaporative demand from the latter cells. Thus, any changes in the aperture of one stoma will necessarily influence the water status and therefore the aperture of surrounding stomata. Experimental evidence for these interactions has been provided by several studies (Mott et al. 1997, 1999; Mott and Franks, in press). Other evidence suggests that changes in hydraulic demand in one region of a leaf can influence stomata in a distant region of the same leaf. When stomatal closure is induced in half of a wheat leaf by decreasing the photon flux density (PFD), stomatal conductance increases in the other half of the same leaf (Buckley and Mott 2000). It has been suggested that such long-distance interactions may help to coordinate whole-leaf gas exchange in the event of localized perturbations in hydraulic supply (by cavitation of minor leaf veins) or heterogeneity in the initial kinetics of stomatal responses to any environmental change (Mott and Buckley 1998, 2000).

3 Stomatal Responses to Hydraulic Environmental Factors

Years of research have substantially clarified the nature of what has historically been termed the 'stomatal response to humidity.' It has been experimentally demonstrated that stomatal conductance does not respond directly to atmospheric humidity, but instead responds to the rate of water loss from the leaf (Mott and Parkhurst 1991). These studies have been supported by a subsequent study (Monteith 1995) showing that stomatal responses to humidity from the literature are consistent with a linear response of conductance to transpiration rate up to some critical transpiration rate above which the response becomes nonlinear. Despite

this progress, no consensus has emerged concerning the mechanism behind this response. It is also clear that stomata can respond to the rate of water supply from the roots (Sperry 2000). Numerous studies have shown that stomatal conductance is proportional to the hydraulic conductivity between the soil and the leaf (e.g., Meinzer and Grantz 1990; Meinzer et al. 1995; Saliendra et al. 1995; Comstock 2000). Also, stomata show rapid, reversible responses to perturbations in the supply of water to the leaf. These perturbations have been effected experimentally by pressurizing roots or by changing the hydraulic conductance between the soil and the leaf (usually by xylem cavitation). When roots are pressurized, stomata open over a period of many minutes, and often display an initial transient closing response similar to that observed when Δw is decreased (Saliendra et al. 1995; Comstock and Mencuccini 1998). Subsequent depressurization causes the opposite response. Furthermore, the stomatal response to humidity can be essentially completely reversed by pressurizing the roots (Saliendra et al. 1995; Comstock and Mencuccini 1998). Reductions in xylem conductivity also cause stomata to close relatively rapidly (Hubbard et al. 2001). The similarities between the stomatal responses to humidity and water supply raise the intriguing possibility that these two responses may be caused by the same mechanism (Saliendra et al. 1995), and this idea is explored in the paragraphs below.

Two possible mechanisms for the stomatal response to water supply are immediately obvious. First, the response could be due to a signal (chemical or other) that is generated by the roots or xylem tissue in response to changes in water availability or water transport rate. Although the effects of chemical signals from the roots that are produced in response to soil drought are well-documented (e.g., Lösch and Schulze 1994), these signals must be carried from the roots to the leaves in the transpiration stream and therefore show a rather slow response time. It seems unlikely that they could be responsible for rapid reversible movements that stomata display in response to root pressurization and xylem cavitation (Sperry 2000). The second possible mechanism is that stomata are responding to the water potential (or turgor pressure) of the leaf, which is controlled by the transpiration rate, the soil water potential, and the hydraulic conductivity between the soil and the leaf. Mesophyll or epidermal cells could be involved in this response, but it is unlikely that guard cells themselves could serve as the sensing site, since they undergo such large changes in turgor pressure independent of those caused by changes in water supply. Therefore, it is necessary to also postulate the existence of some signal, generated by cells in the leaf other than the guard cells, that causes changes in guard cell osmotic concentration. There is some evidence for such an effect (Grantz and Schwartz 1988), and it would explain stomatal responses to several different ex-

perimental perturbations in water supply, and, as we shall see below, it could also explain stomatal responses to humidity.

There are several possible mechanisms by which stomata could respond to the rate of transpiration from the leaf. One possibility is that the stomata could sense transpiration rate via some molecule in the transpiration stream that accumulates at the guard cells in proportion to the transpiration rate. Abscisic acid (ABA) is one possible candidate for such a role (Lösch and Schulze 1994), but it has recently been shown that ABA-insensitive and ABA-deficient mutants of *Arabidopsis* have stomatal responses to humidity that are similar to that of the wild type (Assmann et al. 2000). Ewert et al (2000) have proposed that sucrose could fulfill this role, and they demonstrated that mannitol, applied through the transpiration stream, could accumulate in guard cell walls of a transpiring leaf to a high enough level to substantially lower the turgor pressure in the guard cells. Although attractive in principle, this hypothesis is not consistent with data showing that stomata are capable of responding to humidity in darkness, when sucrose concentrations in the apoplast should be quite low (Kappen and Haeger 1991). It also cannot serve as the mechanism for stomatal responses to cavitation and root pressurization since neither of these stimuli will have a direct effect on transpiration rate and therefore will have no effect on the delivery of the signal molecule to the guard cell. Indeed, as stomata open in response to root pressurization, the transpiration rate is actually increased, which should increase the delivery of the signal molecule to the guard cells and cause stomatal closure.

Another possibility is that stomatal responses to humidity are caused by direct transpiration-induced reductions in the turgor pressure of the guard cells. This hypothesis requires that the water potential drawdown is substantially larger for the guard cells than for the surrounding epidermal cells (to overcome the mechanical advantage of the epidermis), and this assumption in turn demands either that more water evaporates from the guard cells than from the surrounding epidermal cells, or that there is a very low hydraulic conductivity between the guard cells and the surrounding epidermal cells, or both. The evidence concerning these postulates is mixed and contradictory (see Cowan 1994). However, in some species stomatal responses to humidity can take upwards of an hour before they are complete, and it seems unlikely that the hydraulic conductivity between guard and epidermal cells could be low enough to cause this slow a response. If guard cells were the dominant site of evaporation but had such difficulty obtaining water, it seems likely that they would plasmolyze following any substantial increase in the rate of water loss, causing complete and immediate stomatal closure followed by a very slow recovery of aperture. It is also important to note that this mechanism, like the chemical-signal hypothesis discussed above, can not explain the stomatal responses that are observed when leaf water

supply is perturbed. Pressurization of the roots would increase the turgor pressure of both the epidermal cells and the guard cells by roughly equal amounts, and (because of the mechanical advantage of the epidermal cells) this would lead to stomatal closure, rather than stomatal opening. Nevertheless, these counter-arguments are circumstantial and theoretical, and there is no direct and unequivocal evidence to disprove the hypothesis that guard cells are the primary and initial sensor of changes in transpiration rate.

The last possibility that will be considered here is that the stomatal response to humidity is mediated by water potential or turgor pressure somewhere in the leaf other than the guard cells. In this scenario, the stomatal response to humidity would actually be a response to water potential or turgor pressure in specific cells in the leaf. This hypothesis requires that these physical variables be transduced into a signal that is perceptible by guard cells, and although there is no direct evidence for such a signal, there is good circumstantial evidence for such a mechanism. First, although bulk leaf water potential is often relatively constant as Δw is varied, pressure probe studies show that epidermal and mesophyll cells experience rapid changes in turgor pressure in response to changes in transpiration rate (Shackel and Brinckmann 1985; Nonami and Schulze 1989; Nonami et al. 1990; Mott and Franks, in press). Thus, these cells could generate the necessary signal. Second, there is good evidence that most of the stomatal response to humidity is caused by metabolic events in the guard cells rather than purely by hydraulics (see Grantz 1990 for a discussion). Third, this mechanism can accurately account for both the kinetics and steady-state responses of stomata to humidity (Haefner et al. 1997; Jarvis et al. 1999). And fourth, it is possible to explain stomatal responses to perturbations in the supply of water using the same mechanism (Fig. 2), and this provides an attractive unifying theme for how stomata respond over short time periods to balance the supply and loss of water from the leaf.

It has been pointed out that such a mechanism cannot account for stomatal responses that reduce transpiration as Δw is increased (Farquhar 1978). This type of response occurs occasionally at very high values of Δw, and has been termed 'feedforward' (Cowan and Farquhar 1977). Water loss directly from the outside of the guard cells has been proposed as a possible mechanism for this response (see Cowan 1994 for a discussion), but as discussed above, mechanisms based on direct evaporation from the guard cells have severe theoretical limitations. It has also been proposed that these feedforward responses of stomata to humidity are associated with patchy stomatal closure (Mott and Parkhurst 1991). This idea has been challenged by Bunce (1997), who showed that feedforward responses could occur in the absence of patchy stomatal conductance. However, in that study deviations in the relationship between CO_2 assimilation rate (A) vs. intercellular CO_2 (c_i) were used as

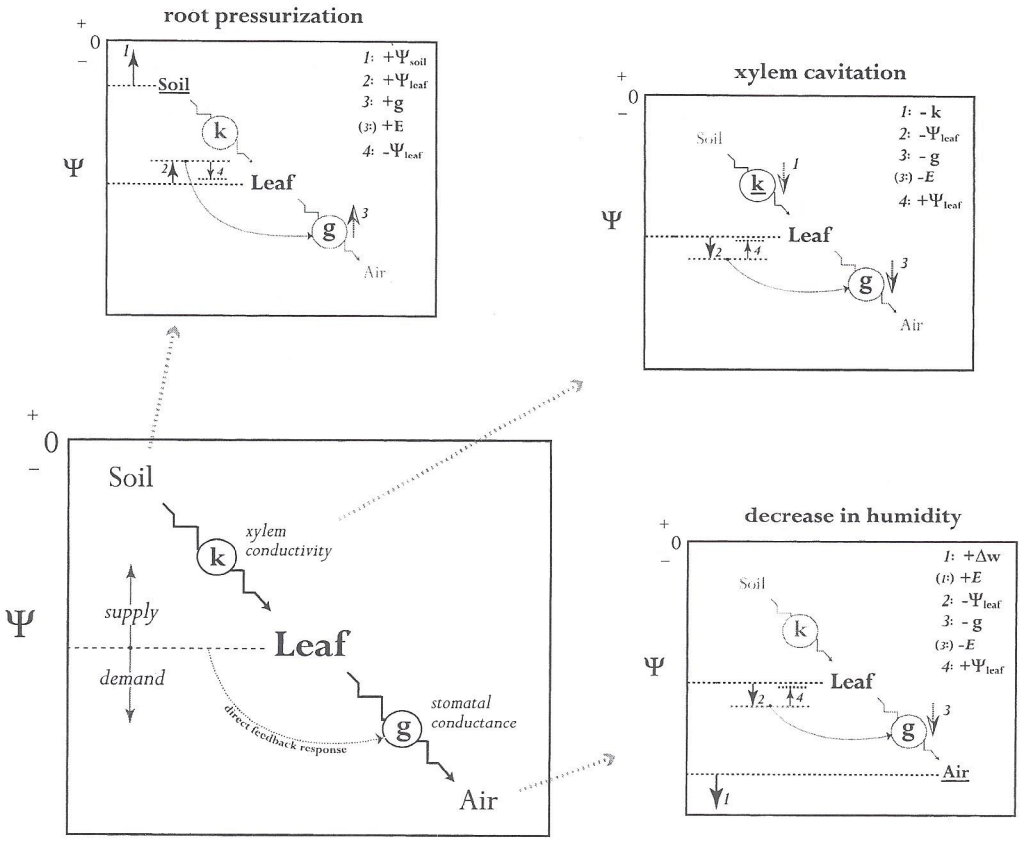

Fig. 2. Diagram showing how the short-term stomatal responses to several hydraulic perturbations (root pressurization, increasing evaporative demand (D), and xylem cavitation) may be synthesized under the auspices of a single mechanism wherein stomata respond by direct feedback to leaf water potential, Ψ (or a surrogate thereof). Leaf water potential is determined by the balance between hydraulic supply (through the xylem, and ultimately from the soil) and demand (by the atmosphere, via transpirational water loss). The sequence of events following each of these three hydraulic perturbations is shown at the *bottom*; in each case, the variable that changes first is *underlined*, shifts in leaf water potential are shown by *solid arrows*, and responses in stomatal conductance (*g*) or xylem conductance (*k*) are shown with *half-shaded arrows* to clarify that those responses do not relate to the vertical axis of the diagram, which represents water potential. In real leaves, these dynamics may involve several cycles of the feedback loop before equilibrium is achieved, but for clarity, only one cycle is shown in each case

an indicator of patchiness, and subsequent studies have shown that most patchy conductance distributions will not cause substantial effects on this relationship (Buckley et al. 1997).

We propose that the 'feedforward' phenomenon may be explained by a feedback response of stomatal conductance to water potential if it is

recognized that (1) not only hydraulic demand (transpiration), but also hydraulic supply through the xylem, can influence water potential, Ψ, (2) changes in xylem hydraulic conductance, K, are generally irreversible in the short term (Sperry 2000), and (3) the response of K to Ψ is sigmoidal (Fig. 3, top right), with no insignificant loss of conductivity above a certain threshold Ψ, but dramatic declines in K for lower values of Ψ. As illustrated heuristically in Fig. 3, there is very little loss of K as water potential declines with increasing Δw for most of the range of Δw. At higher values of Δw, however, xylem cavitation hysteretically changes the relationship between transpiration rate (E) and water potential, and as a result, a plot of E vs. D appears inconsistent with a feedback mechanism (hence the term 'feedforward'). The difficulty is resolved when the movement of the system through a third dimension (K, shown in contour form in the lower two plots of Fig. 3) is considered. This hypothesis is consistent with experimental evidence showing that feedforward responses are irreversible in the short term, and tend to occur only at high values of Δw (Franks et al. 1997; Cowan and Farquhar 1977; Farquhar 1978).

4 Stomata as Integrators of Hydraulic Supply and Demand

Most of this review has focused on identifying a single mechanism that can explain short-term stomatal responses to a variety of hydraulic factors. In this section, we take an entirely different tact and ask *why*, rather than *how*, stomata respond to these factors. We begin with the assumption that the evolution of stomatal function has been driven by the need to satisfy certain ecological 'goals' by precise control of gas exchange. It is important to review our analysis from this perspective for at least two reasons. First, these ecological goals if appropriately framed, are universal, and are thus likely to unify stomatal behavior even when mechanisms differ (as, for example, may be the case for stomatal responses to humidity and gradual soil drought). Second, there are two seemingly disparate ecological 'goals' that may drive short-term stomatal responses to hydraulic perturbations, and it is necessary to determine whether the single mechanism that can reproduce the phenomenology of these responses can also satisfy these distinct, and presumably underlying goals.

Stomatal behavior impacts fitness in several obvious ways. By controlling leaf gas exchange, stomata determine leaf energy balance, mitigate the contingency of xylem cavitation, regulate competition for water, and control the balance between short-term carbon gain and water loss. Traditionally, optimality theory has considered carbon gain to be a bottom line, primarily because carbon can be invested to mitigate these other fitness impacts (for instance, in roots to acquire soil water at the

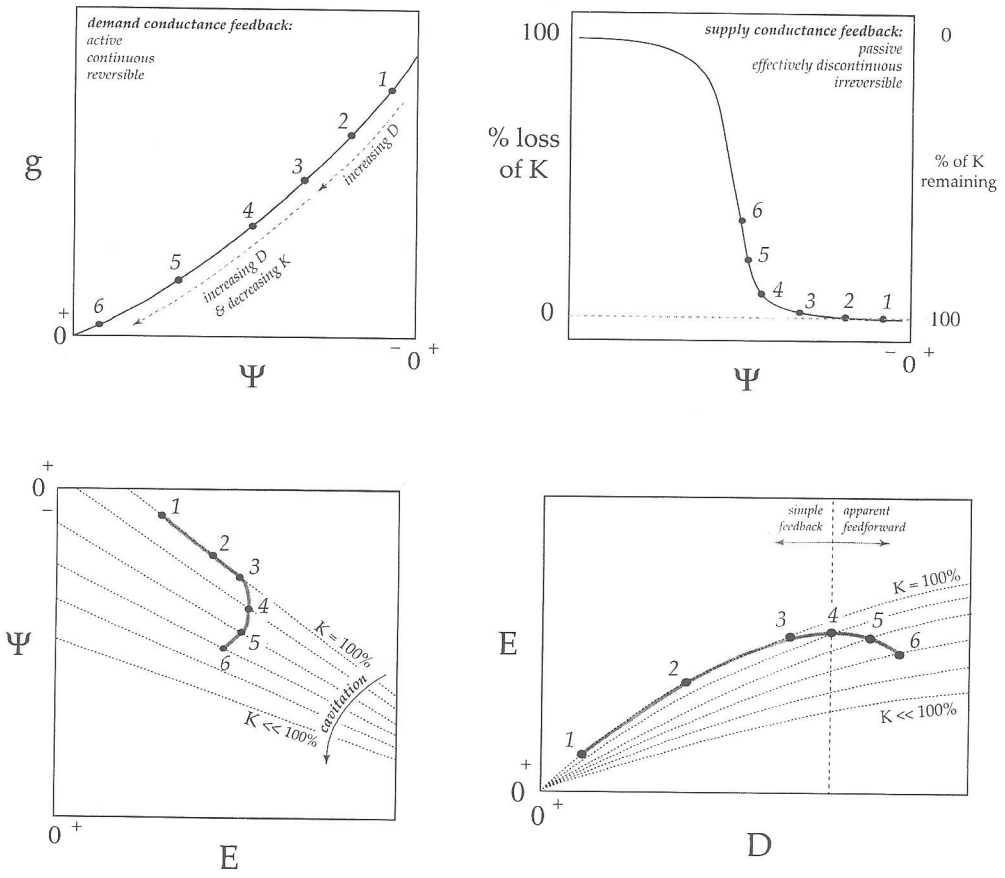

Fig. 3. Qualitative relationships between stomatal conductance (*g*), water potential at the evaporating site (*Y*), and xylem hydraulic conductance (*K*), transpiration rate (*E*), and evaporative demand (*D*), annotated with a hypothetical sequence of six steady-state points along a trajectory of increasing D. The intent of this figure is to illustrate how the 'feedforward response of stomata to humidity,' in which E declines with increasing D at high values of D (as shown in the plot at *lower right*) may be explained by the hypothesis that stomata respond by direct feedback to Y (plot at *upper left*), in conjunction with the observation that K remains nearly constant above a certain threshold Y (plot at *upper right*). Increasing evaporative demand draws down water potential via transpiration (points *1*, *2* and *3* in each plot), but as Y declines further, decreases in K become very substantial, effectively throwing the system onto a series of new trajectories corresponding to progressively lower values of K (points *4*, *5* and *6*). The resulting plot relationship between E vs. D is non-unique, which is inconsistent with a feedback mechanism (hence the term 'feedforward'); however, the true feedback is neither of E to D, nor of E to Y, but of g to Y. The distinction is clarified by showing that the trajectory of E moves through a third dimension (K, shown in contour form in the lower two plots) when plotted against either D or Y

expense of a competitor, in sapwood to increase xylem flow capacity and limit cavitation, and in leaf pubescence to increase reflectivity to reflect excess radiation). Fixed carbon can also, of course, be used to create viable and dispersible seeds, which are the ultimate bottom line for fitness. In this section, we discuss stomatal hydraulics in the context of carbon/water balance and cavitation prevention, assuming for the moment that these are separable ecological goals in their own right. Recent work (see Sperry 2000) has suggested that stomata act to maintain leaf water potential above some critical value to prevent runaway xylem cavitation. The goal in this case is apparently to keep the rate of water loss below a specified discrete maximum value. A reasonable question is then, 'How close to that threshold can stomata go?' A different line of thought preempts that question by suggesting that stomata modulate transpiration rate continuously to keep gas exchange near a mathematically identifiable optimum location – a fixed ratio of the incremental increases in carbon gain and water loss resulting from an increment in conductance. We proceed by identifying precisely what is required, both empirically and mechanistically, for stomatal dynamics to fulfill each role, and then determine whether a single mechanism can meet these requirements.

If the rate of water loss were able to increase without bounds as evaporative demand increased, then water potential throughout the plant's hydraulic continuum could decrease indefinitely. Therefore, in order to prevent runaway xylem cavitation (which would result if xylem water potential were allowed to drop below some critical threshold; Tyree and Sperry (1989)), the transpiration rate must either (1) reach a maximum and subsequently decline with further increases in evaporative demand, (2) reach a maximum and stay there, not responding at all to further increases in Δw, or (3) asymptotically approach a predetermined maximum value as Δw is increased. What underlying mechanistic responses can produce each of these three gas exchange patterns? None of these three options can depend entirely on feedback from xylem conductance (K) or any variables that may be influenced by K, which does not change significantly with increasing Δw until Δw reaches fairly large values (Sperry 2000). Furthermore, cavitation prevention also requires that stomata respond to changes in hydraulic supply (via soil water potential or K) as well as demand. Because this requires stomatal conductance to decline before any change in transpiration rate (E) and without any change in evaporative demand, the mechanism cannot depend entirely on a stomatal response to either E or Δw. Therefore, all three options for the response of transpiration to Δw that satisfy the cavitation-prevention role also require that stomatal conductance be controlled by feedback from hydraulic demand (via E or Δw) under certain conditions, and by feedback from hydraulic supply (K) under other conditions.

Mathematical analysis can reveal patterns of stomatal behavior that maximize carbon gain for a given water supply. These patterns sometimes demand that stomata actually close in the middle of the day, under conditions of high evaporative demand (Cowan and Farquhar 1977). This cannot be achieved with a direct feedback response of stomata to evaporative demand or transpiration rate *per se*, because it requires the 'feedforward' pattern – transpiration must eventually decline and approach zero continuously as Δw increases. However, as discussed above, this response cannot depend entirely on stomatal sensitivity to K either, because K only begins to decline significantly at high values of Δw. Therefore, the continuous-optimization role also demands that stomata respond by direct feedback both to hydraulic supply and demand.

The most parsimonious synthesis of these theoretical and empirical considerations would involve regulation of stomatal conductance by direct, reversible feedback from a single variable that is influenced by both hydraulic supply and demand. Water potential at the evaporating site (or a variable that is directly and reliably linked to that water potential, such as epidermal turgor pressure) is the most obvious candidate for this sensor. By postulating a direct feedback response of stomatal conductance to water potential, the short-term stomatal responses to humidity, root pressurization, and xylem cavitation are unified by a single role for stomata as integrators of hydraulic supply and demand.

5 Concluding Remarks

Stomata are the nexus of hydraulic supply and demand. They bear the heavy ecological burden of integrating all immediate and contingent threats to the continual supply of water, because that supply is critical for carbon acquisition and thus, ultimately, for reproductive success. In the last decade, we have developed a more thorough understanding of the hydraulic aspects of stomatal function at several scales, from single guard and epidermal cells to whole leaves, and this information is critical for interpreting cellular process of guard cells in terms of whole leaf stomatal conductance. Furthermore, we have recognized and substantially characterized the interactions between stomatal behavior and xylem conductance. Analysis of this new knowledge suggests a synthetic theory for the mechanism of stomatal responses to hydraulic perturbations. This theory postulates water potential at the evaporating site as a primary sensor, and we have put forward a version of this mechanism for debate. Although consensus on the fine details of this mechanism remains elusive, the basic theory is compelling for several reasons. First, when placed in the context of recent discoveries about the response of xylem conductance to water potential, this theory may explain the 'feedforward' response of stomata to humidity. Second, the mechanism

appears to explain the short-term stomatal responses to three different factors: humidity, root pressurization, and xylem cavitation. Finally, this mechanism appears to be consistent with seemingly disparate ecological goals postulated for stomatal behavior: optimization of carbon/water balance by continuous response to immediately perceptible environmental conditions, and prevention of the contingent threat, not immediately perceptible by stomata, of runaway xylem cavitation.

References

Assmann SM (1999) The cellular basis of guard cell sensing of rising CO_2. Plant Cell Environ 22:629–637

Assmann SM, Grantz DA (1990) Stomatal response to humidity in sugarcane and soybean: effect of vapour pressure difference in the kinetics of the blue light response. Plant Cell Environ 13:163–169

Assmann SM, Shimazaki K (1999) The multisensory guard cell. Stomatal responses to blue light and abscisic acid. Plant Physiol 119:809–815

Assmann SM, Snyder JA, Lee YJ (2000) ABA-deficient (*aba1*) and ABA-insensitive (*abi1-1, abi2-1*) mutants of *Arabidopsis* have a wild-type stomatal response to humidity. Plant Cell Environ 23:387–395

Blatt MR (2000) Cellular signaling and volume control in stomatal movements in plants. Annu Rev Cell Dev Biol 16:221–241

Buckley TN, Mott KA (2000) Stomatal responses to non-local changes in PFD: evidence for long-distance hydraulic interactions. Plant Cell Environ 23: 301–309

Buckley TN, Farquhar GD, Mott KA (1997) Qualitative effects of patchy stomatal conductance distribution features on gas exchange calculations. Plant Cell Environ 20:867–880

Bunce JA (1997) Does transpiration control stomatal responses to water vapour pressure deficit? Plant Cell Environ 20:131–135

Comstock J, Mencuccini M (1998) Control of stomatal conductance by leaf water potential in *Hymenoclea salsola* (T.&G.), a desert shrub. Plant Cell Environ 21:1029–1038

Comstock JP (2000) Variation in hydraulic architecture and gas-exchange in two desert sub-shrubs, *Hymenoclea salsola* (T.&G.) and *Ambrosia dumosa* (Payne). Oec 125:1–10

Cowan IR (1972) Oscillations in stomatal conductance and plant functioning associated with stomatal conductance: observations and a model. Planta 106:185–219

Cowan IR (1994) As to the mode of action of the guard cells in dry air. In: Schulze E-D, Caldwell MM (eds) Ecophysiology of photosynthesis. Springer, Berlin Heidelberg New York, pp 205–299

Cowan IR, Farquhar GD (1977) Stomatal function in relation to leaf metabolism and environment. Symp Soc Exp Biol 31:471–505

Delwiche MJ, Cooke JR (1977) An analytical model of the hydraulic aspects of stomatal dynamics. J Theor Biol 69:113–141

Ewert MS, Outlaw WH, Zhang S, Aghoram K, Riddle KA (2000) Accumulation of an apoplastic solute in the guard-cell wall is sufficient to exert a significant effect on transpiration in *Vicia faba* leaflets. Plant Cell Environ 23:195–203

Farquhar GD (1978) Feedforward responses of stomata to humidity. Aust J Plant Physiol 5:787–800

Franks PJ, Cowan IR, Tyerman SD, Cleary AL, Lloyd J, Farquhar GD (1995) Guard cell pressure/aperture characteristics measured with the pressure probe. Plant Cell Environ 18:795–800

Franks PJ, Cowan IR, Farquhar GD (1997) The apparent feedforward response of stomata to air vapour pressure deficit: information revealed by different experimental procedures with two rainforest trees. Plant Cell Environ 20:142–145

Franks PJ, Cowan IR, Farquhar GD (1998) A study of stomatal mechanics using the cell pressure probe. Plant Cell Environ 21:94–100

Franks PJ, Buckley TN, Shope JC, Mott KA (2001) Guard cell pressure and volume measured concurrently by confocal microscopy and the cell pressure probe. Plant Physiol (in press)

Grantz DA (1990) Plant responses to atmospheric humidity. Plant Cell Environ 13:667–679

Grantz DA, Schwartz A (1988) Guard cells of *Commelina communis* L. do not respond metabolically to osmotic stress in isolated epidermis: implications for stomatal responses to drought and humidity. Planta 174:166–173

Haefner JW, Buckley TN, Mott KA (1997) A spatially explicit model of patchy stomatal responses to humidity. Plant Cell Environ 20:1087–1097

Hubbard RM, Ryan MG, Stiller V, Sperry JS (2001) Stomatal conductance and photosynthesis vary linearly with plant hydraulic conductance in ponderosa pine. Plant Cell Environ 24:113–121

Iwanoff L (1928) Zur Methodik der Transpirations-bestimmung am Standort. Ber Dtsh Bot Ges 46: 306–310

Jarvis AJ, Young PC, Taylor CJ, Davies WJ (1999) An analysis of the dynamic response of stomatal conductance to a reduction in humidity over leaves of *Cedrella oderata*. Plant Cell Environ 22:913–924

Kappen L, Haeger S (1991) Stomatal responses of *Tradescantia albiflora* to changing air humidity in light and in darkness. J Exp Bot 42:979–986

Klein M, Cheng G, Chung M, Tallman G (1996) Effects of turgor potentials of epidermal cells neighbouring guard cells on stomatal opening in detached leaf epidermis and intact leaflets of *Vicia Faba* L (faba bean). Plant Cell Environ 19:1399–1407

Lösch R, Schulze E-D (1994) Internal coordination of plant responses to drought and evaporational demand. In: Schulze E-D, Caldwell MM (eds) Ecophysiology of photosynthesis. Springer, Berlin Heidelberg New York, pp 185–204

Meinzer FC, Grantz DA (1990) Stomatal and hydraulic conductance in growing sugarcane: stomatal adjustment to water transport capacity. Plant Cell Environ 13:383–388

Meinzer FC, Goldstein G, Jackson P, Holbrook NM, Butierrez MV, Cavelier J (1995) Environmental and physiological regulation of transpiration in tropical forest gap species: the influence of boundary layer and hydraulic conductance properties. Oec 101:514–522

Monteith JL (1995) A reinterpretation of the stomatal response to humidity. Plant Cell Environ 18:357–364

Mott KA, Buckley TN (1998) Stomatal heterogeneity. J Exp Bot 49:407–417

Mott KA, Buckley TN (2000) Patchy stomatal conductance: emergent collective behaviour of stomata. Trends Plant Sci 5:258–262

Mott KA, Franks PJ (2001) The role of epidermal turgor in stomatal interactions following a perturbation in humidity. Plant Cell Environ (in press)

Mott KA, Parkhurst DF (1991) Stomatal responses to humidity in air and helox. Plant Cell Environ 14:509–515

Mott KA, Denne F, Powell J (1997) Interactions among stomata in response to perturbations in humidity. Plant Cell Environ 20:1098–1107

Mott KA, Shope JC, Buckley TN (1999) Effects of humidity on light-induced stomatal opening: evidence for hydraulic coupling among stomata. J Exp Bot 50:1207–1213

Nonami H, Schulze E-D (1989) Cell water potential, osmotic potential, and turgor in the epidermis and mesophyll of transpiring leaves. Planta 177:35–46

Nonami H, Schulze E-D, Ziegler H (1990) Mechanisms of stomatal movement in response to air humidity, irradiance and xylem water potential. Planta 183:57–64

Raschke K (1970) Stomatal responses to pressure changes and interruptions in the water supply of detached leaves of *Zea mays* L. Plant Physiol 45:415–423
Raschke K, Fellows MP (1971) Stomatal movement in *Zea mays*: shuttle of potassium and chloride between guard cells and subsidiary cells. Planta 110:296–316
Saliendra NZ, Sperry JS, Comstock JP (1995) Influence of leaf water status on stomatal response to humidity, hydraulic conductance, and soil drought in *Betula occidentalis*. Planta 196:357–366
Shackel KA, Brinckmann E (1985) In situ measurement of epidermal cell turgor, leaf water potential, and gas exchange in *Tradescantia virginiana* L. Plant Physiol 78:66–70
Sharpe PJH, Wu H, Spence RD (1987) Stomata mechanics. In: Zeiger E, Farquhar GD (eds) Stomatal function. Stanford University Press, Stanford, pp 91–114
Sperry JS (2000) Hydraulic constraints on plant gas exchange. Ag For Meteor 104:13–23
Steudle E, Zimmermann U, Luttge U (1977) Effect of turgor pressure and cell size on the wall elasticity of plant cells. Plant Physiol 59:285–289
Tyree MT, Sperry JS (1989) Vulnerability of xylem to cavitation and embolism. Annu Rev Plant Physiol Molec Biol 40:19–38

Thomas N. Buckley
Department of Forest Resources
Utah State University
Logan, Utah 84322–5215,
e-mail: Tom.Buckley@alumni.jmu.edu

Keith A. Mott
Biology Department
Utah State University
Logan, Utah 84322–5305, USA
Tel.: +01-435-797–3563
Fax: +01-435-797-1575
e-mail: kmott@biology.usu.edu