

Commentary

Stomatal responses to humidity: has the ‘black box’ finally been opened?

Stomata are the sensory nexus of plant–atmosphere carbon and water exchange, yet the mechanisms of stomatal responses to hydraulic perturbations such as soil and atmospheric drought remain unresolved. This might seem surprising at first glance: given that stomatal aperture is controlled by turgor pressure in stomatal guard cells, one might naïvely predict that stomata should close when leaf turgor is reduced by water loss following exposure to dry air. However, this ignores the countervailing effect of turgor in the surrounding epidermal cells, which affects aperture in the opposite manner as guard cell turgor. In angiosperms, those cells have a mechanical advantage over guard cells – that is, equal declines in turgor in both epidermal and guard cells cause stomata to open rather than close (Franks *et al.* 1998). This causes angiosperm stomata to open transiently before closing in dry air (Fig. 1a). Some mechanism is therefore needed to amplify guard cell turgor loss to produce stomatal closure. This still-uncertain mechanism is the ‘black box’ referred to in the title of this Commentary.

Several hypotheses have been proposed to explain how guard cell turgor changes are amplified during humidity responses. Some hypotheses hold that a shift to dry air causes water potential to decline more in guard cells than in epidermal cells, either due to direct evaporation from guard cells (Maier-Maercker 1983) or accumulation of an osmolyte near guard cells in proportion to the transpiration rate (Outlaw & De Vlieghere-He 2001), or because guard cells are in equilibrium with air in the stomatal pore, which experiences far larger changes in water potential in relation to ambient humidity than liquid-phase tissue water (Peak & Mott 2011). However, the first and second of these mechanisms fail to predict transient stomatal opening, and none of them correctly predicts observed responses to changes in water supply (e.g. increased xylem resistance, leaf excision or root pressurization and depressurization), which are qualitatively identical to humidity responses, including the transients (Fig. 1b) (e.g. Comstock & Mencuccini 1998). Furthermore, because these mechanisms are essentially passive, none explains why stomatal responses to humidity have similar kinetics to actively mediated light responses (Grantz & Zeiger 1986).

Other proposed mechanisms are based on active rather than passive amplification of guard cell turgor loss. For example, one hypothesis holds that abscisic acid (ABA) is continuously produced somewhere upstream of guard cells and delivered in the transpiration stream to guard cells, where it is continuously broken down, so that the ABA concentration at guard cells is proportional to the transpiration rate (Tardieu & Davies 1993). Because ABA closes stomata, this mechanism would create a negative feedback response to the transpiration rate. However, like the passive mechanisms described earlier, it fails

to predict stomatal closure in response to short-term changes in reduced water supply.

Another hypothesis, the hydroactive feedback hypothesis (HFH), holds that guard cell osmotic pressure is actively regulated in response to leaf water potential or turgor, rather than humidity or transpiration rate *per se*. Because the change in water status precedes and induces the active response, the latter lags behind the former, producing the transient opening characteristic of stomatal responses to short-term hydraulic perturbations (Figs. 1 and 2). The HFH predicts all the features of stomatal control described earlier (Buckley 2005) and is consistent with data showing a marked reduction in guard cell osmotic content during humidity responses (e.g. Bauer *et al.* 2013, Losch & Schenk 1978). In the HFH, the line between ‘hydraulic’ and ‘chemical’ signals is blurred because even responses to hydraulic signals are actively mediated and thus essentially biochemical in nature. The Achilles heel of the HFH has always been its speculative nature: it requires the existence of a yet-undiscovered signal that transduces changes in leaf water status into actively mediated changes in guard cell osmotic content. Root-derived ABA signals cannot provide the missing signal, because they generally do not reach guard cells quickly enough to explain humidity responses and also because any fundamental role for root signals in stomatal function is questioned by the fact that stomata behave normally in shoots grafted onto rootstock that cannot produce ABA (e.g. Holbrook *et al.* 2002).

McAdam *et al.* (2015) fill this gap and complete the HFH by unequivocally demonstrating a viable mechanism for hydroactive feedback: rapid, *de novo* synthesis of ABA within the leaf following exposure to dry air. These authors found that the rate-controlling enzyme for ABA biosynthesis is up-regulated 14-fold in angiosperm leaves within 20 min of doubling the evaporative gradient and that leaf ABA levels increase accordingly, leading to stomatal closure. This demonstrates an ultimate mechanism (gene regulation), the resulting intermediate signal (ABA) and the proximate effect (stomatal closure), and it does so in wild-type leaves of three species, on timescales relevant to normal humidity responses. This result is all the more incisive because it is not based on comparisons between wild-type and mutant genotypes. Such comparisons are often ambiguous because other, unobserved aspects of leaf physiology may adjust to restore wild-type function in mutants; for example, mutants with impaired capability for ABA biosynthesis exhibit strongly elevated activity of ABA release from conjugated pools compared with wild-type genotypes (Bauer *et al.* 2013). The results of McAdam *et al.* (2015) also dovetail with previous work from the same group, which found that stomata in seedless vascular plants are insensitive to ABA but that because they lack an epidermal mechanical advantage, they do not require an active mechanism to amplify passive

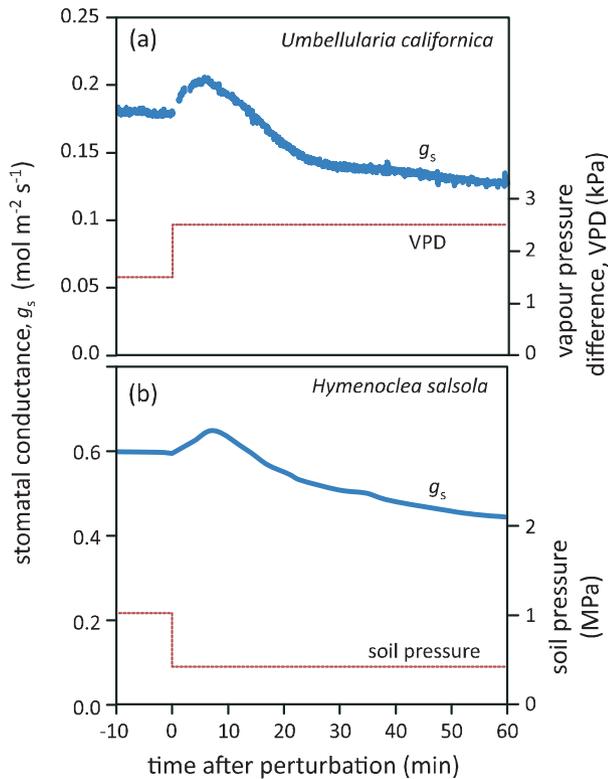


Figure 1. Stomatal responses are similar whether leaf water status is altered via evaporative demand (a) or water supply (b). Data in (b) are reproduced with permission from Comstock and Mencuccini (1998); raw data in (a) are unpublished data from the author's laboratory.

changes in guard cell turgor following hydraulic perturbations (e.g. Brodribb & McAdam 2011).

The sensitivity and timing of hydroactive feedback should depend on the magnitude of changes in water potential, which in turn varies among leaf tissues and especially with distance from minor veins (Mott & Franks 2001). It is thus important to identify exactly where water status is sensed in the leaf. Bauer *et al.* (2013) suggest guard cells themselves are the sensor, although given their small size, this would not explain elevation of whole-leaf ABA levels during humidity responses (McAdam *et al.* 2015) nor the fact that elevation of ABA in the epidermis appears to lag behind that in the bulk leaf (McAdam & Brodribb 2015). Perhaps the guard-cell-endogenous response is initiated by ABA from another source; indeed, exogenous ABA stimulates guard cell ABA biosynthesis in *Arabidopsis* (Bauer *et al.* 2013). It is also unknown whether ABA synthesis is activated by water potential *per se* or instead by osmotic pressure or turgor pressure, although of these three, only turgor pressure would correctly predict both responses to hydraulic perturbations and the effects of osmotic adjustment (gradual accumulation of osmotic solutes during drought), which sustains both turgor and stomatal conductance but not water potential. Clarity on these questions and precise characterization of the feedback response across species will help inform modelling efforts.

The work by McAdam *et al.* (2015) points the way to informed exploration and manipulation of differences in

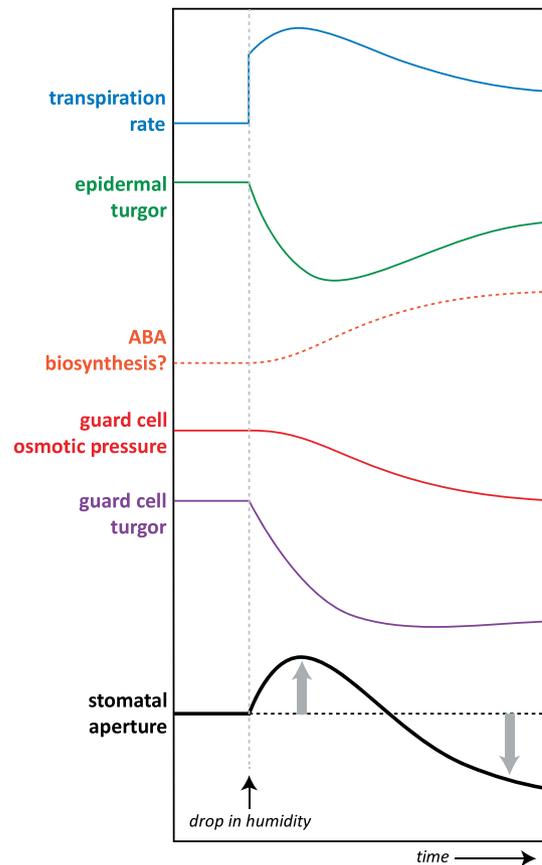


Figure 2. Mechanism of stomatal responses to humidity according to the hydroactive feedback hypothesis. Increased water loss initially reduces epidermal and guard cell turgor by similar amounts, causing transient stomatal opening. Reduced water status then causes biosynthesis of abscisic acid (ABA), which induces solute loss from guard cells, amplifying their turgor loss and causing stomatal closure.

stomatal sensitivity to perturbations of the soil–plant–atmosphere hydraulic continuum, and it provides a rare but valuable direct link between molecular physiology and integrated functioning of the intact leaf in wild-type plants. By moving the HFH a large step closer to the status of theory rather than hypothesis, this result offers hope that we will soon, and finally, have a non-speculative mechanistic model for stomatal behaviour.

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