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Review

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How do stomata respond to water status?

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Summary

Stomatal responses to humidity, soil moisture and other factors that influence plant water status are critical drivers of photosynthesis, productivity, water yield, ecohydrology and climate forcing, yet we still lack a thorough mechanistic understanding of these responses. Here I review historical and recent advances in stomatal water relations. Clear evidence now implicates a metabolically mediated response to leaf water status ('hydroactive feedback') in stomatal responses to evaporative demand and soil drought, possibly involving abscisic acid production in leaves. Other hypothetical mechanisms involving vapor and heat transport within leaves may contribute to humidity, light and temperature responses, but require further theoretical clarification and experimental validation. Variation and dynamics in hydraulic conductance, particularly within leaves, may contribute to water status responses. Continuing research to fully resolve mechanisms of stomatal responses to water status should focus on several areas: validating and quantifying the mechanism of leaf-based hydroactive feedback, identifying where in leaves water status is actively sensed, clarifying the role of leaf vapor and energy transport in humidity and temperature responses, and verifying foundational but minimally replicated results of stomatal hydromechanics across species. Clarity on these matters promises to deliver modelers with a tractable and reliable mechanistic model of stomatal responses to water status.

I. Introduction

Stomata control CO_2 and H_2O exchange between land plants and the atmosphere. Stomatal regulation impacts productivity and growth in both natural systems (Hinckley *et al.*, 1980; Reichstein *et al.*, 2002; Guarín & Taylor, 2005; McDowell, 2011; Choat *et al.*, 2012; Pfautsch & Adams, 2012) and agricultural systems (Fischer *et al.*, 1998; Davies *et al.*, 2002; Blum, 2009; Stewart *et al.*, 2011), drives ecological divergence of species (Anderegg *et al.*, 2016; Adams *et al.*, 2017; Martin-StPaul *et al.*, 2017) and mediates climate feedbacks (Bonan, 2008; Boucher *et al.*, 2009; Cao *et al.*, 2010; de Boer *et al.*, 2011). Despite the obvious importance of stomata for terrestrial plant functioning (Hetherington & Woodward, 2003; Berry *et al.*, 2010), basic questions remain about the

mechanisms by which stomata respond to environmental factors that influence leaf water status, such as humidity and soil moisture. The most simple and intuitively obvious conceptual model of this relationship – that water status controls stomatal conductance (g_{sw}) passively, simply by inflating and deflating stomatal guard cells in relation to the prevailing water potential of the leaf, and thus opening and closing the stomatal pore – is fundamentally incorrect because it ignores the opposing effect of adjacent epidermal cells. In angiosperms, passive inflation of epidermal cells at high water potential pushes back on guard cells, causing stomata to close rather than open. In order for high water potential to open stomata, the epidermal effect must be overcome. One likely mechanism to achieve this is 'hydroactive feedback': the active regulation of guard cell osmotic pressure in relation to leaf water status, triggered by a feedback response to changes in cell turgor or water content somewhere within the leaf. The hydroactive feedback hypothesis parsimoniously unifies stomatal responses to any factor that influences leaf water potential, including changes in humidity, soil moisture and plant water transport, under the umbrella of a single mechanism (Buckley, 2005). The signaling mechanism(s) involved in hydroactive feedback are a subject of ongoing debate, and are one focus of this review.

Stomatal regulation consists of much more than guard cell signaling, however. It also involves tissue- and leaf-scale biophysical factors that translate guard cell function into changes in stomatal conductance (Fig. 1). For example, the water potential of guard cells may be affected by vapor exchange with relatively dry air within the stomatal pore channel (Peak & Mott, 2011), or with relatively moist air in the airspaces between sun-warmed mesophyll cells (Pieruschka *et al.*, 2010). Water status may be actively sensed in guard cells (Bauer *et al.*, 2013), or in other tissues such as mesophyll (McAdam & Brodribb, 2018) or phloem companion cells (Endo *et al.*, 2008), which experience different degrees of water stress. Understanding of stomatal function in intact leaves thus rests not only on guard cell biology, but also on features of leaf and plant biophysics such as fine-scale gradients in temperature and water potential. Some of those features are poorly resolved. Some vary widely across taxa, and may therefore drive diversity in the ecophysiology of gas exchange and stress resilience. My objective here is to stimulate and focus progress on these issues. I begin by summarizing general features of plant water relations and stomatal function, in the context of long-standing theories of how stomata respond to humidity and drought (Section II). Then I discuss recent work and new ideas bearing on our understanding of how stomata in intact leaves respond to changes in soil moisture, evaporative demand, humidity, temperature and water transport (Section III). Finally, I identify several pathways for continuing research that are critical to enabling confident, mechanistic understanding of stomatal responses to water status in intact leaves (Section IV).

II. Background: stomatal water relations

1. What is water status, and what controls it?

Water moves through plants from a moist substrate to a much drier atmosphere. Even at soil moisture thresholds causing plant death, soil is still usually far 'wetter' than the atmosphere, as gauged by water potential (ψ). For example, few plants can survive in soil with a water potential of −3 MPa (−30 bars), yet such soil would still lose water to an atmosphere with 97% relative humidity. This disequilibrium drives water movement and generates gradients in water potential, both among and within plant organs. During steady-state transpiration, water potential at any given point in the plant, ψ_j, is given by

$$\psi_j = \psi_{soil} - r_{sj} E, \quad \text{Eqn 1}$$

(ψ_{soil}, soil water potential; E, transpiration rate of all tissues distal to the point in question; r_{sj}, effective water transport resistance of the pathways proximal to the point in question). Three salient points arise from Eqn 1. First, three factors – soil water potential, transport resistance and transpiration rate – determine the water status of a given tissue. Second, the direct, immediate effect of a decline in soil water potential is an equal decline in water potential at all points throughout the plant. Third, the immediate effect of an increase in water loss is to reduce water potentials throughout the plant, but not uniformly – rather, in proportion to the hydraulic 'distance' (as gauged by r_{sj}) of each tissue from the soil. For instance, soil drought would initially reduce ψ by the same amount in roots and leaves, but increased evaporative gradient would reduce water potential much more steeply in leaves than in roots.

Effects of soil and atmospheric drought are further mediated by five important factors that are not explicit in Eqn 1, but which affect the variables therein. First, stomatal conductance decreases during drought as a result of negative feedbacks that are the focus of this review. Second, stomatal closure reduces evaporative cooling, which can warm the leaf, increasing humidity in the leaf intercellular airspaces and thereby enhancing evaporative demand. Third, transport resistance often increases during soil or atmospheric drought, due to the formation of gas bubbles (emboli) in xylem conduits (Sperry, 2000); this exaggerates any initial decline in water potential caused by drought, but only for tissues downstream from (distal to) the point where resistance has increased. For example, increased root resistance would reduce ψ throughout the plant, whereas increased resistance in the leaf xylem would reduce ψ only in the leaf mesophyll and epidermis. Fourth, vapor transport through the leaf airspaces can be driven by small temperature gradients, independent of water potential, which means that water movement and, hence, water potential of tissues outside the xylem are sensitive to variables that influence temperature gradients, such as light absorption, leaf thickness and airspace fraction (Buckley *et al.*, 2017). Fifth, because stomata may not sense water potential *per se*, but instead a closely related variable such as turgor or cell volume (Sack *et al.*, 2018), I use the more general term 'water status' in this review.

2. Stomatal hydromechanics

Stomatal aperture (a_s) is determined by the displacement of stomatal guard cell walls adjacent to the stomatal pore (the 'ventral' walls). That displacement is caused by deformation of guard cells due to volume changes, but in most species it is counteracted to a

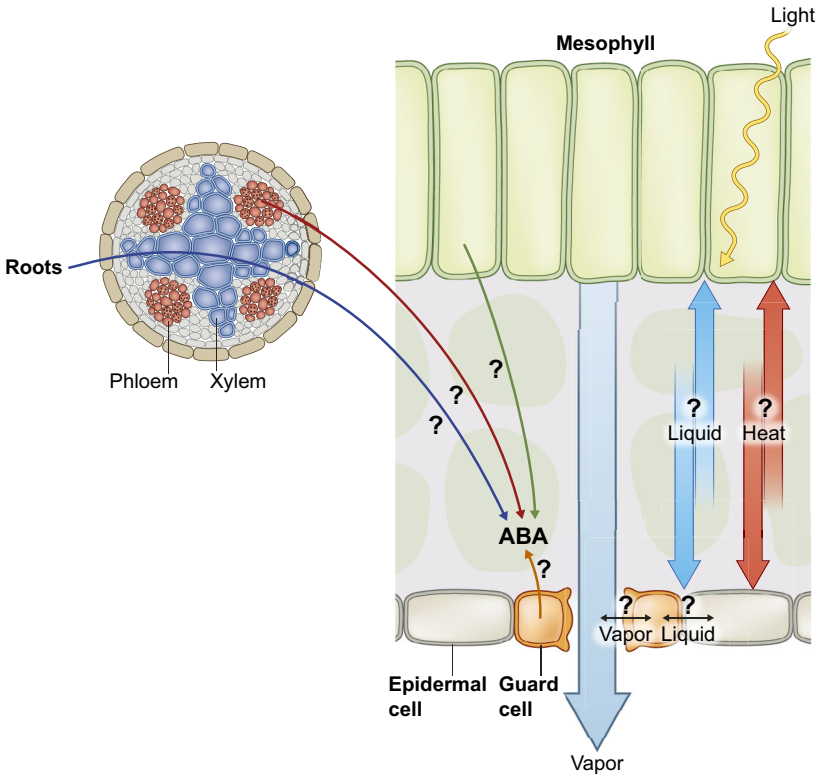

Fig. 1 Stomatal conductance is regulated not only by guard cell biology, which governs guard cell osmotic content, but also by numerous biophysical factors that influence guard and epidermal cell water potentials and link these cells to other tissues across the leaf and plant. Abscisic acid and other signaling compounds may be synthesized in guard cells or synthesized elsewhere and transported to guard cells (Sections III.1, III.2), but may have little impact on stomata in some species (Section III.3). The potential ABA source tissues are located at different positions along the soil–plant–atmosphere continuum, and are thus differentially sensitive to soil drought and evaporative demand (Section III.5). Guard cells may or may not exchange vapor with air in the stomatal pore channel and liquid water with epidermal cells (Section III.4), and heat and liquid water may move in either direction between the mesophyll and epidermis (Sections III.4, III.5).

degree by volume changes in the adjacent epidermal or subsidiary cells. Thus, aperture is related positively to guard cell turgor pressure ($a_s \propto P_g$) and negatively to epidermal turgor ($a_s \propto -P_e$). Direct measurements of the relationships among a_s, P_g and P_e indicate that, in angiosperms, the epidermis has a 'mechanical advantage' over guard cells in determining aperture (Glinka, 1971; DeMichele & Sharpe, 1973; Sharpe *et al.*, 1987; Franks *et al.*, 1995, 1998). That is,

$$m \equiv -\frac{\partial a_s/\partial P_e}{\partial a_s/\partial P_g} > 1, \qquad \text{Eqn 2}$$

where m is the *epidermal mechanical advantage*. The fact that $m > 1$ creates a puzzle: although stomatal apertures are observed to decline when leaf water status declines – for instance, following an increase in transpiration rate or a decrease in source water potential or hydraulic conductance – $m > 1$ implies that stomata should instead *open* in such instances, unless the epidermis is flaccid ($P_e = 0$) (Fig. 2). Both of these predictions – stomatal closure and stomatal opening – are in fact correct: following an increase in water loss or a decrease in water supply, stomata transiently 'pop open' before eventually closing (e.g. Fig. 3). The transient opening is called the 'wrong-way response' (WWR) and the subsequent steady-state closure is called the 'right-way response' (RWR) (the WWR is 'wrong' in adaptive terms: it amplifies any decline in leaf water status). The biphasic WWR/RWR response is observed in angiosperms following any hydraulic perturbation, including a change in evaporative demand (Mott *et al.*, 1997), source water potential (Comstock & Mencuccini, 1998), hydraulic conductance (Saliendra *et al.*, 1995) or leaf excision (Powles *et al.*, 2006). An exception is seedless plants, which appear to lack a WWR and exhibit only a rapid RWR (Lange *et al.*, 1971; Lösch, 1977, 1979; Brodribb & McAdam, 2011). The duration of the WWR varies widely across taxa, from *c.* 2 to 20 min (Buckley *et al.*, 2011). The increase in water loss during the WWR transiently amplifies any initial decline in water potential, as well as any process influenced by water potential (e.g. xylem cavitation or ABA synthesis), although such amplification has not been examined, to my knowledge. WWRs are of interest here mainly because they offer insight about response mechanisms: the WWR is easily explained by 'hydropassive' stomatal function, given $m > 1$, whereas the RWR is not.

3. Hypothesized mechanisms for stomatal responses to water status

Stomata respond to seemingly any environmental perturbation that changes water potential in the plant, with a steady-state response that tends to partially reverse the initial change in water potential. For instance, if increased transpiration reduces leaf water potential, stomata partially close, making the net change in water potential smaller than it would otherwise have been. Two responses have drawn most attention: the response to soil drought, or reduced soil water potential, and the response to atmospheric drought, or reduced air humidity. Historically, two opposing hypotheses have been advanced to explain the steady-state stomatal closure in dry air. One hypothesis assumes that guard cells are hydraulically distal to epidermal cells in the transpiration stream – that is, the total hydraulic resistance from the soil to the guard cells is greater than that from the soil to the epidermis – so that a drop in humidity causes a larger drop or 'drawdown' in water potential in guard cells

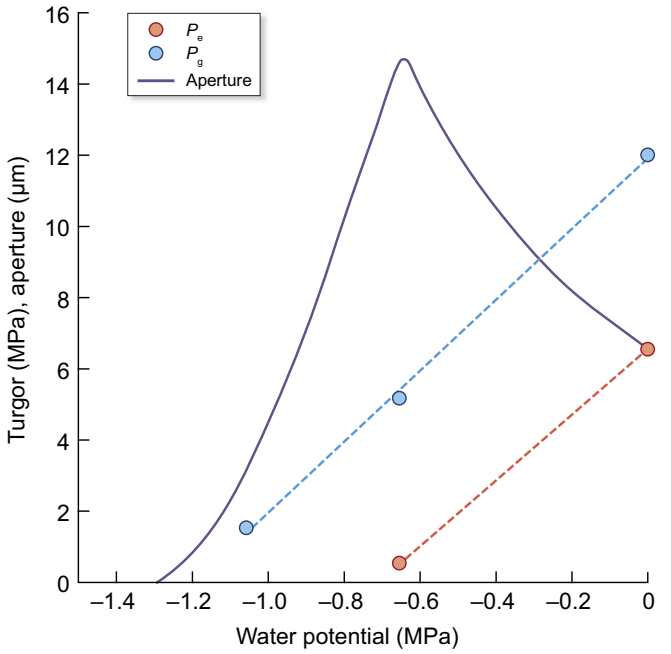

Fig. 2 Effect of uniform changes in water potential in epidermal and guard cells on epidermal turgor, P_e (red circles and line), guard cell turgor, P_g (blue circles and line) and stomatal aperture (solid purple line) observed by Glinka (1971).

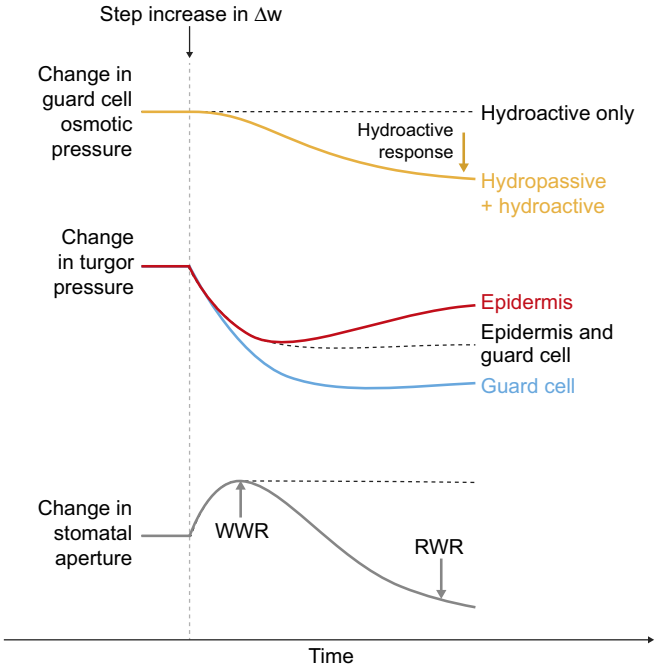

Fig. 3 Illustration of dynamics of stomatal water relations following a step increase in Δw in the absence ('hydropassive'; dashed lines), or in the presence of hydroactive feedback (solid lines). Without a hydroactive response, epidermal and guard cell turgor decline by similar amounts, and stomatal aperture increases and remains elevated (dashed lines; wrong-way response, WWR). If instead guard cell (GC) osmotic pressure is actively reduced by a feedback response to water status, this amplifies the decline in guard cell turgor (blue line), causing a net reduction in stomatal aperture (solid black line, right-way response, RWR). Stomatal closure reduces water loss compared to the hydropassive case, partially mitigating the decline in epidermal turgor (red line).

than in epidermal cells (Farquhar, 1978; Maier-Maercker, 1983; Dewar, 1995). The other hypothesis assumes that guard cells 'hydroactively' release osmotic solutes in response to a drop in water status, much as they actively regulate solute content in response to other environmental factors like light and CO_2 (Darwin, 1898; Stålfelt, 1929; Meidner, 1986; Buckley, 2005). In both hypothesized mechanisms, the epidermal mechanical advantage (Eqn 2) is overcome by a disproportionate drop in guard cell turgor – this is caused by a disproportionate decline in guard cell water potential in the first hypothesis, or in guard cell osmotic pressure in the second hypothesis.

The drawdown hypothesis has several weaknesses. If $m \approx 2$ as indicated by pressure probe data (Franks *et al.*, 1995, 1998), then the resistance between epidermal and guard cells – a pathway spanning mere micrometers and one cell–cell interface – must be at least as large as the resistance from the soil to the epidermis. This seems unlikely given high aquaporin expression (Kaldenhoff *et al.*, 1995; Sun *et al.*, 2001), high water permeability (Grondin *et al.*, 2015) and rapid osmotic water exchange (Shope & Mott, 2006) in guard cells. The epidermis-to-guard cell resistance also must change dynamically during the WWR and RWR to reconcile observed stomatal kinetics with observed pressure–aperture relationships (Buckley & Mott, 2002a). Furthermore, the biphasic WWR/RWR occurs not only in response to evaporative demand, but also in response to water supply, that is, changes in soil moisture, plant hydraulic conductance, or water potential in other regions of the same leaf (Saliendra *et al.*, 1995; Mott *et al.*, 1997; Comstock & Mencuccini, 1998; Buckley & Mott, 2000; Buckley, 2005). But because water supply perturbations affect guard and epidermal turgor equally, the drawdown hypothesis predicts only wrong-way responses to these perturbations.

The 'hydroactive feedback' hypothesis (HFH) parsimoniously explains both WWRs and RWRs, to both evaporative demand and water supply (Buckley, 2005). Moreover, spatially explicit models based on the HFH accurately predict how the behavior of neighboring stomata can become coordinated, producing 'patchy' stomatal conductance and influencing the kinetics of stomatal responses to light (Haefner *et al.*, 1997; Mott *et al.*, 1999; Mott & Buckley, 2000). Until recently, however, there was no established mechanism linking guard cell osmotic content to leaf water status, nor any direct evidence for hydroactive feedback except a single report showing that humidity responses were accompanied by solute loss from guard cells (Losch & Schenk, 1978). Several recent reports have overcome this challenge, by showing that abscisic acid (ABA), which is known to close stomata by inducing solute loss from guard cells, is rapidly synthesized *de novo* within leaves in response to reduced air humidity (Xie *et al.*, 2006; Bauer *et al.*, 2013; McAdam *et al.*, 2016b). Questions that remain concerning the HFH include uncertainty about where exactly water status is sensed, the role of ABA in water status responses, particularly in seedless plants, the possible role of water exchange between guard cells and dry air in the stomatal pore

channel, and how temperature gradients in the leaf and changes in hydraulic conductivity influence hydration of epidermal and guard cells. I address each of these issues in Section III.

II. New ideas and new evidence about stomatal responses to water status

1. Are stomatal responses to soil drought initiated in leaves or roots?

It has long been known that ABA is synthesized in dehydrating tissues (Loveys, 1977) and induces stomatal closure (Kriedemann *et al.*, 1972). For many years, it was thought that ABA generated in drying roots and transported in the xylem to leaves was primarily responsible for stomatal closure during soil drought. That idea arose from experiments in which roots were dehydrated but leaf water status was maintained, either by split-pot treatments or root pressurization, and stomatal closure was reported without any noticeable change having occurred in bulk leaf water potential, ψ_{leaf} (Gollan *et al.*, 1986; Khalil & Grace, 1993). Zhang & Davies (1991) showed further that xylem sap extracted from droughted maize plants caused stomatal closure, and that sap [ABA] predicted the degree of closure in the same fashion as exogenously supplied [ABA]. Borel *et al.* (2001) showed that root-derived ABA in droughted tobacco plants could close stomata in ABA-deficient shoots grafted onto normal rootstock. These results show that droughted roots can contribute ABA to xylem sap, and that ABA in xylem sap can close stomata.

More recent experimental evidence questions whether root-derived ABA is the sole cause, or even the primary cause, of stomatal closure in drying soil. Among plants created by reciprocal grafts of wild-type (WT) and ABA-deficient mutant scions and rootstock, drought responses are determined by the genotype of the scion and not the rootstock, in tomato (Holbrook *et al.*, 2002; McAdam *et al.*, 2016a), pea (McAdam *et al.*, 2016a), sunflower (Fambrini *et al.*, 1995) and Arabidopsis (Christmann *et al.*, 2005, 2007) (Fig. 4). In Arabidopsis seedlings, pressure-probe measurements of mesophyll turgor pressure show that changes in root system water potential are propagated to the leaf within 1–3 min, inducing a decline in stomatal aperture within 10 min and > 90% closure in 45 min (Christmann *et al.*, 2007). If leaf turgor decline is prevented by placing leaves in contact with a solution of high water potential, stomatal closure is prevented, despite persistent exposure of roots to low water potential (Christmann *et al.*, 2007). When leaves and roots of Arabidopsis are isolated from one another and subjected to water stress, ABA concentrations rise greatly in the former but not the latter (Ikegami *et al.*, 2009). Much of the ABA present in roots may in fact originate in leaves (Ikegami *et al.*, 2009; McAdam *et al.*, 2016a; Castro *et al.*, 2019), and ABA synthesis in roots may require precursors transported from the leaves (Ren *et al.*, 2006; Manzi *et al.*, 2015; Zhang *et al.*, 2018). Together, these results suggest that strong stomatal responses to changes in water supply originate primarily in leaves, not in roots.

Other long-distance signaling mechanisms also may contribute to drought responses, although their putative roles remain unresolved. Holbrook *et al.* (2002) reported stomatal closure during soil drying in grafted tomato plants even when shoot water status was maintained by pressurizing roots, and regardless of the scion or rootstock genotype, from which they inferred the existence of a root signal other than ABA. Wilkinson & Davies (1997) showed that xylem sap pH increased during drought, and that increased sap pH caused stomatal closure regardless of drought – perhaps by helping to sequester leaf ABA in the apoplast due to its behavior as a weak acid. In tomato, Visentin *et al.* (2016) reported stomatal closure and enhanced ABA sensitivity in both droughted WT plants and irrigated plants with rootstocks deficient in strigolactone synthesis, leading the authors to speculate that reduced export of strigolactones from roots may 'prime' leaves to be more ABA sensitive in drought. As noted by Tardieu (2016), the effect of any given chemical messenger, including ABA, often varies across species and timescales, so in order to educe a universal model of root-to-shoot signaling, we must accept a 'diversity of hypotheses' that may seem mutually incompatible in the narrow context of a single experimental system.

When interpreting evidence for root–shoot interactions, it is important to recognize that near-invariance in leaf water potential, as reported in split-pot experiments, is entirely consistent with a feedback response to leaf water status. The first effect of reduced soil water potential – before stomata have responded in any way – is an equal decline in water potential throughout the plant, including in the leaves (Eqn 1). This occurs within minutes (Saliendra *et al.*, 1995; Christmann *et al.*, 2007), which is far faster than ABA can be synthesized in roots, let alone transported to leaves. The fact that observed leaf water potentials often do not decline measurably only shows that stomata can respond quickly and sensitively enough to hydraulic signals to achieve near-homeostasis in leaf water potential. For instance, Saliendra *et al.* (1995) showed that stomatal closure following a drop in stem hydraulic conductance occurred

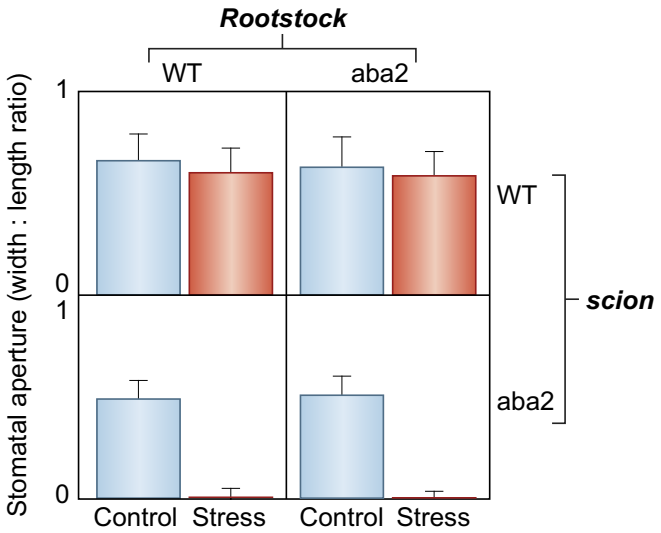

Fig. 4 Genotype of scions, not rootstocks, determines stomatal response to water stress (water potential of medium = 0 (blue bars; control) or −1.0 MPa (red bars; stress)) in grafted Arabidopsis plants. *y*-axes, stomatal aperture (expressed as ratio of pore width to length; range = 0–1 in all panels). Error bars are ± SE. WT, wild type; aba2, ABA-deficient mutants. Adapted from Christmann *et al*. (2007).

within minutes and was strong enough to prevent measurable decline in ψ_{leaf}.

2. Does leaf-endogenous ABA drive humidity responses?

Until fairly recently, there was no direct evidence that leaf ABA metabolism also was involved in the response to evaporative demand. Xie *et al.* (2006) showed that the stomatal response to reduced atmospheric humidity was weaker in Arabidopsis mutants ost1-4 (with a mis-sense mutation for a gene involved in ABA signaling) and aba2-13 (with an insertion mutation for a gene involved in ABA biosynthesis), clearly indicating a role for ABA in the humidity response. Several authors have since demonstrated rapid increases in leaf ABA in response to changes in humidity. In Arabidopsis, Ikegami *et al.* (2009) showed rapid activation of ABA synthesis in leaves but not roots of plants exposed to reduced humidity, and Okamoto *et al.* (2009) reported enhanced expression of enzymes involved in ABA catabolism, and reduced [ABA], in leaves exposed to high humidity. Bauer *et al.* (2013) reported ABA synthesis in Arabidopsis guard cells exposed to reduced humidity, and showed that guard cells expressed all of the genes required for both *de novo* synthesis and release from conjugated forms. McAdam *et al.* (2016b) reported that in WT genotypes of tomato, pea and Arabidopsis, reduced humidity caused expression of a key rate-limiting enzyme in ABA biosynthesis (9-cis-epoxycarotenoid dioxygenase or NCED), leading to rapid increase in leaf ABA concentrations (Fig. 5). That increase was not accompanied by a commensurate drop in concentrations of the conjugated storage form ABA-GE, which suggests that *de novo* synthesis rather than release from stores was the likely source of most of the increase in [ABA] in those experiments. The magnitude and timing of those processes were broadly consistent with subsequent stomatal closure responses. These authors also found that changes in leaf [ABA] and g_{sw} were not significant in ABA-deficient mutants (*wilty* of *P. sativum*, and *sitiens*, *flacca* and *notabilis* of *S. lycopersicum*) (Fig. 5). Similar results were reported for leaves forcibly dehydrated in a pressure chamber (McAdam & Brodribb, 2016; Sussmilch *et al.*, 2017), which suggests leaf ABA synthesis is driven by anything that reduces leaf water content (Sack *et al.*, 2018). Together these data suggest that *de novo* synthesis of ABA in leaves is the mechanism of hydroactive feedback responses to humidity in these species.

That conclusion contrasts with results of Merilo *et al.* (2018), who reported stomatal closure under reduced humidity in a range of ABA mutants, including *wilty* and *flacca*, as well as mutants of Arabidopsis lacking several steps in the ABA synthesis pathway. Rescuing ABA synthesis in guard cells or phloem companion cells restored WT phenotypes, including constitutively lower stomatal conductance. Merilo *et al.* (2018) suggested that ABA influences steady-state g_{sw}, independent of humidity, and hypothesized that the observed humidity responses were 'hydropassive'.

It is unclear how to reconcile these contrasting results. Perhaps ABA synthesis merely occurs at the same time as, but does not cause, humidity responses; however, that is not consistent with the fact that restoring ABA biosynthesis led to reduced g_{sw} in mutants, which implies that any enhancement of leaf ABA synthesis should reduce g_{sw}. Perhaps redundant mechanisms exist for hydroactive humidity responses, as is the case for light and CO_2 responses (Zeiger *et al.*, 2002; Messinger *et al.*, 2006; Lawson, 2009; Lawson *et al.*, 2018). Careful comparison of the phenomenology of humidity responses between mutant and WT plants could help distinguish redundant response mechanisms, given that redundant mechanisms are unlikely to produce quantitatively identical responses.

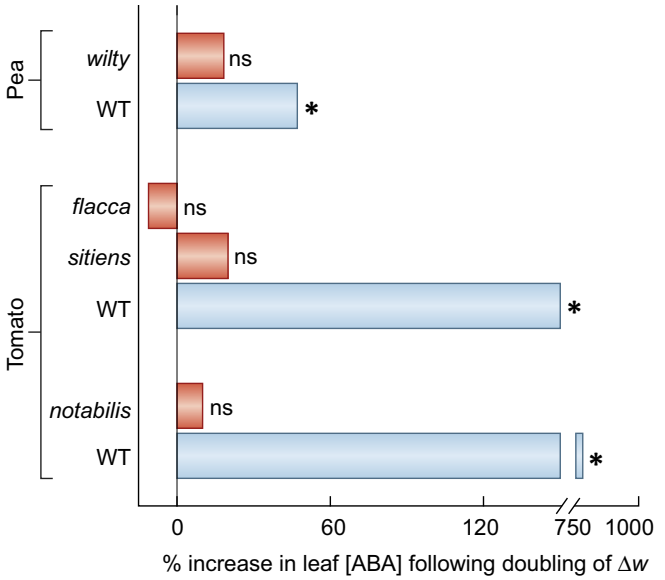

Fig. 5 A step increase in leaf to air water vapor mole fraction gradient (Δw) (from 7 to 15 mmol mol^{-1}) causes *de novo* synthesis of abscisic acid (ABA) in leaves, enhancing [ABA]. Adapted from McAdam *et al*. (2016b). ABA-deficient mutants (red bars; *wilty, flacca, sitiens* and *notabilis*) were compared with similar-background wild-type (WT) genotypes (blue bars) of pea (*Pisum sativum*) and tomato (*Solanum lycopersicum*). Statistical significance: *, $P < 0.05$; ns, not significant ($P > 0.05$).

The suggestion by Merilo *et al.* (2018) that the mutant responses are hydropassive deserves careful scrutiny. Because of the mechanical advantage of the epidermis, responses to humidity in angiosperms cannot be hydropassive unless either (1) guard cells are separated from epidermal cells by a large hydraulic resistance, or (2) the epidermis is constitutively flaccid, so that humidity does not influence P_e. Alternative (1) is the 'drawdown hypothesis' discussed in II.3, and it predicts stomatal opening rather than closure in response to reduced water supply. Alternative (2) is conceivable, given the constitutively high g_{sw} in ABA mutants, but has not been documented. These alternatives could be tested and distinguished by examining stomatal responses to leaf excision: (1) predicts a larger WWR to excision in mutants than in WT plants, because the RWR would only take effect once the epidermis has lost turgor, whereas (2) predicts no WWR at all, because a flaccid epidermis does not influence g_{sw}. Notably, (2) is consistent with the apparent absence of WWRs in most mutant lines (Merilo *et al.*, 2018). It also is noteworthy that a strong hydroactive feedback response to reduced humidity – a decline in guard cell osmotic concentration (in this case K^+) – was observed even in ABA-deficient mutants of Arabidopsis in the experiments of Bauer *et al.* (2013).

Assuming leaf-endogenous ABA drives hydroactive responses to humidity, it is important to determine where and how changes in water status are sensed and transduced into ABA production, and what determines rates of ABA catabolism. Several studies have demonstrated strong upregulation of ABA biosynthetic enzymes in response to water stress in leaf phloem parenchyma cells and stomatal guard cells (Koiwai *et al.*, 2004; Endo *et al.*, 2008; Kuromori *et al.*, 2014). Kuromori *et al.* (2014) suggested that ABA transported from a vascular synthesis site drove subsequent ABA synthesis in guard cells, consistent with Bauer *et al.* (2013)'s finding that exogenous ABA can stimulate ABA synthesis in guard cells. Endo *et al.* (2008) also reported activity of ABA synthesis genes in mesophyll cells, but to a smaller degree and after a longer lag than in vascular tissue. By contrast, McAdam & Brodribb (2018) partitioned leaves into segments dominated by vascular or mesophyll tissue, and found that dehydration generally led to a greater increase in [ABA] in the latter, suggesting a predominantly mesophyll site for ABA synthesis. The latter authors argued that phloem and guard cells are unlikely sites of water status sensing, because cell turgor and volume in both locations is actively regulated in relation to other factors, which should cause ABA concentrations to fluctuate dramatically over the day in ways that are not observed. For example, increased [CO_2] would reduce [ABA] (and hence increase g_{sw}) by enhancing photosynthesis and hence phloem loading and turgor. By contrast, both increased [CO_2] and reduced sink strength (which enhances phloem turgor in source leaves) tend to reduce g_{sw} (Nikinmaa *et al.*, 2013). A vascular site for water status sensing also would lead to much smaller stomatal responses to humidity than to source water status, for a given change in bulk leaf water status, because shifts in humidity cause much larger changes in water potential in the mesophyll than in the vasculature (due to hydraulic resistance distal to the xylem; Scoffoni *et al.*, 2017). The role of ABA catabolism in stomatal responses to humidity is poorly known, except that both short-term exposure to high humidity (Okamoto *et al.*, 2009) and growth in sustained high humidity stimulate ABA catabolism (Arve *et al.*, 2015).

3. Are stomatal responses to water status passive in seedless plants?

It is currently unresolved whether, as in seed plants, ABA also drives water status responses in seedless plants. Some evidence suggests that active responses are not necessary in seedless plants because their epidermis lacks a mechanical advantage. For example, a few early studies (Lange *et al.*, 1971; Lösch, 1977, 1979) reported that ferns lacked a WWR to humidity; however, those measurements were performed in epidermal peels, in which the mechanical advantage might have been eliminated by breakage of epidermal cells during peeling. Franks & Farquhar (2007), however, verified that the epidermis had little effect on stomatal aperture in the lycopod *Huperzia prolifera* and the fern *Nephrolepis exaltata*, and Brodribb & McAdam (2011) demonstrated the functional corollary that WWRs were absent in seedless plants (including three lycophytes and eight ferns) following either reduced humidity or leaf excision. The latter authors also reported that seedless plants were insensitive to ABA (Brodribb & McAdam, 2011; McAdam & Brodribb, 2012b). Together, this evidence suggests that the short-term hydroactive feedback response to leaf water status is weak or absent in seedless plants, and is stronger in angiosperms due to the need to overcome a greater mechanical advantage (Franks, 2013). Brodribb & McAdam (2011) speculated further that stomatal sensitivity to ABA evolved in angiosperms for this reason. That hypothesis appears difficult to reconcile with other evidence. For example, of the genetic machinery needed for ABA signaling and biosynthesis is present in most ferns (Cai *et al.*, 2017) and at least some mosses (Chater *et al.*, 2011; Lind *et al.*, 2015), and other authors have shown that exogenous ABA can close stomata in seedless plants (Ruszala *et al.*, 2011; Hõrak *et al.*, 2017). Yet there is a distinction between the potential, in principle, for ABA to influence stomata in seedless plants, and the realized functional significance of ABA responses *in vivo*. Three salient questions arise:

1 **How much of the observed stomatal response to water status in seedless plants is accounted for by the hydropassive mechanism**? The evidence is mixed on this point. Hõrak *et al.* (2017) found that the half-time for stomatal closure in low humidity was larger (i.e. responses were slower) in leaves of the fern *Athyrium filix-femina* in which stomatal conductance had been increased by exposure to low [CO_2]; a strictly passive mechanism would predict the opposite, because increased conductance should lead to faster water loss and thus faster hydropassive changes in guard cell turgor. By contrast, Cardoso *et al.* (2019) found that a passive model based on leaf hydraulic resistance and capacitance (which control the kinetics of passive changes in guard cell volume) in *A. filix-femina* could accurately predict humidity responses in both ambient and reduced [CO_2]. Further close examination of the dynamic humidity response across seedless taxa is needed to resolve the matter.
2 **Are stomata in seedless plants and seed plants equally sensitive to exogenous ABA**? The data are mixed on this point as well. 10 μM ABA reduced stomatal conductance by 8–24% in three fern species (*A. filix-feminata, Dryopteris carthusiana* and *D. filix-max*) grown in growth cabinets, but had little effect (ranging from a 14% decrease to a 6% increase in conductance) when the same species were grown in growth rooms (Hõrak *et al.*, 2017). By comparison, the same treatments reduced conductance by 43–56% in Arabidopsis. 50 μM ABA reduced apertures by 21% in the fern *Polystichum proliferum* and 23% in *Nephrolepis exaltata* after an hour of exposure; 200 μM ABA was required to reduce apertures by 32% and 45%, respectively (Cai *et al.*, 2017). In epidermal peels of the lycophyte *Selaginella uncinata*, 25 μM ABA inhibited light-induced stomatal opening by 50% and reduced previously opened apertures by 16%; in intact *Selaginella* leaves, 1000 μM ABA reduced stomatal conductance by 15%. Grantz *et al.* (2019) found that up to 100 μM ABA reduced steady-state stomatal conductance by 18–24% in *N. exaltata* (although the effect was statistically insignificant at the $P = 0.05$ level). By contrast, a literature survey of 15 angiosperm species by Cardoso *et al.* (2019) found that 10 μM ABA reduced stomatal opening by 33–100% (median 66%). Thus, although seedless plants are responsive to

exogenous ABA, it appears that they typically require higher ABA concentrations than seed plants to close stomata.

3 **Are observed stomatal responses to water status in seedless plants consistent with a dominant role for endogenous ABA?** Few studies have examined this question directly, but the few that have suggest endogenous ABA does not drive stomatal responses to water status in seedless plants. Leaf ABA concentrations do not appear to increase in response to reduced humidity (McAdam & Brodribb, 2015). Dehydration does lead to increased ABA concentrations in fern and lycophyte leaves, but when these leaves are rehydrated, stomata open despite the presence of high concentrations of endogenous ABA (McAdam & Brodribb, 2012a); by contrast, in angiosperms, ABA produced during water stress or low humidity causes hysteresis in stomatal responses (McAdam & Brodribb, 2015).

In summary, it appears that seedless plants can synthesize and respond to ABA, but these responses are weaker than in seed plants, and the available evidence suggests that endogenous ABA may play little role in stomatal responses to water status in intact seedless plants. To test the generality of this conclusion, additional research should focus on comparing endogenous and physiologically active concentrations of ABA across diverse seedless plant taxa, and performing more extensive analyses of the kinetics of water status responses, as have Hõrak *et al.* (2017) and Cardoso *et al.* (2019).

It also is important to clarify here the distinction between hydroactive responses to water status, which are the focus of this section, and other 'hydroactive' stomatal responses (i.e. responses in which guard cell volume and hence stomatal aperture are driven by actively mediated changes in guard cell osmotic content). Seedless plants clearly possess actively mediated responses to light (Doi *et al.*, 2015), CO_2 (Franks & Britton-Harper, 2016), and drought and high-light stress (Zhao *et al.*, 2019), although the generality of active stomatal movements in some bryophytes remains unclear (Renzaglia *et al.*, 2017; Duckett & Pressel, 2018; Duckett *et al.*, 2018).

4. Do stomata sense humidity via the air in the pore channel?

A seminal experiment by Mott & Parkhurst (1991), in which humidity and transpiration rate were decoupled by replacing N_2 with helium in air (which speeds up vapor diffusion), was thought to have shown that stomata respond not to ambient humidity *per se*, but rather to the rate of leaf water loss. Other evidence suggests that stomatal responses to humidity and Δw (the leaf to air water vapor mole fraction gradient) involve other processes, however. First, transpiration rate sometimes declines at high Δw. This is called the 'feedforward' response because it is superficially incompatible with a feedback response of g_{sw} to ψ_{leaf} (declining E should increase ψ_{leaf}, whereas a feedback response can, at best, produce near homeostasis in ψ_{leaf}). Second, a few published reports (Hall & Kaufmann, 1975; Hall *et al.*, 1975; Ball *et al.*, 1987; Fredeen & Sage, 1999; Mott & Peak, 2010) suggest that stomata open in response to leaf warming when Δw is held constant, but only Δw is nonzero (Mott & Peak, 2010). Third, stomatal conductance and epidermal turgor on one surface of an amphistomatous leaf are minimally affected when Δw is changed at the opposite leaf surface (Mott, 2007), which suggests that the Δw response is mediated by a very localized mechanism.

These observations led Peak & Mott (2011) to propose a new model for stomatal function (hereafter the PM model), based on three hypotheses: (1) guard cells are hydraulically isolated from other leaf tissues but in equilibrium with the air in the stomatal pore channel; (2) mesophyll evaporating sites cool, relative to the guard cells and epidermis, in proportion to transpiration rate; and (3) the epidermis is in water potential equilibrium with the mesophyll evaporating sites. Because the humidity in the pore channel is poised between ambient and intercellular values, a drop in ambient humidity reduces the pore-channel humidity, which in turn causes a large drop in guard cell water potential (e.g. by 1.4 MPa for a 1% drop in relative humidity). Thus, Hypothesis (1) predicts stomatal closure in dry air. It also predicts a feedforward response and explains highly localized responses to humidity, because pore-channel humidity affects only guard cells. Hypotheses (2) and (3) predict stomatal closure in response to increasing water loss, because latent cooling at the evaporating sites reduces intercellular humidity, and with it, pore-channel humidity. Hypotheses (1) and (2) predict stomatal opening as the leaf warms at constant Δw, because in order to keep Δw constant as temperature rises, ambient humidity must increase, which increases pore-channel humidity.

Despite these successes, the PM model has some difficulties. It predicts only wrong-way responses to source water status and plant hydraulic conductance. It also contains a contradiction: Hypotheses (2) and (3) together imply vapor concentration is lower in the mesophyll than near the epidermis, which would drive net vapor diffusion into the leaf – causing condensation in the mesophyll (contradicting Hypothesis (2)) and requiring liquid flow from the mesophyll to the epidermis (contradicting Hypothesis (3)) (see Supporting Information Notes S1) (Fig. 6). Hypothesis (2) also contradicts physical models of heat, liquid and vapor transport in leaves, which predict that the epidermis is cooler than the mesophyll and that more evaporation usually occurs from the epidermis than from the mesophyll (Rockwell *et al.*, 2014; Buckley *et al.*, 2017) (Fig. 7). Hypothesis (1) assumes the guard cell membrane and cell wall are impervious to water everywhere except adjacent to the pore, which is difficult to reconcile with evidence that guard cell membrane water permeability is large and similar to that of other leaf cells (Grondin *et al.*, 2015).

Some of the observations cited by Peak & Mott (2011) also can be explained by known phenomena. For example, the feedforward response can arise from anything that hysteretically amplifies stomatal closure at high Δw, such as a decline in hydraulic conductance (Oren *et al.*, 1999; Buckley & Mott, 2002b) or slow catabolism of accumulated ABA. A transpiration-dependent temperature response could reflect a temperature dependency of water transport or ABA metabolism, and a very localized response to Δw at one leaf surface is consistent with simulations of water potential gradients within leaves (Buckley *et al.*, 2015).

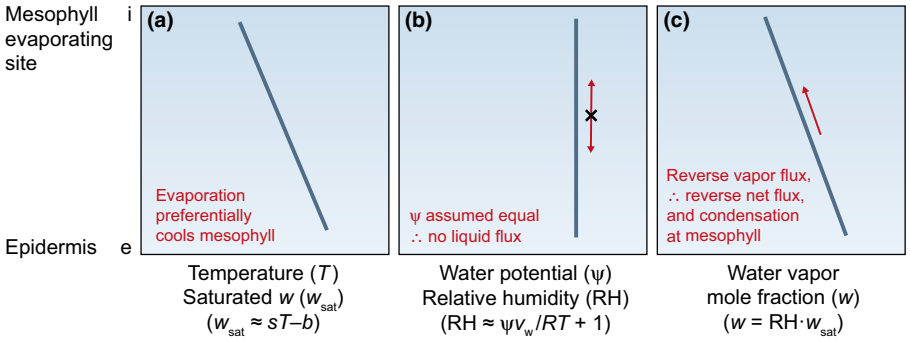

Fig. 6 Illustration of the contradiction in the model of Peak & Mott (2011). The model assumes that evaporation cools an evaporating site in the mesophyll (denoted 'i') relative to the epidermis ('e'), which means the saturation water vapor mole fraction, w_{sat}, is lower in the mesophyll airspaces than near the epidermis (a). The model also assumes that the mesophyll evaporating site and epidermis are at equal water potential (ψ), and therefore do not exchange liquid water (b). Those two assumptions together imply that water vapor mole fraction (w) is lower in the mesophyll airspaces than near the epidermis (c). That would drive net vapor flux from the epidermis to the mesophyll, which in turn would require liquid flux in the opposite direction to sustain mass balance (more so if transpiration rate is significant), contradicting the assumption of equal water potentials. In (a), s denotes the sensitivity of w_{sat} to temperature, and b denotes minus the intercept of the tangent line to the relationship of w_{sat} vs T. In (b), v_w is the molar volume of liquid water and R is the gas constant.

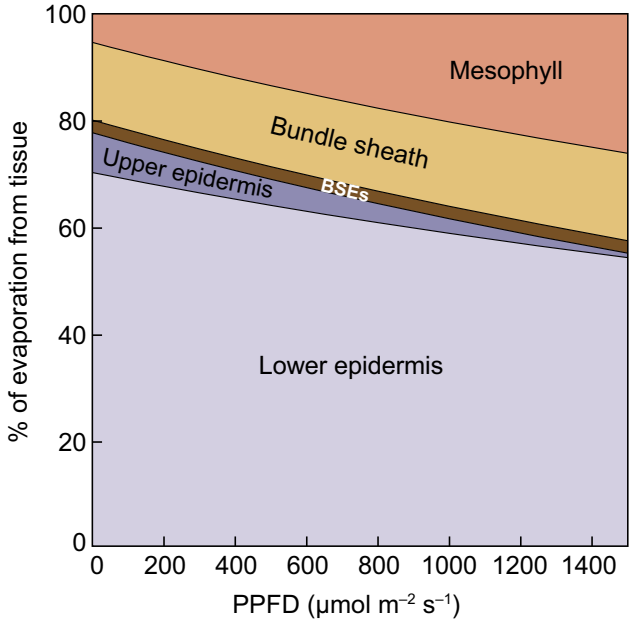

Fig. 7 More evaporation within hypostomatous leaves is predicted to occur from the abaxial (lower) epidermis than from the mesophyll, although the epidermal fraction of evaporation declines as photosynthetic photon flux density (PPFD) increases. Simulations from MOFLO 2.0 model, using average anatomical parameter values for 14 broadleaf angiosperm species; adapted from Buckley *et al*. (2017) with permission (© American Society of Plant Biologists, www.plantphysiol.org). BSEs, bundle sheath extensions.

5. How do changes in water transport drive stomatal movements?

Saliendra *et al.* (1995) showed that stomata respond to changes in hydraulic conductance (K) of stems in a manner consistent with a feedback response to leaf water potential. Seasonal shifts in K also clearly contribute to changes in stomatal conductance (Rodriguez-Dominguez *et al.*, 2016). However, it is unclear whether naturally occurring diurnal changes in K influence stomata. K decline due to xylem cavitation does not typically begin until stomata are already mostly closed (Bartlett *et al.*, 2016; Martin-StPaul *et al.*, 2017). Hydraulic conductance outside the leaf xylem (K_{ox}) apparently declines with dehydration before turgor loss, if measured by the evaporative flux method (Hernandez-Santana *et al.*, 2016; Scoffoni *et al.*, 2017, 2018), although leaf hydraulic conductance (K_{leaf}) measured by rehydration kinetics methods generally does not show such an effect (Brodribb *et al.*, 2014, 2016; Skelton *et al.*, 2017). The mechanism of putative K_{ox} decline with dehydration is unknown, and may involve effects of ABA on aquaporin function (Shatil-Cohen *et al.*, 2011; Pantin *et al.*, 2013a). If such declines are not instantaneously reversible, they could contribute to stomatal responses to water status, including apparent feedforward responses to Δw (Oren *et al.*, 1999; Buckley & Mott, 2002b; Buckley, 2005).

By contrast, Simonin *et al.* (2014) reported a positive correlation between transpiration rate (E) and K_{leaf} among leaves. They suggested K_{leaf} increases with E within a leaf, through a yet-unidentified mechanism. A correlation between E and K_{leaf} may arise from concurrent effects of light on E (via stomatal opening) and on K_{leaf} (Scoffoni *et al.*, 2008) within individual leaves, or from covariation between stomatal and hydraulic conductances among leaves. Resolving this would require repeated measurements of changing K_{leaf} and E within a single leaf over time, which is not possible with current destructive methods to measure K_{leaf}.

Changes in leaf vapor transport also may impact stomatal behavior. For example, temperature gradients can drive vapor transport within leaves without contributing to water potential gradients (Rockwell *et al.*, 2014; Buckley, 2015). This could help to hydrate the epidermis, potentially driving stomatal opening via hydroactive feedback. Pieruschka *et al.* (2010) reported that stomata opened in proportion to total absorbed radiation; because the effect was independent of wavelength, the authors attributed the radiation effect to temperature-driven vapor transport rather than to a photochemical mechanism. Mott & Peak (2011) repeated those experiments and found the effect disappeared when leaf temperature was corrected for thermocouple errors, however. Thermocouples measure a weighted average of leaf and air

temperature, so leaf temperature can be systematically underestimated as the leaf warms relative to the air.

The idea that temperature-driven vapor transport could drive stomatal opening also has been explored using models of energy and water transport within leaves, with varying results. Rockwell *et al.* (2014) simulated a key experiment of Pieruschka *et al.* (2010) in which g_{sw} increased by *c.* 11% after a 25% increase in absorbed radiation in sunflower (*Helianthus annuus*) while leaf temperature was held constant by adjusting air temperature. The simulations validated the concept in principle, although the net effect on g_{sw} was small (blue symbols in Fig. 8) and was nullified by allowing leaf temperature, and hence Δw, to vary naturally (red symbols in Fig. 8). I extended those simulations across a wider range of radiation absorption using a similar model (MOFLO 2.0; Buckley *et al.*, 2017), which predicted an increase of only 5% in g_{sw} from darkness to 200 W m^{-2} when leaf temperature was allowed to vary (dashed red line in Fig. 8; see Notes S2 for details of these simulations). These simulations used parameter values that enhance the effect of radiation on vapor transport, such as large airspace fraction (80%) and leaf thickness (*c.* 400 μm) and low cell thermal conductivity (0.2 W m^{-1} K^{-1}; cf. *c.* 0.6 for pure water). Using parameter values given by Buckley *et al.* (2017) for *H. annuus* (43% airspace, 182 μm leaf thickness and 0.45 W m^{-1} K^{-1} thermal conductivity, respectively), MOFLO 2.0 predicted a 21% decline in g_{sw} (solid line in Fig. 8). These results suggest that temperature-driven vapor transport may induce small stomatal movements, particularly in thick, porous leaves, but that the net effect of radiation could go in either direction, and may be difficult to detect from measurements g_{sw} estimated using leaf thermocouples.

The location of hydroactive sensing has implications for the mechanistic and adaptive impact of changes in leaf water transport. For example, if sensing occurs within the vasculature, then a decrease in outside-xylem conductance would dehydrate the epidermis without inducing hydroactive feedback, so it would cause hydropassive stomatal opening rather than closure. Differences in the location of hydroactive sensing also may enable plants to independently 'tune' their stomatal sensitivities to water supply and evaporative demand. For example, increased water loss causes a greater drop in water potential in guard cells than in the mesophyll, whereas reduced soil water potential affects both locations equally. Understanding the functional and adaptive roles of water transport in stomatal function thus requires knowledge of where water status is sensed, and whether or how that location varies across taxa.

IV. Synthesis and suggestions for continuing research

Current evidence strongly suggests that stomatal responses to changes in both evaporative demand and soil moisture involve an actively mediated feedback response to leaf water status (hydroactive feedback), possibly involving *de novo* synthesis of ABA in leaves (Sections III.1, III.2). Although hydropassive responses may dominate stomatal function in seedless plants (Section III.3), hydroactive feedback remains the only proposed mechanism that can explain rapid responses to both water supply and evaporative demand, including both transient wrong-way and steady-state

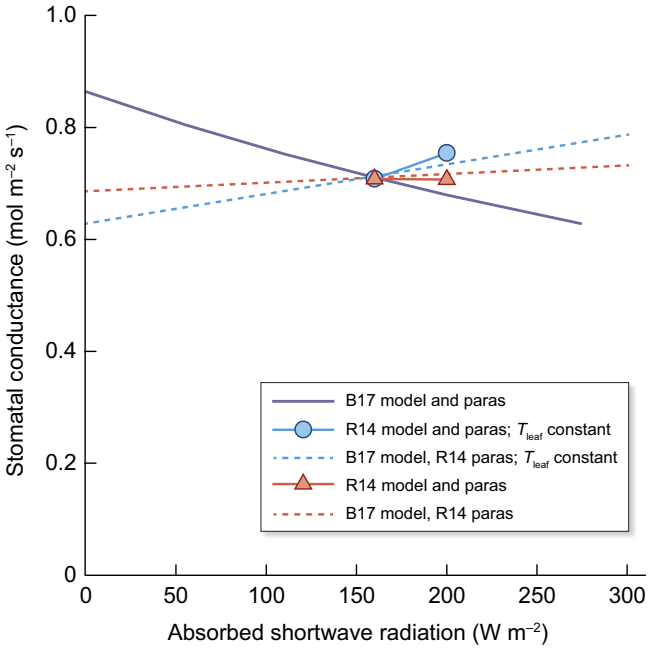

Fig. 8 Predicted effect of absorbed shortwave radiation on stomatal conductance via hydroactive response to hydration of the abaxial epidermis. Simulations using the MOFLO 2.0 model and/or parameter values ('paras'; Supporting Information Table S1) of Buckley *et al*. (2017) ('B17') or Rockwell *et al*. (2014) ('R14'), with the temperature of the lower leaf surface (T_{leaf}) either allowed to vary naturally, or held constant ('T_{leaf} constant').

right-way responses, in plants in which the epidermis has a mechanical advantage. Other mechanisms may complement or enhance hydroactive feedback, including interactions with root signals other than ABA (Section III.1), coupling of guard cell water status to air in the stomatal pore channel (Section III.4), and modulation of epidermal water status by changes in leaf water transport in relation to dehydration, radiation absorption and perhaps ABA itself.

Below I discuss three key areas for continuing research: verifying the generality of seminal results that underpin current theoretical understanding of stomatal water relations, quantifying the phenomenology of hydroactive feedback and the traits that govern it, and validating and quantifying the importance of other mechanisms. Table 1 lists open questions arising in these areas.

1. Generalizing key results related to stomatal responses to water status

Current understanding of stomatal responses to water status in intact leaves is based heavily on the conclusions of a few key seminal experiments that have been poorly replicated. Foremost is the idea that epidermal cells have a mechanical advantage over guard cells in angiosperms ($m > 1$; Eqn 2), which was based on pioneering pressure-probe studies of stomatal pressure–aperture relations (Meidner & Bannister, 1979; Franks *et al.*, 1995, 1998; Franks & Farquhar, 2007). Two other key findings are the conclusion that stomata respond not to ambient humidity *per se* but to the rate of water loss from leaves, which was based on Mott & Parkhurst's (1991) helox study, and the idea that stomatal responses to water

Table 1. Key questions and suggested avenues of experimentation in stomatal responses to water status.

Question	Suggested approach	Challenge for suggested approach
What is the form of the relationship between water content of sensory tissues and ABA concentration?	Determine water content and ABA content *in vivo* through standard destructive methods, use microscopy to quantify tissue volume changes	Identifying common basis for leaf ABA and tissue volume measurements
	Characterize dynamic regulation of both ABA synthesis and catabolism in leaves	Existing methods of ABA analysis do not enable repeated measurement of a tissue over time
In what tissue(s) does hydroactive sensing of leaf water status occur?	Analysis of ABA synthesis in isolated tissues	Not feasible in many species
	Manipulation of ψ in xylem and outside-xylem tissues via source ψ and evaporative demand (see Section IV.2)	Requires confidence in values of xylem and outside-xylem hydraulic resistance
Are guard cells hydraulically isolated from the epidermis and coupled to the air in the stomatal pore channel?	Compare stomatal sensitivity to supply vs demand perturbations that produce similar changes in ψ at site of water status sensing	Requires knowledge of site of hydroactive water status sensing
	Test for hydrophobic barrier in guard cell walls and variable aquaporin activity in relevant areas of guard cell membrane	
How general are key experimental results of stomatal water relations?	Replicate seminal experiments across taxa	Some experiments require control of gas content and/or temperature in ways that are not straightforward with commercial gas exchange systems
	Replicate seminal experiments and publish results	Disincentive to publish 'replicatory' work. Sound-science journals and preprint archives are possible solutions
Does the epidermis really have a net mechanical advantage ($m > 1$) in most angiosperms?	Additional pressure probe experiments along the lines of the work of Franks *et al*. (1998)	Time consuming, low success rate within species, impractical for many species
	Observe effects of imposed atmospheric pressure on stomatal aperture (see Section IV.1)	Performing microscopy within pressure chamber

ABA, abscisic acid; ψ, water potential; m, mechanical advantage of the epidermis.

supply and evaporative demand are fundamentally symmetrical, which is based on a few reports showing transient wrong-way and steady-state right-way stomatal responses to water potential in distant tissues, analogous to Δw responses (Raschke, 1970; Saliendra *et al*., 1995; Comstock & Mencuccini, 1998; Buckley & Mott, 2000). It is important to repeat such foundational results, and even more so to verify that they hold across major taxonomic groups. A good example of the potential value of replicating key results across taxa is Franks & Farquhar's (2007) finding that differences in stomatal anatomy among seedless plants, grasses and dicots lead to important differences in stomatal function.

The helox and supply–demand results described above could be repeated and extended using widely available portable gas exchange systems, with straightforward modifications. Verifying that the epidermis has a net mechanical advantage, however, and quantifying how it varies within and across species, is far more challenging. The presence or absence of transient wrong-way responses has been used as evidence for $m > 1$ or $m < 1$, respectively (Brodribb & McAdam, 2011), but the WWR also could arise, in theory, if guard cells equilibrated more slowly than epidermal cells following a change in local water status. If so, the time constant for changes in guard cell volume would be much greater than that for epidermal cell volume but similar to that for right-way stomatal responses. Some meager data are available to test this prediction: for example, osmotically induced guard cell volume changes are complete in *c.* 1 min in *Vicia faba* (Shope & Mott, 2006), whereas the WWR alone lasts *c.* 9 min (Buckley *et al*., 2011). Extending such comparisons across species could validate the use of WWRs as a probe for $m > 1$. Another way to verify $m > 1$ would be to enclose a leaf in a pressure chamber with the petiole protruding out, and use an applied air pressure of δP to forcibly reduce epidermal and guard cell volumes by amounts equivalent to turgor pressure declines of δP. This would change aperture by an amount, δa_s, given by

$$\delta a_s \approx \frac{\partial a_s}{\partial P_g} \cdot (-\delta P) + \frac{\partial a_s}{\partial P_e} \cdot (-\delta P) = \frac{\partial a_s}{\partial P_g}(m-1) \cdot \delta P. \qquad \text{Eqn 3}$$

Because $\partial a_s / \partial P_g \geq 0$, leaf pressurization would open stomata if $m > 1$ and close them otherwise. Stomatal apertures could be observed in the chamber using a long working-distance microscope objective aimed through a chamber window composed of a strong, transparent material such as synthetic sapphire.

2. Quantifying the phenomenology of hydroactive feedback

Hydromechanical models of stomatal function typically assume that guard cell and epidermal turgor pressures affect stomatal aperture linearly, and that hydroactive feedback manifests as a linear sensitivity of guard cell osmotic pressure to leaf turgor (Buckley *et al*., 2003; Rodriguez-Dominguez *et al*., 2016). These linear proportionalities were chosen strictly for convenience – they permit analytical solution of the underlying models – not because data indicate the true relationships were linear. Indeed, the available data suggest that aperture depends nonlinearly on P_g and P_e (Franks *et al*., 1998), and that hydroactive feedback is nonlinear (Deans *et al*., 2017) and involves sensing of cell or tissue volume rather than turgor (Sack *et al*., 2018). It is important to more accurately quantify these relationships in order to validate, and if necessary modify or abandon, analytical modeling approaches based on linear responses.

The shape of the hydroactively driven steady-state response of stomatal conductance to water status depends on the shapes of three underlying relationships: the effect of guard cell osmotic pressure

(π_g) on stomatal conductance, the response of [ABA] to water status, and the response of π_g to [ABA]. The shapes of these three effects are poorly known. Although π_g affects P_g linearly for a given water potential, the resulting effect on stomatal aperture also depends on the adjacent epidermal or subsidiary cells. Franks & Farquhar (2007) reported that subsidiary cell osmotic pressure and volume decline steeply when π_g increases in wheat, which should accelerate stomatal movements, and Raissig *et al.* (2017) verified that stomatal responses are slower and weaker in wheat lacking normal subsidiary cells. These results should be verified and extended to other grass species. Although metabolic effectors often exhibit a saturating effect due to mass action and saturation of sensory binding sites (Ap Rees & Hill, 1994), this may not apply to effects of ABA synthesis on $\delta\pi_g$, because of positive feedbacks mediated by ABA synthesis within guard cells themselves (Bauer *et al.*, 2013) and the influence of ABA import, export and catabolism on [ABA]. It is essential to quantify each of these factors in intact leaves. As discussed in Section III.5, hydroactive feedback responses to Δw also depend on where exactly water status is actively sensed, and on properties of water transport proximal to that location. Attempts to identify the sensing site using tissue dissection or cell-specific promoters have thus far produced divergent conclusions, implicating either the vascular tissue (Kuromori *et al.*, 2014), the mesophyll (McAdam & Brodribb, 2018) or guard cells themselves (Bauer *et al.*, 2013). An *in vivo* alternative to these approaches would be to manipulate water potentials independently in the leaf xylem and outside-xylem tissues by combining shifts in Δw with root pressurization (see Notes S3 for a description of how this could be achieved). A thorough quantitative understanding of hydroactive feedback is not possible until these factors are characterized in detail.

The importance of leaf and plant anatomy in stomatal function also has been highlighted by the discovery of fundamental differences in stomatal function between major taxonomic groups as a result of differences in stomatal anatomy (Franks & Farquhar, 2007; Brodribb & McAdam, 2011), and by growing awareness of the impact of leaf hydraulics and capacitance on stomata (Buckley *et al.*, 2011; Deans *et al.*, 2017; Scoffoni *et al.*, 2018). These insights need to be further developed and extended across diverse taxa. It also is essential to develop a quantitative understanding of how stomatal sensitivity to water status changes over longer timescales of seasons and years, and how these shifts are driven by changes in the underlying control parameters. For example, stomatal sensitivity to ABA is modulated by changes in humidity experienced during growth (Pantin *et al.*, 2013b). Increased leaf osmotic pressure can sustain stomatal opening during soil drought (Turner *et al.*, 1978), and conversely, reduced hydraulic conductance at low water potentials can enhance stomatal closure during drought (Rodriguez-Dominguez *et al.*, 2016). These longer-term dynamics must be characterized thoroughly for any practical modeling based on mechanistic understanding of stomatal control.

3. Exploring additional mechanisms and stomatal responses linked to water status

Further research is needed to validate the hypothesized mechanisms, described in Sections III.4 and III.5, that may complement or modulate hydroactive feedback, and to quantify their importance in intact leaves. Numerous experimental approaches are possible. For example, water exchange between guard cells and air in the stomatal pore channel should make Δw responses stronger than water supply responses for a given change in bulk leaf water potential; this could be used to quantify the importance of the pore-air effect hypothesized by Peak and Mott (Section III.4). Detailed microscopy also may help to visualize the distribution of aquaporins across guard cell membranes, and of hydrophobic substances in guard cell walls, to validate the PM model's premise that guard cells exchange water only in the vicinity of the stomatal pore channel. The PM mechanism also demands further theoretical analysis and extension to resolve the contradictions raised in Section III.4. Likewise, the effect of light on vapor transport hypothesized by Pieruschka *et al.* (2010) relies on the hypothesis that hydroactive sensing occurs predominantly in the epidermis. As discussed earlier, some evidence implicates hydroactive sensing in the mesophyll or vasculature, and it is unclear whether those tissues would be hydrated by light absorption; if they are not, then the Pieruschka effect would induce hydropassive stomatal closure rather than hydroactive opening. This again underscores the need to determine where water status sensing occurs.

Surprisingly few studies have documented the direct stomatal response to temperature (i.e. the response at constant Δw) (Hall & Kaufmann, 1975; Hall *et al.*, 1975; Ball *et al.*, 1987; Fredeen & Sage, 1999; Mott & Peak, 2010). Shifts in Δw in nature can be driven by temperature, humidity or both, so relationships between g_{sw} and Δw under natural conditions include an unknown and probably variable superposition of at least two distinct responses: one involving Δw *per se* and another involving T *per se*. Confidence in mechanistic models depends on filling this critical knowledge gap. The first step is to characterize the direct T response across species. This can be achieved, in principle, with modern commercial gas exchange systems by controlling T and Δw simultaneously, although most chambers have a very limited range of T control. Imposing high chamber T at low Δw also risks condensation in sample lines downstream of the leaf chamber; this could be addressed by heating the entire gas exchange system, including the chamber, gas lines and gas analyzer, simultaneously. Alternatively, the T response could be inferred by difference, by comparing T- and humidity-driven Δw responses, or by interpolation between Δw responses measured at different temperatures. The second step is to explore the mechanism. This is more difficult, given the multitude of potential influences of temperature on stomatal function (e.g. via photosynthesis-related signals, guard cell metabolism itself, temperature effects on liquid and vapor phase water transport, and the Peak–Mott mechanism). One approach could be to study the effect of T hierarchically – first studying guard cell responses in epidermal peels, then quantifying photosynthesis-driven effects in nontranspiring leaves (in which water transport properties would have no effect), and finally quantifying transpiration-linked effects.

V. Conclusion

Plant biology is within reach of a rigorously mechanistic understanding of stomatal responses involving water status, which can then be translated into useful quantitative models, by building on

existing approaches (Buckley *et al.*, 2003; Franks 2004; Peak & Mott, 2011). Achieving such an understanding is vital if we are to move beyond empirical models for predicting and interpreting stomatal responses to a changing environment. It also is feasible: efforts in recent years have identified a narrowing set of clear questions (Table 1) that must now be addressed experimentally, validated across species and incorporated into existing hydromechanical modeling frameworks.

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References

Adams HD, Zeppel MJ, Anderegg WR, Hartmann H, Landhäusser SM, Tissue DT, Huxman TE, Hudson PJ, Franz TE, Allen CD. 2017. A multi-species synthesis of physiological mechanisms in drought-induced tree mortality. *Nature Ecology & Evolution* **1**: 1285.

Anderegg WR, Klein T, Bartlett M, Sack L, Pellegrini AF, Choat B, Jansen S. 2016. Meta-analysis reveals that hydraulic traits explain cross-species patterns of drought-induced tree mortality across the globe. *Proceedings of the National Academy of Sciences, USA* **113**: 5024–5029.

Ap Rees T, Hill S. 1994. Metabolic control analysis of plant metabolism. *Plant, Cell & Environment* **17**: 587–599.

Arve LE, Kruse OMO, Tanino KK, Olsen JE, Futsæther C, Torre S. 2015. Growth in continuous high air humidity increases the expression of CYP707A-genes and inhibits stomatal closure. *Environmental and Experimental Botany* **115**: 11–19.

Ball JT, Woodrow IE, Berry JA. 1987. A model predicting stomatal conductance and its contribution to the control of photosynthesis under different environmental conditions. In: Biggens J, ed. *Progress in photosynthesis research.* Leiden, the Netherlands: Martinus Nijhoff, 221–224.

Bartlett MK, Klein T, Jansen S, Choat B, Sack L. 2016. The correlations and sequence of plant stomatal, hydraulic, and wilting responses to drought. *Proceedings of the National Academy of Sciences, USA* **113**: 13098–13103.

Bauer H, Ache P, Lautner S, Fromm J, Hartung W, Al-Rasheid KA, Sonnewald S, Sonnewald U, Kneitz S, Lachmann N. 2013. The stomatal response to reduced relative humidity requires guard cell-autonomous ABA synthesis. *Current Biology* **23**: 53–57.

Berry JA, Beerling DJ, Franks PJ. 2010. Stomata: key players in the earth system, past and present. *Current Opinion in Plant Biology* **13**: 232–239.

Blum A. 2009. Effective use of water (EUW) and not water-use efficiency (WUE) is the target of crop yield improvement under drought stress. *Field Crops Research* **112**: 119–123.

de Boer HJ, Lammertsma EI, Wagner-Cremer F, Dilcher DL, Wassen MJ, Dekker SC. 2011. Climate forcing due to optimization of maximal leaf conductance in subtropical vegetation under rising CO_2. *Proceedings of the National Academy of Sciences, USA* **108**: 4041–4046.

Bonan GB. 2008. Forests and climate change: forcings, feedbacks, and the climate benefits of forests. *Science* **320**: 1444–1449.

Borel C, Frey A, Marion-Poll A, Tardieu F, Simonneau T. 2001. Does engineering abscisic acid biosynthesis in *Nicotiana plumbaginifolia* modify stomatal response to drought? *Plant, Cell & Environment* **24**: 477–489.

Boucher O, Jones A, Betts RA. 2009. Climate response to the physiological impact of carbon dioxide on plants in the Met Office Unified Model HadCM3. *Climate Dynamics* **32**: 237–249.

Brodribb TJ, McAdam SA. 2011. Passive origins of stomatal control in vascular plants. *Science* **331**: 582–585.

Brodribb TJ, McAdam SA, Jordan GJ, Martins SC. 2014. Conifer species adapt to low-rainfall climates by following one of two divergent pathways. *Proceedings of the National Academy of Sciences, USA* **111**: 14489–14493.

Brodribb TJ, Skelton RP, McAdam SA, Bienaimé D, Lucani CJ, Marmottant P. 2016. Visual quantification of embolism reveals leaf vulnerability to hydraulic failure. *New Phytologist* **209**: 1403–1409.

Buckley TN. 2005. The control of stomata by water balance. *New Phytologist* **168**: 275–292.

Buckley TN. 2015. The contributions of apoplastic, symplastic and gas phase pathways for water transport outside the bundle sheath in leaves. *Plant, Cell & Environment* **38**: 7–22.

Buckley TN, John GJ, Scoffoni C, Sack L. 2015. How does leaf anatomy influence water transport outside the xylem? *Plant Physiology* **168**: 1616–1635.

Buckley TN, John GP, Scoffoni C, Sack L. 2017. The sites of evaporation within leaves. *Plant Physiology* **173**: 1763–1782.

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

Buckley TN, Mott KA. 2002a. Dynamics of stomatal water relations during the humidity response: implications of two hypothetical mechanisms. *Plant, Cell & Environment* **25**: 407–419.

Buckley TN, Mott KA. 2002b. Stomatal water relations and the control of hydraulic supply and demand. *Progress in Botany* **63**: 309–325.

Buckley TN, Mott KA, Farquhar GD. 2003. A hydromechanical and biochemical model of stomatal conductance. *Plant, Cell & Environment* **26**: 1767–1785.

Buckley TN, Sack L, Gilbert ME. 2011. The role of bundle sheath extensions and life form in stomatal responses to leaf water status. *Plant Physiology* **156**: 962–973.

Cai S, Chen G, Wang Y, Huang Y, Marchant B, Wang Y, Yang Q, Dai F, Hills A, Franks PJ. 2017. Evolutionary conservation of ABA signaling for stomatal closure in ferns. *Plant Physiology* **174**: 732–747.

Cao L, Bala G, Caldeira K, Nemani R, Ban-Weiss G. 2010. Importance of carbon dioxide physiological forcing to future climate change. *Proceedings of the National Academy of Sciences, USA* **107**: 9513–9518.

Cardoso AA, Randall JM, McAdam SAM. 2019. Hydraulics regulate stomatal responses to changes in leaf water status in the fern *Athyrium filix-femina. Plant Physiology* **179**: 533–543.

Castro P, Puertolas J, Dodd IC. 2019. Stem girdling uncouples soybean stomatal conductance from leaf water potential by enhancing leaf xylem ABA concentration. *Environmental and Experimental Botany* **159**: 149–156.

Chater C, Kamisugi Y, Movahedi M, Fleming A, Cuming AC, Gray JE, Beerling DJ. 2011. Regulatory mechanism controlling stomatal behavior conserved across 400 million years of land plant evolution. *Current Biology* **21**: 1025–1029.

Choat B, Jansen S, Brodribb TJ, Cochard H, Delzon S, Bhaskar R, Bucci SJ, Feild TS, Gleason SM, Hacke UG. 2012. Global convergence in the vulnerability of forests to drought. *Nature* **491**: 752.

Christmann A, Hoffmann T, Teplova I, Grill E, Müller A. 2005. Generation of active pools of abscisic acid revealed by *in vivo* imaging of water-stressed Arabidopsis. *Plant Physiology* **137**: 209–219.

Christmann A, Weiler EW, Steudle E, Grill E. 2007. A hydraulic signal in root-to-shoot signalling of water shortage. *The Plant Journal* **52**: 167–174.

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

Darwin F. 1898. Observations on stomata. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* **190**: 531–621.

Davies WJ, Wilkinson S, Loveys B. 2002. Stomatal control by chemical signalling and the exploitation of this mechanism to increase water use efficiency in agriculture. *New Phytologist* **153**: 449–460.

Deans RM, Brodribb TJ, McAdam SA. 2017. An integrated hydraulic-hormonal model of conifer stomata predicts water stress dynamics. *Plant Physiology* **174**: 478–486.

DeMichele DW, Sharpe PJH. 1973. An analysis of the mechanics of guard cell motion. *Journal of Theoretical Biology* **41**: 77–96.

Dewar RC. 1995. Interpretation of an empirical model for stomatal conductance in terms of guard cell function. *Plant, Cell & Environment* **18**: 365–372.

Doi M, Kitagawa Y, Shimazaki K-i. 2015. Stomatal blue light response is present in early vascular plants. *Plant Physiology* **169**: 1205–1213.

Duckett JG, Pressel S. 2018. The evolution of the stomatal apparatus: intercellular spaces and sporophyte water relations in bryophytes: two ignored dimensions. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* **373**: 20160498.

Duckett JG, Pressel S, Renzaglia KS, Clymo RS. 2018. Hornwort stomata do not respond actively to exogenous and environmental cues. *Annals of Botany* **122**: 45–57.

Endo A, Sawada Y, Takahashi H, Okamoto M, Ikegami K, Koiwai H, Seo M, Toyomasu T, Mitsuhashi W, Shinozaki K. 2008. Drought induction of *Arabidopsis* 9-cis-epoxycarotenoid dioxygenase occurs in vascular parenchyma cells. *Plant Physiology* **147**: 1984–1993.

Fambrini M, Vernieri P, Toncelli ML, Rossi VD, Pugliesi C. 1995. Characterization of a wilty sunflower (*Helianthus annuus* L.) mutant: III. Phenotypic interaction in reciprocal grafts from wilty mutant and wild-type plants. *Journal of Experimental Botany* **46**: 525–530.

Farquhar GD. 1978. Feedforward responses of stomata to humidity. *Australian Journal of Plant Physiology* **5**: 787–800.

Fischer R, Rees D, Sayre K, Lu Z-M, Condon A, Saavedra AL. 1998. Wheat yield progress associated with higher stomatal conductance and photosynthetic rate, and cooler canopies. *Crop Science* **38**: 1467–1475.

Franks PJ. 2013. Passive and active stomatal control: either or both? *New Phytologist* **198**: 325–327.

Franks PJ, Britton-Harper ZJ. 2016. No evidence of general CO_2 insensitivity in ferns: one stomatal control mechanism for all land plants? *New Phytologist* **211**: 819–827.

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

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 & Environment* **18**: 795–800.

Franks PJ, Farquhar GD. 2007. The mechanical diversity of stomata and its significance in gas-exchange control. *Plant Physiology* **143**: 78–87.

Fredeen A, Sage R. 1999. Temperature and humidity effects on branchlet gas-exchange in white spruce: an explanation for the increase in transpiration with branchlet temperature. *Trees* **14**: 161–168.

Glinka Z. 1971. The effect of epidermal cell water potential on stomatal response to illumination of leaf discs of *Vicia faba*. *Physiologia Plantarum* **24**: 476–479.

Gollan T, Passioura J, Munns R. 1986. Soil water status affects the stomatal conductance of fully turgid wheat and sunflower leaves. *Australian Journal of Plant Physiology* **13**: 459–464.

Grantz DA, Linscheid BS, Grulke NE. 2019. Differential responses of stomatal kinetics and steady state conductance to abscisic acid in a fern: comparison with a gymnosperm and an angiosperm. *New Phytologist* **222**, 1883–1892.

Grondin A, Rodrigues O, Verdoucq L, Merlot S, Leonhardt N, Maurel C. 2015. Aquaporins contribute to ABA-triggered stomatal closure through OST1-mediated phosphorylation. *Plant Cell* **27**: 1945–1954.

Guarín A, Taylor AH. 2005. Drought triggered tree mortality in mixed conifer forests in Yosemite National Park, California, USA. *Forest Ecology and Management* **218**: 229–244.

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

Hall AE, Camacho-B SE, Kaufmann MR. 1975. Regulation of water loss by citrus leaves. *Physiologia Plantarum* **33**: 62–65.

Hall AE, Kaufmann MR. 1975. Stomatal response to environment with *Sesamum indicum* L. *Plant Physiology* **55**: 455–459.

Hernandez-Santana V, Rodriguez-Dominguez CM, Fernández JE, Diaz-Espejo A. 2016. Role of leaf hydraulic conductance in the regulation of stomatal conductance in almond and olive in response to water stress. *Tree Physiology* **36**: 725–735.

Hetherington AM, Woodward FI. 2003. The role of stomata in sensing and driving environmental change. *Nature* **424**: 901–908.

Hinckley T, Duhme F, Hinckley A, Richter H. 1980. Water relations of drought hardy shrubs: osmotic potential and stomatal reactivity. *Plant, Cell & Environment* **3**: 131–140.

Holbrook NM, Shashidhar VR, James RA, Munns R. 2002. Stomatal control in tomato with ABA-deficient roots: response of grafted plants to soil drying. *Journal of Experimental Botany* **53**: 1503–1514.

Hõrak H, Kollist H, Merilo E. 2017. Fern stomatal responses to ABA and CO_2 depend on species and growth conditions. *Plant Physiology* **174**: 672–679.

Ikegami K, Okamoto M, Seo M, Koshiba T. 2009. Activation of abscisic acid biosynthesis in the leaves of Arabidopsis thaliana in response to water deficit. *Journal of Plant Research* **122**: 235.

Kaldenhoff R, Kölling A, Meyers J, Karmann U, Ruppel G, Richter G. 1995. The blue light-responsive AthH2 gene of Arabidopsis thaliana is primarily expressed in expanding as well as in differentiating cells and encodes a putative channel protein of the plasmalemma. *The Plant Journal* **7**: 87–95.

Khalil A, Grace J. 1993. Does xylem sap ABA control the stomatal behaviour of water-stressed sycamore (*Acer pseudoplatanus* L.) seedlings? *Journal of Experimental Botany* **44**: 1127–1134.

Koiwai H, Nakaminami K, Seo M, Mitsuhashi W, Toyomasu T, Koshiba T. 2004. Tissue-specific localization of an abscisic acid biosynthetic enzyme, AAO3, in *Arabidopsis*. *Plant Physiology* **134**: 1697–1707.

Kriedemann P, Loveys B, Fuller G, Leopold A. 1972. Abscisic acid and stomatal regulation. *Plant Physiology* **49**: 842–847.

Kuromori T, Sugimoto E, Shinozaki K. 2014. Inter-tissue signal transfer of abscisic acid from vascular cells to guard cells. *Plant Physiology* **164**: 1587–1592.

Lange OL, Lösch R, Schulze E-D, Kappen L. 1971. Responses of stomata to changes in humidity. *Planta* **100**: 76–86.

Lawson T. 2009. Guard cell photosynthesis and stomatal function. *New Phytologist* **181**: 13–34.

Lawson T, Terashima I, Fujita T, Wang Y 2018. Coordination between photosynthesis and stomatal behavior. In: Adams WW III, Terashima I, eds. *The leaf: a platform for performing photosynthesis*. Cham, Switzerland: Springer, 141–162.

Lind C, Dreyer I, López-Sanjurjo EJ, von Meyer K, Ishizaki K, Kohchi T, Lang D, Zhao Y, Kreuzer I, Al-Rasheid KA. 2015. Stomatal guard cells co-opted an ancient ABA-dependent desiccation survival system to regulate stomatal closure. *Current Biology* **25**: 928–935.

Lösch R. 1977. Responses of stomata to environmental factors-experiments with isolated epidermal strips of *Polypodium vulgare*. *Oecologia* **29**: 85–97.

Lösch R. 1979. Responses of stomata to environmental factors-experiments with isolated epidermal strips of *Polypodium vulgare*. *Oecologia* **39**: 229–238.

Losch R, Schenk B. 1978. Humidity responses of stomata and the potassium content of guard cells. *Journal of Experimental Botany* **29**: 781–787.

Loveys B. 1977. The intracellular location of abscisic acid in stressed and non-stressed leaf tissue. *Physiologia Plantarum* **40**: 6–10.

Maier-Maercker U. 1983. The role of peristomatal transpiration in the mechanism of stomatal movement. *Plant, Cell & Environment* **6**: 369–380.

Manzi M, Lado J, Rodrigo MJ, Zacarías L, Arbona V, Gómez-Cadenas A. 2015. Root ABA accumulation in long-term water-stressed plants is sustained by hormone transport from aerial organs. *Plant Cell Physiology* **56**: 2457–2466.

Martin-StPaul N, Delzon S, Cochard H. 2017. Plant resistance to drought depends on timely stomatal closure. *Ecology Letters* **20**: 1437–1447.

McAdam S, Brodribb T. 2018. Mesophyll cells are the main site of abscisic acid biosynthesis in water-stressed leaves. *Plant Physiology* **177**: 911–917.

McAdam SA, Brodribb TJ. 2012a. Fern and lycophyte guard cells do not respond to endogenous abscisic acid. *Plant Cell* **24**: 1510–1521.

McAdam SA, Brodribb TJ. 2012b. Stomatal innovation and the rise of seed plants. *Ecology Letters* **15**: 1–8.

McAdam SA, Brodribb TJ. 2015. The evolution of mechanisms driving the stomatal response to vapor pressure deficit. *Plant Physiology* **167**: 833–843.

McAdam SA, Brodribb TJ. 2016. Linking turgor with ABA biosynthesis: implications for stomatal responses to vapour pressure deficit across land plants. *Plant Physiology* **171**: 2008–2016.

McAdam SA, Brodribb TJ, Ross JJ. 2016a. Shoot-derived abscisic acid promotes root growth. *Plant, Cell & Environment* **39**: 652–659.

McAdam SA, Sussmilch FC, Brodribb TJ. 2016b. Stomatal responses to vapour pressure deficit are regulated by high speed gene expression in angiosperms. *Plant, Cell & Environment* **39**: 485–491.

McDowell NG. 2011. Mechanisms linking drought, hydraulics, carbon metabolism, and vegetation mortality. *Plant Physiology* **155**: 1051–1059.

Meidner H. 1986. Cuticular conductance and the humidity response of stomata. *Journal of Experimental Botany* **177**: 517–525.

Meidner H, Bannister P. 1979. Pressure and solute potentials in stomatal cells of *Tradescantia virginiana*. *Journal of Experimental Botany* **30**: 255–265.

Merilo E, Yarmolinsky D, Jalakas P, Parik H, Tulva I, Rasulov B, Kilk K, Kollist H. 2018. Stomatal VPD response: there is more to the story than ABA. *Plant Physiology* **176**: 851–864.

Messinger SM, Buckley TN, Mott KA. 2006. Evidence for involvement of photosynthetic processes in the stomatal response to CO_2. *Plant Physiology* **140**: 771–778.

Mott KA. 2007. Leaf hydraulic conductivity and stomatal responses to humidity in amphistomatous leaves. *Plant, Cell & Environment* **30**: 1444–1449.

Mott KA, Buckley TN. 2000. Patchy stomatal conductance: emergent collective behaviour of stomata. *Trends in Plant Science* **5**: 258–262.

Mott KA, Denne F, Powell J. 1997. Interactions among stomatal in response to perturbations in humidity. *Plant, Cell & Environment* **20**: 1098–1107.

Mott KA, Parkhurst DF. 1991. Stomatal responses to humidity in air and helox. *Plant, Cell & Environment* **14**: 509–515.

Mott KA, Peak D. 2010. Stomatal responses to humidity and temperature in darkness. *Plant, Cell & Environment* **33**: 1084–1090.

Mott KA, Peak D. 2011. Alternative perspective on the control of transpiration by radiation. *Proceedings of the National Academy of Sciences, USA* **108**: 19820–19823.

Mott KA, Shope JC, Buckley TN. 1999. Effects of humidity on light-induced stomatal opening: evidence for hydraulic coupling among stomata. *Journal of Experimental Botany* **336**: 1207–1213.

Nikinmaa E, Hölttä T, Hari P, Kolari P, Mäkelä A, Sevanto S, Vesala T. 2013. Assimilate transport in phloem sets conditions for leaf gas exchange. *Plant, Cell & Environment* **36**: 655–669.

Okamoto M, Tanaka Y, Abrams SR, Kamiya Y, Seki M, Nambara E. 2009. High humidity induces abscisic acid 8′-hydroxylase in stomata and vasculature to regulate local and systemic abscisic acid responses in *Arabidopsis*. *Plant Physiology* **149**: 825–834.

Oren R, Sperry JS, Katul GG, Pataki DE, Ewers BE, Phillips N, Schafer KVR. 1999. Survey and synthesis of intra- and interspecific variation in stomatal sensitivity to vapour pressure deficit. *Plant, Cell & Environment* **22**: 1515–1526.

Pantin F, Monnet F, Jannaud D, Costa JM, Renaud J, Muller B, Simonneau T, Genty B. 2013a. The dual effect of abscisic acid on stomata. *New Phytologist* **197**: 65–72.

Pantin F, Renaud J, Barbier F, Vavasseur A, Le Thiec D, Rose C, Bariac T, Casson S, McLachlan DH, Hetherington AM. 2013b. Developmental priming of stomatal sensitivity to abscisic acid by leaf microclimate. *Current Biology* **23**: 1805–1811.

Peak D, Mott KA. 2011. A new, vapour-phase mechanism for stomatal responses to humidity and temperature. *Plant, Cell & Environment* **34**: 162–178.

Pfautsch S, Adams MA. 2012. Water flux of *Eucalyptus regnans*: defying summer drought and a record heatwave in 2009. *Oecologia* **172**: 317–326.

Pieruschka R, Huber G, Berry JA. 2010. Control of transpiration by radiation. *Proceedings of the National Academy of Sciences, USA* **107**: 13372–13377.

Powles JE, Buckley TN, Nicotra AB, Farquhar GD. 2006. Dynamics of stomatal water relations following leaf excision. *Plant, Cell & Environment* **29**: 981–992.

Raissig MT, Matos JL, Gil MXA, Kornfeld A, Bettadapur A, Abrash E, Allison HR, Badgley G, Vogel JP, Berry JA. 2017. Mobile MUTE specifies subsidiary cells to build physiologically improved grass stomata. *Science* **355**: 1215–1218.

Raschke K. 1970. Stomatal responses to pressure changes and interruptions in the waer supply of detached leaves of *Zea mays* L. *Plant Physiology* **45**: 415–423.

Reichstein M, Tenhunen JD, Roupsard O, Ourcival J-m, Rambal S, Miglietta F, Peressotti A, Pecchiari M, Tirone G, Valentini R. 2002. Severe drought effects on ecosystem CO_2 and H_2O fluxes at three Mediterranean evergreen sites: revision of current hypotheses? *Global Change Biology* **8**: 999–1017.

Ren H, Gao Z, Chen L, Wei K, Liu J, Fan Y, Davies WJ, Jia W, Zhang J. 2006. Dynamic analysis of ABA accumulation in relation to the rate of ABA catabolism in maize tissues under water deficit. *Journal of Experimental Botany* **58**: 211–219.

Renzaglia KS, Villarreal JC, Piatkowski BT, Lucas JR, Merced A. 2017. Hornwort stomata: architecture and fate shared with 400-million-year-old fossil plants without leaves. *Plant Physiology* **174**: 788–797.

Rockwell FE, Holbrook NM, Stroock AD. 2014. The competition between liquid and vapor transport in transpiring leaves. *Plant Physiology* **164**: 1741–1758.

Rodriguez-Dominguez CM, Buckley TN, Egea G, Cires A, Hernandez-Santana V, Martorell S, Diaz-Espejo A. 2016. Most stomatal closure in woody species under moderate drought can be explained by stomatal responses to leaf turgor. *Plant, Cell & Environment* **39**: 2014–2026.

Ruszala EM, Beerling DJ, Franks PJ, Chater C, Casson SA, Gray JE, Hetherington AM. 2011. Land plants acquired active stomatal control early in their evolutionary history. *Current Biology* **21**: 1030–1035.

Sack L, John GP, Buckley TN. 2018. ABA accumulation in dehydrating leaves is associated with decline in cell volume, not turgor pressure. *Plant Physiology* **176**: 489–495.

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.

Scoffoni C, Albuquerque C, Brodersen C, Townes SV, John GP, Bartlett MK, Buckley TN, McElrone AJ, Sack L. 2017. Outside-xylem vulnerability, not xylem embolism, controls leaf hydraulic decline during dehydration. *Plant Physiology* **173**: 1197–1210.

Scoffoni C, Albuquerque C, Cochard H, Buckley TN, Fletcher LR, Caringella MA, Bartlett M, Brodersen CR, Jansen S, McElrone AJ. 2018. The causes of leaf hydraulic vulnerability and its influence on gas exchange in *Arabidopsis thaliana*. *Plant Physiology* **178**: 1584–1601.

Scoffoni C, Pou A, Aasamaa K, Sack L. 2008. The rapid light response of leaf hydraulic conductance: new evidence from two experimental methods. *Plant, Cell & Environment* **31**: 1803–1812.

Sharpe PJH, Wu H, Spence RD. 1987. Stomatal mechanics. In: Zeiger E, Farquhar GD, Cowan IR, eds. *Stomatal function*. Stanford, CA, USA: Stanford University Press, 91–114.

Shatil-Cohen A, Attia Z, Moshelion M. 2011. Bundle-sheath cell regulation of xylem-mesophyll water transport via aquaporins under drought stress: a target of xylem-borne ABA? *The Plant Journal* **67**: 72–80.

Shope JC, Mott KA. 2006. Membrane trafficking and osmotically induced volume changes in guard cells. *Journal of Experimental Botany* **57**: 4123–4131.

Simonin KA, Burns E, Choat B, Barbour MM, Dawson TE, Franks PJ. 2014. Increasing leaf hydraulic conductance with transpiration rate minimizes the water potential drawdown from stem to leaf. *Journal of Experimental Botany* **66**: 1303–1315.

Skelton RP, Brodribb TJ, McAdam SA, Mitchell PJ. 2017. Gas exchange recovery following natural drought is rapid unless limited by loss of leaf hydraulic conductance: evidence from an evergreen woodland. *New Phytologist* **215**: 1399–1412.

Sperry JS. 2000. Hydraulic constraints on gas exchange. *Agricultural and Forest Meteorology* **104**: 13–23.

Stålfelt MG. 1929. Die abhngigkeit der spaltoffnungsreaktionen von der wasserbilanz. *Planta* **8**: 287–296.

Stewart WC, Fulton A, Krueger WH, Lampinen BD, Shackel KA. 2011. Regulated deficit irrigation reduces water use of almonds without affecting yield. *California Agriculture* **65**: 90–95.

Sun M-H, Xu W, Zhu Y-F, Su W-A, Tang Z-C. 2001. A simple method for *in situ* hybridization to RNA in guard cells of *Vicia Faba* L.: the expression of aquaporins in guard cells. *Plant Molecular Biology Reporter* **19**: 129–135.

Sussmilch FC, Brodribb TJ, McAdam SA. 2017. Up-regulation of NCED3 and ABA biosynthesis occur within minutes of a decrease in leaf turgor but AHK1 is not required. *Journal of Experimental Botany* **68**: 2913–2918.

Tardieu F. 2016. Too many partners in root–shoot signals. Does hydraulics qualify as the only signal that feeds back over time for reliable stomatal control? *New Phytologist* **212**: 802–804.

Turner N, Begg J, Tonnet M. 1978. Osmotic adjustment of sorghum and sunflower crops in response to water deficits and its influence on the water potential at which stomata close. *Functional Plant Biology* **5**: 597–608.

Visentin I, Vitali M, Ferrero M, Zhang Y, Ruyter-Spira C, Novák O, Strnad M, Lovisolo C, Schubert A, Cardinale F. 2016. Low levels of strigolactones in roots as a component of the systemic signal of drought stress in tomato. *New Phytologist* **212**: 954–963.

Wilkinson S, Davies WJ. 1997. Xylem sap pH increase: a drought signal received at the apoplastic face of the guard cell that involves the suppression of saturable abscisic acid uptake by the epidermal symplast. *Plant Physiology* **113**: 559–573.

Xie X, Wang Y, Williamson L, Holroyd GH, Tagliavia C, Murchie E, Theobald J, Knight MR, Davies WJ, Leyser HO *et al.* 2006. The identification of genes involved in the stomatal response to reduced atmospheric relative humidity. *Current Biology* **16**: 882–887.

Zeiger E, Talbott LD, Frechilla S, Srivastava A, Zhu J. 2002. The guard cell chloroplast: a perspective for the twenty-first century. *New Phytologist* **153**: 415–424.

Zhang F-P, Sussmilch F, Nichols DS, Cardoso AA, Brodribb TJ, McAdam SA. 2018. Leaves, not roots or floral tissue, are the main site of rapid, external pressure-induced ABA biosynthesis in angiosperms. *Journal of Experimental Botany* **69**: 1261–1267.

Zhang J, Davies WJ. 1991. Antitranspirant activity in xylem sap of maize plants. *Journal of Experimental Botany* **42**: 317–321.

Zhao C, Wang Y, Chan KX, Marchant DB, Franks PJ, Randall D, Tee EE, Chen G, Ramesh S, Phua SY. 2019. Evolution of chloroplast retrograde signaling facilitates green plant adaptation to land. *Proceedings of the National Academy of Sciences, USA* **116**: 5015–5020.

Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Notes S1 Proof and explanation of contradiction between two assumptions of the model of Peak & Mott (2011).

Notes S2 Simulations of the effect of within-leaf vapor transport on stomatal conductance.

Notes S3 Manipulating leaf xylem and outside-xylem water potential independently.

Table S1 Parameter values used in the simulations described in Notes S2.

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