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Xylem and stomata, coordinated through time and space.

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ABSTRACT

Land plants exhibit a degree of homeostasis in leaf water content to protect against damage to photosynthetic and xylem tissues, and to maintain an efficient allocation of resources. This is achieved by a strong coordination between the systems regulating water delivery (xylem) and water loss (stomata). This review discusses evolution in xylem and stomatal function, specifically focussing on the interactions between them.

Summary statement:

Vascular plants incur penalties for allowing leaf hydration to range outside strict limits of hydration. Xylem and stomatal tissues regulate the acquisition and loss of water in leaves, and they must work together to maintain a safe level of leaf hydration. This review examines how xylem and stomatal tissues are coordinated to achieve this important role.

Introduction

Water courses from the soil, upwards through dead xylem tubes into the crowns of trees, under the pull of low water potential in leaves. This elegant solution to the problem of replacing rampant water loss from photosynthetic surfaces does not require any input of biological energy, yet there is considerable danger involved in using capillary force generated in the leaves to drag water from the soil. Water under tension is metastable and vulnerable to the invasion of air embolisms once a certain threshold in water tension is exceeded ([Tyree & Zimmermann 2002](#)).

Early in the evolution of land plants the cost of bubble embolism in the vasculature was relatively small due to the tiny stature and likely desiccation tolerance of species (Raven 1977). In such plants the rapid desiccation of leaves after embolism-failure of water transport is non-lethal, and water transport is rapidly repairable by capillary collapse of embolisms upon rewetting. However, the evolution of vascular plants has seen a loss of desiccation tolerance in vegetative tissues, leading to a much greater cost associated with catastrophic xylem embolism. In the majority of vascular sporophytes, uncontrolled xylem embolism would rapidly cause leaf death by desiccation.

Stomatal pores in the leaf surface thus provide a highly effective way for plants to significantly delay the onset of xylem embolism by closing to dramatically reduce leaf water loss prior to the initiation of xylem embolism ([Brodrribb *et al.* 2016](#)). Such a role has been proposed to explain the original proliferation of stomata on the leaves of early vascular plants ([Edwards *et al.* 1998](#)). Support for the proposition, that stomata fulfil an ancestral role in tracheophytes as a protective mechanism against xylem embolism, is displayed in the extraordinary intimacy between xylem and stomatal function and anatomy in the leaves of vascular plants. In this review we examine some key aspects of xylem-stomatal coordination, illustrating how the evolution of stomatal control revolves around a communication of xylem water potential with the closure of the pore using passive and active modes of signalling. Interactions between stomatal and xylem physiology are discussed in terms of the effect on plant ecology. Finally we discuss the anatomical and developmental principles behind xylem-stomatal coordination in leaves.

Stomatal and water transport physiology of the earliest vascular plants

A primary link between desiccation-induced xylem embolism and stomatal closure exists through the common dependence of both these processes upon leaf water content. The aperture of the stomatal pore is regulated by two flanking, adjustable, guard cells; when the turgor of these guard cells increases they deform to open the pore and conversely when guard cell turgor falls these cells collapse towards each other and close the pore (von Möhl 1856, Heath 1938). This link between guard cell turgor and stomatal aperture provides a very economic and efficient means of linking stomatal behaviour with xylem water status and embolism. If guard cell turgor could change passively in unison with leaf water status, then as leaf water status declines so too does guard cell turgor, closing the stomatal pore, thereby reducing plant water loss.

In ferns and lycophytes, which are modern day representatives of some of the earliest vascular land plants to evolve ([Kenrick & Crane 1997](#)), this simple linkage between leaf water status and stomatal aperture is one of the most conspicuous features of stomatal behaviour. In these two most basal groups of extant vascular land plants the highly predictable stomatal responses to changes in leaf water status were recognised in some of the earliest studies of stomatal responses in these lineages ([Lange *et al.* 1971](#), [Löscher 1977](#), [1979](#)). In formative work into the control of the stomata by humidity, [Lange *et al.* \(1971\)](#) selected the fern species *Polypodium vulgare* as an experimental model and documented stomatal responses to changes in humidity and temperature (or more precisely the vapour pressure deficit between the leaf and the atmosphere (VPD)) that were rapid and highly repeatable, opening at low VPD and closing at high VPD. The rapidity and repeatability of these stomatal responses was attributed to VPD influencing guard cell turgor via a direct linkage between leaf water status and guard cell turgor in these plants.

The readily observable and highly predictable stomatal responses to VPD led [Lange *et al.* \(1971\)](#) to develop a model for stomatal responses that could accurately predict leaf gas exchange in *P. vulgare*. This “hydropassive” model was grounded in their observations that guard cell turgor in this fern species was influenced directly by, and passively responsive to, changes to atmospheric water status. The predictable and passive nature of stomatal responses to changes in leaf water status has since been described in a wide diversity of species spanning these two most basal extant lineages of vascular land plants ([Brodrribb & McAdam 2011](#), [Martins *et al.* 2016](#)). Under controlled conditions, as well as in field settings, the stomatal responses to changes in leaf water status in these species can, with high accuracy, be predicted by a simple hydraulic model that assumes guard cell turgor, although higher than epidermal turgor in the light, is directly affected by leaf water potential such that stomatal aperture is directly responsive to biophysical processes of leaf water supply, including leaf hydraulic capacitance, hydraulic conductance and transpiration rate ([Brodrribb & McAdam 2011](#), [Husby *et al.* 2014](#), [Martins *et al.* 2014](#)). It has been argued recently that the hydropassive responses of stomata found in the two most basal lineages of vascular land plants, ferns and lycophytes represent the ancestral mechanism for regulating leaf hydration in vascular land plants ([Brodrribb & McAdam 2011](#)). Evolution in both the control of water loss by stomata, and the efficiency of water supply to the photosynthetic tissue, appears to have had a major influence over the ecological diversity and evolutionary success of these vascular plant lineages.

The rise of lycophytes (~410 million years ago) marked a major transition in the evolution of land plants, with this group diversifying over the following 100 million years to form the world’s first forests in the Devonian to mid-Carboniferous ([Phillips & DiMichele 1992](#), [Rickards 2000](#)). The first vascularized leaves of lycophytes contributed to a major surge in plant productivity on land, yet lycophyte leaves are simple and, with only a few rare exceptions ([Wagner *et al.* 1982](#)), single-veined ([Banks 2009](#)). A single vein places major hydraulic constraints on the theoretical maximum width and thickness of leaves because the

high resistance to water flow through the mesophyll tissue causes a rapid decline in water potential with hydraulic distance from the xylem tissue during transpiration ([Zwieniecki et al. 2004](#), [Brodribb et al. 2007](#)) preventing stomatal opening. Evidence for this hydraulic constraint on leaf size and morphology is vivid in the lycophyte clade with the majority of aerial leaves from the lycophyte fossil record, as well as in living representatives of this clade (including *Selaginella*, *Lycopodium* and *Huperzia*), being small (< 2cm) and linear in form ([Boyce 2010](#)). One of the largest aerial lycophyte leaves documented in the fossil record was over 76 cm long, yet had a maximum width less than 6 mm ([Kosanke 1979](#)). Very soon after the divergence of lycophytes, the earliest ferns evolved reticulate venation in leaves, a patterning that allowed homogeneous irrigation of a broad lamina, eliminating the hydraulic constraint of a single vein on leaf morphological diversity ([Boyce 2005](#)). This evolutionary adaptation in vein development led to the radiation of a huge diversity of leaf sizes and morphologies in ferns ([Vasco et al. 2013](#)). Plasticity in leaf size was presumably an adaptation to environments with variable light, with broad leaves being far more efficient at harvesting light under a canopy ([Boyce & Knoll 2002](#)).

While the topology of leaf venation and leaf morphology is diverse across lycophytes and ferns, the ecological diversity of these two lineages remains highly constrained. The apparent ecological conservatism in ferns and lycophytes has been traditionally attributed to an ephemeral reproductive stage (the gametophyte) that is dependent on liquid water for fertilisation to occur ([Campbell 1905](#)). However, more recently this idea has been challenged by the observation that gametophytes of many fern species can persist for many years in hostile environments as well as through cycles of extreme desiccation, suggesting instead that the limited ecological diversity in these lineages may be due to features of the sporophyte ([Watkins et al. 2007](#), [Watkins et al. 2010](#)). In order to survive periods of soil water stress fern and lycophyte species rely on significant modifications to morphology or xylem physiology, including (i) an ability to resprout when leaves die from drought ([McAdam & Brodribb 2013](#), [Baer et al. 2016](#)), (ii) a high capacitance which prolongs the time between

stomatal closure and xylem embolism (a strategy particularly employed by epiphytes (McAdam & Brodribb 2013)), (iii) desiccation-tolerance (Hietz 2010) or (iv), in rare cases, embolism-resistant xylem ([Pittermann *et al.* 2011](#), [Baer *et al.* 2016](#)). It has been suggested that this narrow suite of adaptations to surviving water shortage in ferns and lycophytes is constrained by the reliance on a simple hydropassive mechanism of stomatal response to leaf water potential in these lineages (McAdam & Brodribb 2013).

SEED PLANTS- A third-party connection between leaf water status and stomatal aperture

While major morphological or physiological adaptations are the only means by which fern and lycophyte species can survive prolonged periods of water deficit, seed plants have completely changed that way their stomata respond to water, using active signalling rather than passive hydraulic processes ([McAdam & Brodribb 2012b](#)). Explaining this transition away from ancestral hydropassive stomatal control in basal vascular plants, to active control in angiosperms remains controversial, but may be related to a change in stomatal anatomy, from large relatively immobile stomata in ferns to small, highly porous stomata in angiosperms. Unlike ferns, the pores in angiosperm stomata are able to open to form an almost (circular) aperture. This circular aperture provides the greatest efficiency in terms of enabling maximum CO₂ entry per pore area ([Franks & Beerling 2009](#)), but it also requires that guard cells interact mechanically with the encircling epidermal cells to accommodate their movement. Thus the epidermal cells surrounding the guard cells (subsidiary cells) exert a turgor-dependent back-pressure that limits the aperture of the stomatal pore under most circumstances ([DeMichele & Sharpe 1973](#)). A potentially disastrous by-product of the mechanical advantage of the epidermis is that as evaporation increases, epidermal back pressure declines, causing stomata to transiently open (a “wrong way response”) when leaf

water content declines ([Buckley 2005](#)). Thus an active mechanism for stomatal closure is required to drive stomatal responses of angiosperm species to changes in water content because the passive response of angiosperm stomata to increasing evaporation is an opening feedback that would lead to uncontrollable water loss.

The presence of an endogenous chemical signal involved in regulating stomatal behaviour in angiosperms was hinted at in some of the earliest work on stomata, with Stahl (1894) documenting significant differences in the stomatal response to dry air in leaves that had experienced water stress compared to plants that had not. This non-hydraulic signal for stomatal regulation has since been widely observed in field settings ([O'Grady *et al.* 1999](#)). Indeed when the earliest, hydropassive model for a stomatal response to humidity, based on the fern *P. vulgare*, was tested in the field in an angiosperm, it failed ([Schulze *et al.* 1974](#)). In particular, the fern-based model [Schulze *et al.* \(1974\)](#) used could not explain the distinct lack of recovery in stomatal aperture that occurred in the afternoon following midday, high VPD conditions in *Prunus ameniaca*. This hysteresis in the stomatal response to VPD, independent of leaf water status, is a defining feature of angiosperm stomatal behaviour ([Franks *et al.* 1998](#), [O'Grady *et al.* 1999](#), [Gwenzi *et al.* 2012](#), [Zheng *et al.* 2014](#), [McAdam & Brodribb 2015](#)). The simplest explanation for such stomatal behaviours is the presence of an endogenous chemical signal that is synthesized in response to falling leaf water potential, but that has the potential to overshoot or accumulate in the leaf, delaying stomatal recovery. A clear candidate for such a signal is the phytohormone abscisic acid (ABA). Other explanations for apparently non-hydropassive stomatal control in angiosperms invoke changes in the hydraulic conductance of leaves ([Buckley 2005](#)), but evidence or mechanisms for such dynamic variation in leaf hydraulic conductance remains uncertain. Reports of declining K_{leaf} as leaves become moderately dehydrated (during stomatal closure) have been attributed to the effect of bundle sheath turgor ([Brodribb & Holbrook 2006](#)), ABA ([Pantin *et al.* 2013](#)) or aquaporin expression ([Scoffoni *et al.* 2012](#)), and each of these could potentially mimic or augment the effect of ABA on stomata. Close examination of leaf

hydration during VPD transitions is needed to assess the possible contribution of changing K_{leaf} to the stomatal VPD response (McAdam & Brodribb 2015).

ABA signalling stomata

Abscisic acid closes stomata by activating anion channels in the guard cell membrane causing an efflux of ions and subsequently a reduction in guard cell turgor ([MacRobbie 1981](#)). The molecular processes by which ABA causes stomatal closure are extremely well described thanks to countless studies characterising genes in model angiosperms, particularly *Arabidopsis thaliana* ([Geiger et al. 2009](#)). The closure of stomata by ABA was realised very soon after the discovery of this hormone ([Mittelheuser & Van Steveninck 1969](#)), and the link between ABA levels and stomatal closure during drought soon followed ([Wright & Hiron 1969](#)). Functionally relevant increases in ABA levels have been widely documented in angiosperms exposed to high VPD ([Bauerle et al. 2004](#), [McAdam & Brodribb 2015](#)), as well as soil water stress ([Zeevaart 1971](#), [Zabadal 1974](#)). This increase in the level of ABA is regulated by a single enzyme, Nine-*cis*-epoxycarotenoid cleavage dioxygenase (NCED), responsible for catalysing the rate limiting step in the ABA biosynthetic pathway, the cleavage of the carotenoid violaxanthin to xanthoxin ([Qin & Zeevaart 1999](#), [Thompson et al. 2000](#)). The biosynthesis of ABA is thought to be triggered by a decline in the turgor of ABA biosynthetic tissues ([Pierce & Raschke 1980](#), [Davies et al. 1981](#), [McAdam & Brodribb 2016](#)). This decline in cell turgor has been shown to trigger an increase in the expression of *NCED* following exposure to increased evaporative demand or soil water stress ([McAdam & Brodribb 2016](#)).

While the primary location for ABA biosynthesis remains debatable, a substantial body of evidence suggests that ABA is synthesised in the leaf vascular tissue ([Holbrook et al. 2002](#), [Christmann et al. 2005](#), [Kuromori et al. 2014](#), [Manzi et al. 2015](#), [McAdam & Brodribb 2015](#)), although other sites have been proposed ([Zhang et al. 1987](#), [Bauer et al. 2013](#)). A location

of ABA biosynthesis in or around the vascular tissue is intuitive as these cells should experience a much more uniform reduction in water potential during transpiration than mesophyll cells, which may be subject to strong gradients (McAdam *et al.* 2016). In angiosperms, during exposure to high VPD, leaf turgor declines rapidly over minutes to the point at which ABA synthesis is triggered resulting in a rapid increase in ABA levels and stomatal closure. Upon returning to low VPD there is a delay in the reduction of ABA levels due to the apparently slower process of ABA catabolism ([Zeevaart 1980](#)) causing pronounced hysteresis in the reopening of stomata (McAdam & Brodribb 2015). This classic, hysteretic response of angiosperm stomata to changes in VPD contrasts strongly with the passively controlled stomatal responses to VPD in ferns and lycophytes which are non-hysteretic (Figure 1). The central role of ABA in regulating angiosperm stomata in response to changes in leaf water status is highlighted by the dysfunctional stomatal responses to VPD and drought reported in ABA biosynthetic and signalling mutants ([Tal & Nevo 1973](#), [Koorneef *et al.* 1984](#), [Mustilli *et al.* 2002](#), [Xie *et al.* 2006](#), [Merilo *et al.* 2015](#)). Understanding why ABA signalling evolved as a “third party” linkage between leaf water status and stomatal aperture is challenging, but one explanation is that it provides gymnosperms and angiosperms with the evolutionary potential for a far more diverse array of stomatal response to changes in leaf water status. One of the most widely documented variations in stomatal response to leaf water status in seed plants is the continuum between iso- or anisohydric responses to leaf water potential ([Klein 2014](#)). These two extremes have been intimately linked to differences in the regulation of stomata by ABA dynamics ([Tardieu & Simonneau 1998](#), [Brodribb & McAdam 2013](#)). The two contrasting strategies are characterised by stomata that close abruptly on reaching a specific leaf water potential, closing over a very small window of leaf water potential (isohydric species) or stomata that gradually close as leaf water potential declines (anisohydric species) ([Tardieu & Simonneau 1998](#)). In order to close rapidly upon reaching a specific leaf water potential isohydric species must rely on ABA to close stomata when leaves reach a particular leaf water

potential, in contrast ABA production in anisohydric species may peak then decline under prolonged water stress ([Brodrribb *et al.* 2014](#)).

Gymnosperms as ABA intermediates

While debate remains as to whether ferns and lycophytes have signalling components required for guard cells to respond to ABA ([Brodrribb & McAdam 2011](#), [Ruszala *et al.* 2011](#)), it is clear that endogenous ABA plays no role in stomatal closure during drought stress in these species ([McAdam & Brodrribb 2012a](#)) (Fig. 2). Furthermore diurnal stomatal regulation of ferns and lycophytes also appears to be independent of ABA levels with ABA biosynthesis in these plant groups not occurring over a timeframe that is relevant to a stomatal response to VPD ([McAdam & Brodrribb 2015](#), [McAdam & Brodrribb 2016](#)). The most basal extant seed plants, the gymnosperms, represents an interesting group in the context of stomatal evolution as the first group of extant land plants to possess functionally relevant stomatal responses to ABA ([Jackson *et al.* 1995](#)). Gymnosperms are phylogenetically intermediate between ferns (with purely passive stomatal responses to changes in leaf water status) and angiosperms (with stomata that are controlled predominantly by ABA levels) and appear to have a stomatal control system in response to changes in water status that is intermediate between these two groups. In gymnosperms, like ferns and lycophytes, ABA synthesis occurs at a much slower rate than angiosperms, presumably because of a reliance on non-specific enzymes catalysing the final two steps of the ABA biosynthetic pathway ([Hanada *et al.* 2011](#), [McAdam *et al.* 2015](#)). As a consequence of this slower biosynthesis, ABA levels do not change during diurnal fluctuations in VPD, but despite this the stomata of gymnosperms respond predictably and hydropassively to changes in VPD, with no hysteresis observed during a reversible change in VPD under controlled conditions ([McAdam & Brodrribb 2014](#), [Martins *et al.* 2016](#)) (Fig.1) or during stomatal responses to changing atmospheric conditions in the field ([Saugier *et al.* 1997](#)). While stomatal responses to rapid, VPD driven changes in

leaf water status appear to be passively regulated by leaf water status in gymnosperms, stomatal closure during soil water stress in this clade is strongly regulated by ABA, with ABA levels having a major influence over stomatal aperture in drought stressed plants ([Jackson et al. 1995](#), [Hoffman et al. 1999](#), [Wang et al. 1999](#), [Perks et al. 2002](#), [Zuccarini et al. 2011](#), [Brodrigg et al. 2014](#)). Thus the gymnosperms appear to represent transitional characteristics in stomatal control between the hydropassive ferns and the active angiosperms. They respond to short term changes in VPD similarly to hydropassive ferns, but possess active ABA-mediated responses to longer term changes in leaf water status produced by soil dehydration (Fig. 3).

Physiological implications of stomatal-xylem co-evolution

It has long been recognized that the stomata of different species can exhibit distinct responses to plant hydration, particularly during the latter stages of water stress ([Tardieu & Simonneau 1998](#)). These differences have important implications not only for the rate at which plants desiccate, but also for the extent to which assimilation can be maintained during extended water stress. As mentioned above, terms such iso and aniso-hydric stomatal regulation are used to provide a qualitative description of species with stomata that are more (iso) or less (aniso) sensitive to declining water potential ([Tardieu & Simonneau 1998](#)). Despite the rather imprecise nature of these terms, the contrast between these “types” can be stark in dry environments. The best characterized example of the ecological impact of distinct stomatal behaviours is the Pinon-Juniper conifer community in the USA, where the Pinon pines close their stomata long before their Juniper neighbours ([Breshears et al. 2005](#)). These differences in the timing of stomatal closure are associated with very significant differences in the vulnerability of the xylem of these two species to water stress-induced embolism, and are thought to have implications for carbon balance during extended drought ([Limousin et al. 2013](#)). An interesting theory suggests that highly embolism-resistant xylem

in the *Juniper* allows this species to continue low rates of gas exchange during drought without incurring damaging xylem embolism. By contrast it is hypothesized that neighbouring pines have much more vulnerable xylem forcing them to close stomata and cease photosynthesis early during drought ([McDowell et al. 2008](#)). Recent studies demonstrate that contrasting stomatal behaviours such as those of Pinon pines and Junipers is a consistent feature across the entire conifer clade. Associations between stomatal sensitivity and xylem vulnerability have been found across a diverse sample of most conifer clades, with strong interactions found between the dynamics of ABA synthesis, stomatal closure and xylem vulnerability ([Brodribb et al. 2014](#)).

As mentioned in the previous section, the stomata of conifers are responsive to both hydropassive closure signals from tissue dehydration and active closure produced by ABA production. In some species with resistant xylem, the water potential of the leaf can fall sufficiently low during water shortage to cause stomatal closure by passive dehydration of the guard cells, while in other species such as pines, ABA plays a major role in closing stomata before water potentials fall low enough to cause xylem embolism. Divergent strategies of stomatal closure in conifers ([Brodribb & McAdam 2013](#)) appear to have evolved as a result of close linkages between the physiology of the xylem in terms of embolism resistance during water stress and stomatal physiology. Stomatal behaviours such as isohydry and anisohydry, as well as their associated ecologies are also evident in angiosperm species ([Klein 2014](#)) and are likely to also reflect linkages between hydraulic and stomatal physiologies.

Coordination of hydraulic supply and evaporative demand.

Perhaps the most telling example of functional intimacy between xylem and stomata is seen in the high degree of coordination between investment and spatial positioning of these two important tissues on leaves. The planar nature of most leaves creates a situation where the density of and arrangement of veins and stomata determine the maximum conductances of liquid (in terms of veins) and vapour phase (in terms of stomata). The water transport efficiency of leaves is largely dependent upon the distance water must traverse outside the xylem as it moves to the sites of evaporation near the stomata (Sack & Holbrook 2006). This distance is determined by the branching density of the leaf vein network ([Wylie 1939](#), [Boyce *et al.* 2009](#)). Similarly, the porosity of the epidermis to water vapour is determined by the density and size of stomatal pores on the leaf surface (Sack and Buckley, 2016). The size and spacing of stomatal pores appears to be under tight selection in angiosperms, producing an optimal use of epidermal area ([de Boer *et al.* 2016](#)), while evolution in vein density is related to changes in the gas exchange capacity of leaves ([Brodribb *et al.* 2007](#), [McElwain *et al.* 2016](#), [Scoffoni *et al.* 2016](#)). These fascinating evolutionary patterns suggest coordination between stomata and xylem in leaves may be a universal constraint in plant evolution.

Constructing leaves that uphold a conservative ratio between vein density (total vein length per unit area) and stomatal density (total stomata per unit area) ensures that a balance is struck between the rate of water supply to evaporative surfaces near the stomata and the transpirational demand for water generated by stomata. This means that stomata are supplied with the minimum amount of water required to allow them to open to an optimal aperture set by leaf photosynthetic biochemistry (Cowan & Farquhar 1977, Medlyn *et al.* 2011) under well-watered conditions, and that investment in vein infrastructure does not exceed the minimum required for optimal stomatal conductance. This view is supported by observations that vein and stomatal densities are proportional within individual plants, within species, among populations of the same species and among species in a wide range of

woody and herbaceous angiosperms and ferns ([Tanaka & Shiraiwa 2009](#), [Brodrribb & Jordan 2011](#), [Carins Murphy *et al.* 2012](#), [Zhang *et al.* 2012](#), [Brodrribb *et al.* 2013](#), [Martins *et al.* 2014](#), [Yang *et al.* 2014](#), [Zhang *et al.* 2014](#), [Fiorin *et al.* 2016](#), [Carins Murphy *et al.* in press](#)).

A constant ratio could be maintained between vein and stomatal density by one of two mechanisms (or a combination of both). Either there is synchronous differentiation of xylem and guard cells throughout leaf development, or alternatively, xylem and guard cells differentiate in the early stages of leaf development and cell expansion in the surrounding tissue augments their density simultaneously. Coordinating vein and stomatal density via similar rates of differentiation seems unlikely because the development of stomata and xylem cells appears to be governed by distinct processes ([Lau & Bergmann 2012](#), [Lucas *et al.* 2013](#)). On the other hand, there is some evidence that vein and stomatal densities peak well before final leaf size is reached and then decline as leaf expansion proceeds ([Pantin *et al.* 2012](#), [Sack *et al.* 2012](#)). Thus, assuming that leaf expansion is driven by cell expansion rather than cell initiation then the development of vein and stomatal density may be coordinated via their 'passive dilution' by leaf expansion.

In accordance with this hypothesis, shade leaves of woody angiosperms are often larger and have lower densities of veins and stomata than sun leaves ([Bergen 1904](#), [Wylie 1949](#), [Wylie 1951](#), [Abrams & Kubiske 1990](#)). Furthermore, a shade-induced increase in leaf size (mediated by epidermal cell size) in the subtropical angiosperm tree *Toona ciliata* was associated with a proportional and coordinated decrease in vein and stomatal density ([Carins Murphy *et al.* 2012](#)). However, a coordinated shade-induced decrease in vein and stomatal density was only partially related to leaf size in the temperate angiosperm tree *Nothofagus cunninghamii* ([Brodrribb & Jordan 2011](#)). Recent research also found that leaf size can be modified independently of epidermal cell size in woody angiosperms (Carins

[Murphy *et al.* 2014](#)) and that vein and stomatal densities are strongly correlated with cell size but not leaf size across Proteaceae species ([Brodrigg *et al.* 2013](#)).

In support of these previous studies, Carins Murphy *et al.* (in press) found that variation in leaf size in response to light intensity and among species was largely independent of epidermal cell size in a diverse range of woody and herbaceous angiosperms. Consequently, the geometric relationships between vein density, stomatal density and cell size were examined (using the same species) by modelling the relationships between these traits based on the assumption that veins and stomata respond passively to epidermal cell expansion (Carins Murphy *et al.* in press). Modelled relationships agreed closely with observed relationships suggesting that the 'passive dilution' of veins and stomata by differential epidermal cell expansion maintains a constant ratio between vein and stomatal density during leaf development (Fig. 4) (Carins Murphy *et al.* in press). These results suggest that, under glasshouse conditions at least, differential rates of stomatal initiation (reflected in the stomatal index) have only a minor influence on stomatal density. Thus, because leaf hydraulic conductance is closely related to vein density ([Brodrigg *et al.* 2007](#)) and stomatal conductance to stomatal density ([Franks & Beerling 2009](#)) a developmental link between leaf veins and stomata via the process of cell expansion effectively balances leaf water supply with potential transpirational demand.

Conclusion

Since their evolution, the fortunes of stomata and xylem have been closely linked. Without xylem, plants would be unable to lift their canopies beyond the boundary layer of the soil, but without stomata to control embolism, xylem tissue would only function in ever-wet soils. Thus we see evidence of a 400 million year history of collaboration between these critical tissues in vascular plants. Here we have discussed evidence of functional and anatomical coordination, but there remain many questions as to the developmental mechanisms responsible for maintaining the strong linkages evident among all lineages of land plants. Great advances in the understanding of both vein and stomatal development have been made in the last decade, but there is a surprising lack of intersection between the pathways identified in the differentiation of these two tissues. This apparent developmental independence is remarkable in the context of such close functional coordination, but may also reflect a layer of control that is yet to be discovered, such as common control by cell size or cell number. Developmental mutants in stomatal and vein anatomy will provide new insights into the fascinating question of how veins and stomata communicate.

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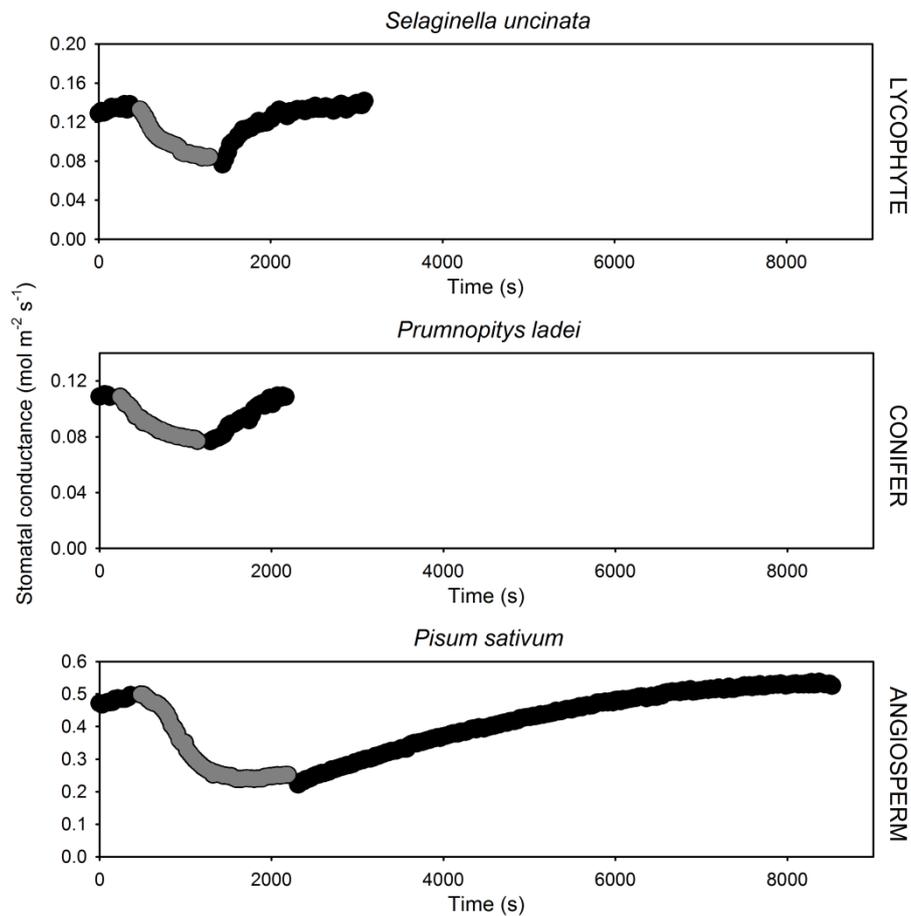


Figure 1. Stomatal responses to changes in vapour pressure deficit are rapid and highly predictable, with no hysteresis in species from basal, non-angiosperm lineages of land plants including lycophytes, ferns and conifers. In representative species area adjusted stomatal conductance was monitored through a reversible step change in VPD increasing from 0.7 kPa to 1.5 kPa (marked in grey) followed by a return to 0.7 kPa (in black). Data are taken from McAdam and Brodribb (2016) published at (www.plantphysiol.org) and are Copyright of the American Society of Plant Biologists.

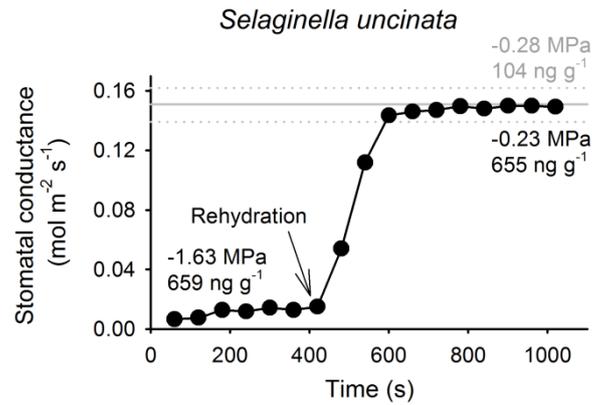


Figure 2. Endogenous ABA plays no role in closing the stomata of ferns and lycophytes during soil water stress (McAdam & Brodribb 2012a). The stomata of the lycophyte *Selaginella uncinata* close when drought stressed as the leaf experiences both low leaf water potentials and high foliar ABA levels. Upon instantaneous rehydration of the stem stomata in this species rapidly reopen despite ABA levels remaining high. Grey lines and lettering indicate mean stomatal conductance ($n = 3, \pm SE$), leaf water potential and foliar ABA level in the same plant prior to water stress.

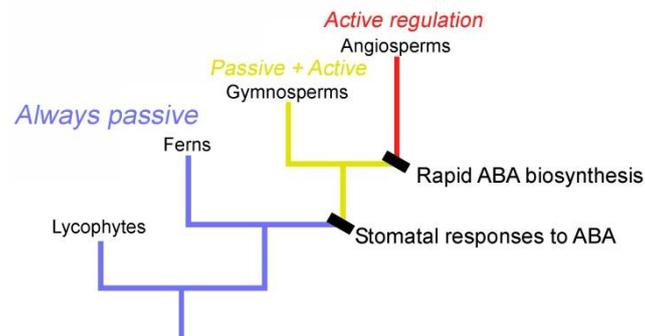


Figure 3. Differences in the mode of stomatal control used by vascular plants to regulate leaf hydration. Ferns and lycophytes respond hydropassively to leaf water content while angiosperms rely on active ABA-mediated control of stomata. Gymnosperms are responsive to ABA, but rely on hydropassive regulation in short term responses to humidity.

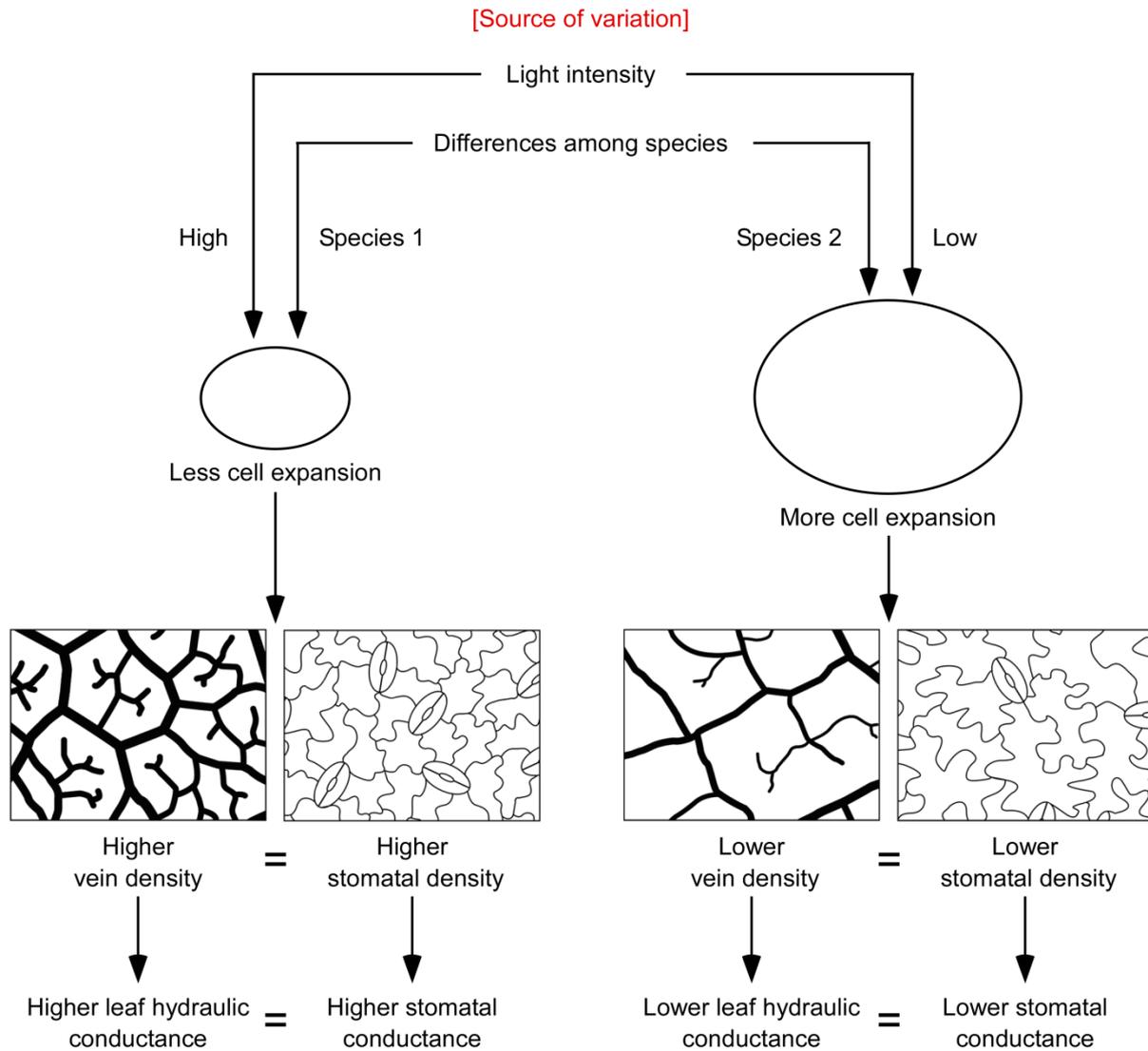


Figure 4. Differential cell expansion in response to variation in light intensity, or variation among species, ‘dilutes’ leaf veins and stomata in unison. This results in proportional co-variation of vein and stomatal density with cell size and a balance between water supply (leaf hydraulic conductance) and transpirational demand (stomatal conductance).