The evolution of water transport in plants: an integrated approach

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ABSTRACT

This review examines the evolution of the plant vascular system from its beginnings in the green algae to modern arborescent plants, highlighting the recent advances in developmental, organismal, geochemical and climatological research that have contributed to our understanding of the evolution of xylem. Hydraulic trade-offs in vascular structure–function are discussed in the context of canopy support and drought and freeze–thaw stress resistance. This qualitative and quantitative neontological approach to palaeobotany may be useful for interpreting the water-transport efficiencies and hydraulic limits in fossil plants. Large variations in atmospheric carbon dioxide levels are recorded in leaf stomatal densities, and may have had profound impacts on the water conservation strategies of ancient plants. A hypothesis that links vascular function with stomatal density is presented and examined in the context of the evolution of wood and/or vessels. A discussion of the broader impacts of plant transport on hydrology and climate concludes this review.

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INTRODUCTION

Plants can control climate as well as respond to it. Recent models supported by independent isotopic evidence suggest that it was the evolution of large, Devonian land plants that may have contributed to the dramatic reductions in atmospheric CO$_2$ in the Late Devonian (Beerling & Berner, 2002; Berner, 2005, 2006) while progressively deeper and more complex root systems accelerated pedogenesis and carbon sequestration (Retallack, 1997; Algeo & Scheckler, 1998; Raven & Edwards, 2001; Berner, 2005). Although the contribution of plants to atmospheric and biogeochemical processes continues to be well explored (Pataki et al., 2003; Bonan, 2008; Malhi et al., 2008), the role of plant water transport in responding to or acting on both of these components has been somewhat overlooked.

There is a direct link, however, between plant water transport and climate. Indeed, important inferences about the levels of palaeoatmospheric CO$_2$ have been based on measurements of stomatal distributions in fossil plants. Stomata are microscopic pores on surfaces of green leaves and stems that allow CO$_2$ to diffuse into photosynthetic tissue, while letting water escape. Analysis of fossil plant material across the Phanerozoic suggests that low stomatal densities reflect elevated palaeoatmospheric CO$_2$ levels, whereas the opposite is true for high stomatal densities (McElwain & Chaloner, 1996; Chaloner & McElwain, 1997; McElwain et al., 1999; Retallack, 2001; Beerling, 2002; Roth-Nebelsick, 2005; Kurschner et al., 2008). Apparently, a greater number of smaller stomata are needed to counter CO$_2$ deficits at low ambient CO$_2$, rather than fewer, but larger stomata (Franks & Beerling, 2009a). However, stomata must also regulate water loss from the leaf because, for each CO$_2$ molecule fixed by the plant, 200–400 molecules of water may escape through the stomatal pore. At any point in time, the stomata must optimize plant water use efficiency, which is the carbon gain for a given amount of water lost. This trade-off and its implications on plant water balance, hydraulics and structure–function may have been apparent early in the evolution of vascular land plants.

What are the climatic consequences of transpiration for atmosphere–biosphere interactions and does the answer lie once again in the stomatal impressions of ancient leaves? How has the evolution of water transport impacted the overall design of both ancient and modern flora? The goal of this review is to address these issues in an interdisciplinary and accessible manner that draws not only on the fossil record, but also on the recent advances in molecular, physiological and evolutionary plant biology. Parts I and II introduce the basics of the cohesion–tension (C–T) theory of water transport and examine the evolutionary and developmental transitions that selected for this ubiquitous and broadly accepted mechanism.
from the earliest vascular land plants to trees. Part III highlights recent advances in our understanding of the structure and function of plant vascular tissue (xylem), and its potential utility for the reconstruction of water transport in extinct taxa from climates that were radically different from today. The review concludes with a discussion of the significance of plant water transport on ecosystem and global hydrology.

PART I: THE EVOLUTION OF WATER TRANSPORT IN TERRESTRIAL PLANTS

Much of the information in Part I draws from the fossil record, which is both rich with information yet fundamentally incomplete. Indeed, the fossil record has limited resolution with respect to the physiological, biochemical and life-history traits that are integral to understanding the transition of photosynthesis from an aquatic to a terrestrial setting. Computer models, systematics and developmental approaches can fill some of these gaps, but the veracity of these hypotheses is ultimately tested against fossils.

Thus, fossil specimens are scrutinized with much caution. The majority of fossils are preserved in riverine depositional environments, meaning that they may have travelled great distances from potentially very different habitats before settling permanently (Stewart & Rothwell, 1993; Taylor et al., 2009). Furthermore, fragmentation can disassociate fragile structures such as leaves from the rest of the plant body, thereby obscuring relationships between various tissue samples and potentially inflating species numbers. For instance, it was not until the discovery of an intact specimen bearing both wood and foliage that Archaeopteris was treated as a single species rather than two separate genera (Beck, 1960; Meyer-Berthaud et al., 1999). Lastly, diagenesis, that is the chemical and physical alteration of fossil and sediment during the conversion to rock, can also cause substantial changes to the structure and character of a specimen (Taylor et al., 2009).

Sampling biases may affect approximations of species diversity in the fossil record leading to errors in estimates of the rates of evolution, particularly if workers focus on particular localities or intervals. In general, if diversity is constant, sampling intensity will not affect results (Raymond & Metz, 1995; Tarver et al., 2007). However, evidence suggests that earlier estimates of diversity of Mid-Silurian through Late Devonian land plants may be higher than expected due to sampling bias, usually attributed to workers’ preferential interest in one era or locality over another (Raymond & Metz, 1995).

Fortunately, plant vasculature is often the most abundant and best preserved plant tissue. Indeed, some xylem samples from the earliest land plants appear as though they were frozen in time, with little distortion to the cell structure. Remarkably, even the chemical composition of early vascular tissue can be discerned to the point where we can reasonably speculate about the biomechanical properties of the stem, as well physiological ecology of the plant (Kenrick & Crane, 1991; Bateman et al., 1998; Boyce et al., 2003; Wilson et al., 2008). For this reason, macroevolutionary and neontological approaches can be fruitfully applied to research addressing evolution of plant water transport, or the relationships of fossil plants to their climates.

The mechanism of water transport in plants

Water transport in plants is not difficult to understand, but neither is it inherently intuitive. In his book ‘Vegetable Staticks’ (1727), the English physiologist Stephen Hales first remarked that water is released from a plant by ‘perspiration’. He thus captured an important element of the C–T theory, which requires that water is lost from the plant by evaporation in order for bulk flow to occur. The C–T theory was fully developed by Dixon and Joly (1894), and remains to this day the most parsimonious and experimentally supported explanation of plant hydration. The fundamental tenet of the C–T mechanism is that the water column can sustain a very high degree of tension without ‘breaking’. This phenomenon is entirely consistent with the properties of water, yet so unusual that a few workers find the C–T mechanism disturbing. Despite recent controversy, the C–T mechanism has withstood vigorous experimental scrutiny and remains the most widely accepted explanation of water movement in land plants (Pockman et al., 1995; Cochard et al., 2001; Angeles et al., 2004).

Water may be transpired at a high rate from the stomatal pores, so effective hydraulic transport must efficiently replace this lost water. Failure to supply water to the shoot as quickly as it is lost results in the familiar phenomenon of wilting followed by damage to cell membranes and ending with death (McDowell et al., 2008). Roots are a crucial component for water entry into the plant yet for the most part, it is the aerial tissues that drive water movement, much like cut flowers in a vase.

The process of water transport begins when water is lost from the mesophyll cell walls located in the interior of the leaf. Plant cell walls are a tight but porous weave of cellulose and pectin that act much like a wick. This evaporation creates capillary suction on the menisci within the cell wall pores, causing tension to be transmitted down the water column in the xylem (Pickard, 1981). The xylem tension exists because water is released from a plant by ‘perspiration’. He thus captured an important element of the C–T theory, which requires that water is lost from the plant by evaporation in order for bulk flow to occur. The C–T theory was fully developed by Dixon and Joly (1894), and remains to this day the most parsimonious and experimentally supported explanation of plant hydration. The fundamental tenet of the C–T mechanism is that the water column can sustain a very high degree of tension without ‘breaking’. This phenomenon is entirely consistent with the properties of water, yet so unusual that a few workers find the C–T mechanism disturbing. Despite recent controversy, the C–T mechanism has withstood vigorous experimental scrutiny and remains the most widely accepted explanation of water movement in land plants (Pockman et al., 1995; Cochard et al., 2001; Angeles et al., 2004).

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(i.e. tension) created by evaporation from the cell wall pores (Sperry, 2000; Tyrre & Zimmermann, 2002; Taiz & Zeiger, 2006).

Because transpiration is chiefly driven by sunlight, water movement through plants is a passive and therefore cheap process that selected for low resistance, efficient transport conduits (Sperry, 2003). These are dead, empty, require little to no metabolic input and can provide a network that allows water to reach tree canopies over 100 m in height (Koch et al., 2004; Burgess et al., 2006; Domec et al., 2008). Underlying this transport capacity is a fundamentally simple mechanism whose origins required little more than a hydrophilic cell wall to move water by capillarity. Green algae possess such a cell wall for the purpose of osmoregulation, so the framework for the evolutionary transition from wicking water over the surface of a cell to bulk flow within the specialized conduits of vascular plants was realized well before the colonization of land (Sperry, 2003; Franks & Brodribb, 2005). In addition to the cell wall, the key innovations that allowed plants to maintain hydration and adequate rates of gas exchange on land were the cuticle, the stomata and the evolution of vascular tissue. The functional significance, evolutionary history and developmental elements of these traits will be addressed below.

The green algae: earth’s first land plants

The connection between land plants and green algae (Chlorophyta) has been apparent since centuries before Darwin’s time, and there is no doubt that embryophytic land plants are descendants of freshwater green algae (Graham, 1993; Graham et al., 2000; Lewis & McCourt, 2004; McCourt et al., 2004). Specifically, it is believed that for the Charophytic green algae lacking bicarbonate pumps, competition for CO₂ may have been the driving force behind their venture onto land (Graham, 1993). Clearly, the photosynthetic benefits of fourfold increased CO₂ diffusivity and higher light levels outweighed the costs of exposure to UV radiation and dessication (Denny, 1993; Graham, 1993). A complete discussion of the morphological, biochemical and reproductive adaptations predisposing Charophytic algae to life on land is beyond the scope of this review, but excellent treatments can be found in Graham (1993), McCourt et al. (2009), Gensel (2008) and Gensel & Edwards (2001). Because the fossil record of the green algae is fragmentary and incomplete (Graham, 1993), the discussion will be limited to recent progress regarding the algal ancestry of land plants.

The green algae display a surprising array of cellular and colonial morphologies, and some species such as Chara braunii may superficially resemble plants (Mccourt et al., 2004). Although green algae are typically associated with aquatic ecosystems, they can also be indigenous and highly speciose elements of desert soil microbiotic communities (Evans & Johansen, 1999). Although the most widely recognized transition of green algae to land has been the one leading to land plants, recent evidence indicates that the green algae have colonized terrestrial surfaces on at least a dozen separate occasions (Lewis & Lewis, 2005). Molecular clock hypotheses suggest that the first green algae may have appeared in the Mid-to-Late Mesoproterozoic (1.4–1.0 billion years ago; Yoon et al., 2004) but the date of their venture onto land is unclear due to poor preservation (Taylor et al., 2009). The oldest observed silicaceous or calcium carbonate remains of green algae are from the Upper Silurian (Feist et al., 2005).

Molecular phylogenies show high support for the two Chlorophyce and Charophyce lineages of the green algae (Lewis & McCourt, 2004; Fig. 1), and there is a general consensus for the placement of the Charophytic lineage as ancestral to land plants because of the high number of shared derived molecular, biochemical, reproductive, developmental and morphological traits between these groups (Graham, 1996; Raven & Edwards, 2004). Nonetheless, the issue of which Charophytic group is most closely related to terrestrial plants remains somewhat controversial primarily because of different approaches to gene and taxon sampling (Qiu et al., 2006, Qiu, 2008; McCourt et al., 2004; Gensel, 2008; see also Turmel et al., 2007).

Despite the progress in elucidating the phylogenetic relationships of the green algae and their affinities to land plants, the shared physiological and biochemical elements of dessication tolerance have attracted less attention (Graham & Gray, 2001). This comes despite Raven’s early efforts to provide such a context for the evolution of Charophytes and his misgivings about the suitability of Charales to life on land based on latter group’s physiology and morphology (Raven, 1977). Perhaps, the plasticity of algal forms (Bhattacharya & Medlin, 1998), combined with the antiquity of the Charophyte to embryophyte divergence, hopelessly complicates such a mapping exercise (Graham & Gray, 2001) but it could nonetheless inform the plausibility of current evolutionary scenarios because environmental water stress exerts some of the strongest selective pressures on terrestrial (and marine) biota (Yancey et al., 1982).

Graham & Gray (2001) argue that the closest Charophytic ancestors of land plants would have been dessication-tolerant and occupied ephemeral, ‘variably unfavourable’ aquatic habitats. These habitats acted as sites of strong evolutionary pressures related to the effects of water deficit, and thus provided the Charophytes with an impetus towards terrestrialization (Graham & Gray, 2001). Consequently, the water status of terrestrial Charophyceans would have fluctuated in response to water availability (so-called poikilohydry), and the evolution of dessication tolerance would have been essential in allowing them to recover from otherwise fatal (80–90%) protoplasmic water loss. Dessication tolerance is a common phenomenon in basal plant lineages characterized by small-statured phenotypes such as the algae, bryophytes and some ferns (Proctor & Tuba, 2002). This trait has been lost, however, in more
derived plants due to structural constraints that prevent the collapse and refilling of cells, as well as the high cost of solute production required to minimize drought injury to cell membranes and metabolic components (Beck et al., 2007).

The significance of the plant cuticle

Although multicellularity reduces water loss by reducing total surface to volume ratios, it is unlikely that an algal colony larger than few hundred cells could have maintained adequate hydration for long periods of time without a means of restricting water loss, or efficiently replacing lost water. Additionally, because a water film represents a significant barrier to CO₂ diffusion, such a simple multicellular arrangement may have significantly constrained photosynthesis (Franks & Brodribb, 2005). All land plants must then reconcile two opposing biophysical constraints. On the one hand, photosynthesis requires large surface areas to facilitate light capture and CO₂ diffusion, whereas on the other, water conservation favours low surface/volume ratios. Three critical innovations – the cuticle, the stomata and water-conducting tissues – allowed plants to resolve these conflicting requirements. Of these three traits, the cuticle is the oldest. We currently know nothing about the evolution of plant homoihydry (that is the ability to maintain hydration over a range of external conditions of water availability; Raven & Edwards, 2004; Raven, 1984) nor the plant fossil record (Willis & McElwain, 2002; Wellman et al., 2003; Montanez et al., 2007). There is good fossil evidence to suggest that liverworts may have been present as early as the Late Ordovician (dotted line; Wellman et al., 2003).

Fig. 1 The phylogeny of land plants in the context of palaeoclimate and clade radiations over Phanerozoic time, updated from Sperry (2003). The graph indicates modelled atmospheric CO₂ concentrations according to Berner (2000), as well as corresponding large-scale climatic patterns (Zachos et al., 2001; Willis & McElwain, 2002; Berner, 2005; Huey & Ward, 2005; Brezninski et al., 2008; Trotter et al., 2008). The phylogeny is from Sperry (2003) with updates from Burleigh & Mathews (2004) and Qiu (2008). Important morphological features relevant to the evolution of plant water relations are indicated on the phylogeny according to Sperry (2003) with several updates (Proctor, 1981; Franks & Farquhar, 2007; VanAller Hemick et al., 2008). Presence in the fossil record is denoted by thick black line. Relative tracheophyte abundances were estimated or re-drawn from Niklas (1992, 1997), Willis & McElwain (2002), Singh (2004) and Montanez et al. (2007). There is good fossil evidence to suggest that liverworts may have been present as early as the Late Ordovician (dotted line; Wellman et al., 2003).
cuticle can significantly restrict water loss, it is unlikely that the colonization of land by plants would have occurred without the evolution of this layer (Niklas, 1992; Raven & Edwards, 2004). Waxes are the key constituents of the cuticle barrier but it is the cutin fraction that is most resilient to degradation and thus best conserved in the fossil record (Schreiber & Riederer, 1996). It is noteworthy that some Charophytic algae can synthesize a wax-free extracellular layer that may allow a limited amount of water and CO₂ diffusion (Graham, 1993; Edwards et al., 1996).

Historically, the usefulness of fossilized cuticle lay primarily in the patterns of preserved stomatal impressions whose size, distribution and abundance are used to infer palaeo-atmospheric CO₂ concentrations (McElwain & Chaloner, 1996; McElwain et al., 1999; Royer et al., 2001; Retallack, 2001; Kouwenberg et al., 2003). Unfortunately, cuticle thickness alone is a poor predictor of species’ drought tolerance because cuticle permeability and thickness may not be related (Edwards et al., 1996; McElwain & Chaloner, 1996). However, emerging work has shown that cuticle alkanes record the deuterium isotope signatures (δD) of meteoric water and can serve as accurate biomarkers for palaeoclimatic reconstruction, particularly if isotopic models take into account the relationships between leaf wax δD patterns and latitude, plant form and photosynthetic pathway (Sachse et al., 2004; Liu & Yang, 2008). Because deuterium enrichment is also partially controlled by transpiration and soil evaporation, n-alkanes may also be useful in constraining estimates of transpiration or evapotranspiration from well-preserved fossil material (Smith & Freeman, 2006).

The evolution of stomata

The cuticle imposes a high degree of resistance on gaseous diffusion, so it must be perforated in order for CO₂ to enter. These perforations take the form of stomata, the 30- to 80-μm long epidermal pores that are formed by the aperture of two adjacent elongated guard cells and found on surfaces of most photosynthetic tissue (Hetherington & Woodward, 2003; Taylor et al., 2009). It is the turgor-mediated opening and closing of the guard cells in response to leaf hydration, light levels, atmospheric CO₂, abscisic acid and soil water availability that controls the diffusion of CO₂ and water vapour between the leaf and the atmosphere (Davies & Zhang, 1991; Assmann & Wang, 2001; Buckley, 2005; Messinger et al., 2006; Ainsworth & Rogers, 2007). Stomatal conductance is tightly coupled to hydraulic transport in land plants (Jones & Sutherland, 1991; Brodribb & Holbrook, 2004), so stomatal evolution may have occurred closely in time with the vascular system (Kenrick & Crane, 1997; Sperry, 2003).

The position of stomata on the land plant phylogeny is decided by the placement of ancestral taxa such as liverworts and bryophytes, the standing of which has been called into question (Raven, 2002). Some species of liverworts and mosses exhibit what appear to be proto- or pseudo-stomata but as the function of these pores in gas exchange is unclear (see below; Ducquet et al., 2009; Proctor, 1981), they may be unsuitable candidates for stomatal precursors. Hence, true stomata are currently accepted to be monophyletic, with their origins in the hornworts (Raven, 2002).

Unfortunately, the fossil record is silent with regard to changes in epidermal cells that may have led to the formation of stomata, but simple perforations are apparent in the cuticles of taxonomically mysterious taxa such as Spongiophyton (Gensel et al., 1991) and Nematothallus from the Lower Devonian (Edwards et al., 1998; Taylor et al., 2009). These organisms had thick cuticles, so epidermal pores would have significantly reduced resistance for gaseous diffusion. Similar perforations found on the thalli of the extant liverwort, Conocephalum conicum probably function in gas exchange (Proctor, 1981; Raven & Edwards, 2004), but it is unlikely that they exert dynamic control over water loss. Without active regulation courtesy of the guard cells, these plants passively and continually lose water in response to the atmospheric vapour pressure deficit.

The oldest unequivocal stomatal specimens have been described from unidentified cuticle and tissue fragments from the Pridoli epoch (Upper Silurian, 416–418.7 Mya, Upper Silurian, Edwards et al., 1996, 1998). In these samples, the stomatal apparatus appears nearly circular, consisting of two thick guard cells that create a lenticular pore. Perhaps due to poor preservation and small fragment size, there is no evidence for a substomatal chamber and in several of these Silurian samples, epidermal cells appear to underlie the guard cells. Small substomatal cavities are found, however, in samples of pro-tracheophytes such as Rhynia gwynne-vaughanii, Aglaophyton major, Hornophyton lignieri and the lycophyte Asteroxylon mackiei from the Lower Devonian (397.5–416 Mya; Edwards et al., 1998). These taxa generally have low stomatal frequencies, and some such as Asteroxylon have sunken stomata –features that are consistent with the idea that water stress was the key abiotic variable acting on early terrestrial vegetation (Gray et al., 1985).

Based on our understanding of stomatal behaviour in modern taxa, it has long been accepted that the evolutionary pressures that drove water conservation strategies favoured the coupling of the cuticle with stomata (Raven, 2002; Franks & Brodribb, 2005). However, the underlying assumption that the regulation of water loss was the impetus for the evolution of stomata (especially in early land plants) has recently been challenged by Ducquet et al. (2009) who argue that the ‘pseudostomata’ of the Sphagnum moss evolved to facilitate dehydration in sporophytic capsules, rather than conserve water. In this plant, it is dehiscence and collapse of guard cells that opens the stomatal aperture, rather than an increase in guard cell pressure, as typically observed in higher land plants. Ducquet et al.’s results leave the evolution of stomata open to interpretation. One could argue that stomata initially
appeared in mosses for the purpose of dehydration and were subsequently adapted for gas exchange in higher plants, but alternatively stomata could have evolved to facilitate gas exchange on land, but were co-opted by the mosses for the purpose of dehydration. A final option is that, in *Sphagnum*, the stomata fulfill both functions over time (Duckett *et al.*, 2009).

Duckett *et al.*’s study changes our perspective on the evolution of stomata and will undoubtedly motivate future work on stomatal structure and function in modern taxa. Despite the fact that stomatal design has remained fundamentally unchanged, notable differences in size, density, shape and ornamentation are apparent across the major plant groups (Ziegler, 1987; Franks & Farquhar, 2007). The authors argue that faster response times of graminoid stomata may have increased water-use efficiency during the global aridification that peaked 45 to 30 Mya, and thus conferred the grasses with a competitive advantage.

Others have applied finite element analysis to explore the relationships between stomatal structure and function to show that sunken stomata, a cuticular lining, small substomatal chamber size and reduced stomatal aperture, each have the potential to greatly decrease stomatal conductance (Roth-Nebelsick, 2007). How variation in the size of the substomatal chamber relates to stomatal size is empirically unknown, as are the consequences of this allometry for photosynthesis, but Roth-Nebelsick’s model suggests that transpiration estimates based solely on fossil stomatal density and aperture size may overestimate stomatal conductance.

Along these lines, the gas-exchange response of Rhyniophytic plants has received considerable attention because the morphological detail of their tissues is well preserved, and their terrestrial habit represents the beginning of the evolution of modern plant physiology. It appears that Rhyniophytes colonized diverse habitats with variations in temperature (Edwards *et al.*, 2001) and water availability due to periodic flooding (Rice *et al.*, 2002; Trewin *et al.*, 2003), thereby providing the context in which the ecophysiology of these taxa can be examined. The cuticle of Rhyniophytic taxa is characterized by low stomatal densities suggesting that in the CO₂-rich climate of the Lower Devonian, these plants would have exhibited high water-use efficiencies (Edwards *et al.*, 1998). Computer simulations suggest that transpiration and photosynthesis were greatest in *Nothia aphylla*, a ‘leafy’ Rhyniophyte with relatively high stomatal densities (El-Saadawy & Lacey, 1979), when compared with the more conservative *Rhynia* and *Aglaophyton* (Konrad *et al.*, 2000; Roth-Nebelsick & Konrad, 2003). The stricter water-use strategies of the latter two taxa probably reflect the absence of a well-developed root system (Roth-Nebelsick & Konrad, 2003).

Lastly, increased water use efficiency may have been the driving force behind selection for presumably ‘optimized’, i.e. rapid, stomatal responses of angiosperms to short-term changes in CO₂, relative to conifers and ferns (Brodribb *et al.*, 2009). Considering that water use efficiency positively affects species’ reproductive output (Farris & Lechowicz, 1990; Dudley, 1996; Ackerly *et al.*, 2000), one could argue that the evolution of stomata single-handedly provided ancestral land plants with a competitive advantage, and thus helped transform the terrestrial landscape into a three-dimensional mosaic of vegetation, rather than a dense film of liverworts.

### The transition to plant vascular tissue

With the cuticle and the stomata in place, the evolution of water-conducting cells completed the basic bauplan needed for plant homoiohydry (Raven & Edwards, 2004). What are the transitional steps from undifferentiated mesophyll cells to a bundle of directionally aligned hollow tubes, and what are the biotic and abiotic signals responsible for this pattern in the first place? Understanding vascular tissue ontogeny may inform our interpretation of fossil material and its relevance to the evolution of xylem.

Our current outlook on the differentiation of unspecified plant cells into elongated tracheary elements is shaped by model systems such as *Zinnia*, *Arabidopsis* and *Populus* (Tuskan *et al.*, 2006; Turner *et al.*, 2007). In plants with a meristematic, procambial region, cambial cells are programmed to differentiate into either phloem or vascular tissue. However, under appropriate culture conditions, mesophyll cells can also be made to de-differentiate and develop into tracheary elements (Fig. 2). Auxin, the newly discovered xylem hormone and other signalling compounds control these changes and much progress has been made in elucidating the genetic regulation of cell differentiation (Roberts & McCann, 2000; Chaffey, 2002). What makes this particularly relevant in an evolutionary context is that the totipotency of plant cells can be so easily manipulated with the appropriate signalling compounds, most of which are produced by neighbouring cells (Nieminen *et al.*, 2004). In other words, the design of even the most basic of vascular systems belonging to liverworts (Ligrone & Duckett, 1996) and bryophytes (Proctor & Tuba, 2002) required little more than the appropriate signal to upregulate a suite of genes that control cell differentiation.

One of the critical steps to the formation of vascular tissue may have been the establishment of a polar auxin gradient because auxin stimulates the differentiation, patterning and elongation of tracheary elements (McCann, 1997; Sieburth & Deyholos, 2006). An auxin gradient can also arise due to wounding, and generate a response characterized by cell rotation in the new tissue formed near the injury (Kramer *et al.*, 2008). Interestingly, evidence for this rotated cell arrangement was recently discovered in secondary vascular tis-
sue belonging to an *Archeopteris* (upper Devonian, 375 Mya; Rothwell & Lev-Yadun, 2005) as well as in wood of tree-sized fossil equisetophytes and arborescent lycophytes belonging to different lineages (Rothwell *et al.*, 2008). In these plants, the independent origins of secondary vascular tissue suggest a role for the parallel evolution of regulation by auxin in each clade (Rothwell *et al.*, 2008).

There is evidence that vascular tissue differentiation is a homologous phenomenon in land plants. Indeed, molecular data indicate that auxin metabolic pathways are present in basal plant lineages such as the Charales, liverworts and bryophytes (Cooke *et al.*, 2002). Of further interest are computer models showing close correspondence between predicted procambial vascular patterns based on theoretical input auxin concentrations, and their close correspondence to actual stelar patterns observed in the Early Devonian *Psilophyton* and several members of the pro-gymnosperm group, the Aneurophytales (Stein, 1993). Altogether, there is growing evidence for the contribution of auxin to the evolution of vascular plants, and the subsequent diversification of Silurian and Devonian plant lineages.

Following cell differentiation, the process of expansion is driven by mechanical forces that are a function of osmotic pressurization, which in mature plant cells can generate pressures ranging from 0.1 to 3 MPa (Somerville *et al.*, 2004; Taiz & Zeiger, 2006). brassinosteroid hormones are thought to play a role in the complex co-ordination of the simultaneous expansion of multiple cells, although several auxin-mediated enzymes acidify and loosen the fibrous, cellulose cell wall matrix (Wang & He, 2004; Turner *et al.*, 2007). Importantly, a class of proteins known as expansins regulates the loosening of the cell wall (Cosgrove, 2005; Popper, 2008). Expansins are not only ubiquitous across the land plant taxa, but also thought to be integral to the development of xylem and evolution of the plant vascular system (Popper, 2008). The genetic toolbox required for cell wall expansion is present in algae, so the only other key innovation in the evolution of plant vascular tissue would have been the development of the cellulosic secondary cell wall for increased rigidity. In plants, this secondary wall is deposited interior to the primary cell wall following cell expansion, but excluded from the vessel endwall and the pitted regions of the conduit that allow water to move.
from one cell to another (Somerville et al., 2004; Choat et al., 2008).

The rigidification of cell walls with polyphenols probably occurred synchronously, or nearly so with the evolution of early plant vascular systems (see next section). Developmentally, the deposition of lignin or its precursors such as lignan into the cellulose, pectin and protein wall matrix of the secondary wall is the final step towards increasing the stiffness and compressive resistance of the conducting cell as well as its impermeamility to water (Niklas, 1992; Lewis, 1999; Koehler & Telewski, 2006). For example, Arabidopsis and Nicotiana mutants with disrupted lignin synthesis not only exhibit malformed vasculature, but also show a high frequency of crimped or collapsed conduits (Turner & Somerville, 1997; Piquemal et al., 1998; Jones et al., 2001).

The complex genetic and chemical components of lignification have been identified (Boerjan et al., 2003; Peter & Neale, 2004) so a solid grasp of the functional implications of the process on plant hydraulics is in the near future. Recently, 12 genes were targeted as the probable candidates of vascular lignification in Arabidopsis (Raes et al., 2003) whereas the Black Cottonwood genome-sequencing project uncovered a high number of gene families responsible for ligno-cellulosic wall formation (Tuskan et al., 2006). However, the genetics of herbaceous model systems such as those of Arabidopsis and Zinnia are easily manipulated (McCann, 1997; Roberts & McCann, 2000) so, at this point in time, we know more about tracheary element formation from these plants than from woody taxa. Interestingly, evidence from the Zinnia model of tracheary element formation suggests that lignification follows cell death, which means that surrounding parenchyma tissue may be responsible for the synthesis and release of lignin precursors (Turner et al., 2007). Certainly, once the digestion of cell contents begins, cell growth has ceased so lignin deposition is the logical, final stage of creating a functioning, but dead water-filled xylem conduit.

Lignins were generally thought to be absent from the algae, but the cell walls of the Charophyte Coleochaete were found to contain lignin-like compounds, adding further support for their close association with land plants (Delwiche et al., 1989). True lignin was recently discovered in the flexible, joint-like tissue; the red algae Calliarthron, prompting the suggestion that lignin biosynthetic pathways may have had an early function in biomechanics, in addition to protecting cells from microbial attack and UV damage (Martone et al., 2009; see also Niklas, 1992). Both studies indicate that the fundamental pathways of lignin biosynthesis are deeply conserved, so the lignification of xylem was essentially a function of time and increased tissue specificity.

On a final note, the biosynthesis of lignin is dependent on oxygen, leading some workers to speculate that elevated O2 produced by the abundant land plants during the Mid-Devonian to the Early Carboniferous, may have spurred the evolution of xylem lignification (Graham et al., 1995; Berner, 2006; Berner et al., 2007). In the proposed sequence of events, a rise in O2 levels due to photosynthesis facilitated lignification, which in turn supported the evolution of arborescence, further increasing photosynthetic biomass in a feedforward cycle. Interestingly, some would argue that lignin is not even a prerequisite for conferring stiffness to the plant body per se and is merely a secondary requirement for arborescence because non-lignified tissues can impart rigidity to the plant body (Rowe & Speck, 2004), thereby eliminating the necessity for the role of oxygen in the first place.

The hydraulic advantage of wide, hollow conduits is evident in the context of the Hagen–Poiseuille
equation, which predicts water-transport efficiency through smooth-walled capillaries. Here, the flow rate of water, \( Q \), with a viscosity \( \eta \), passing through a capillary with a diameter of \( D \) is proportional to the pressure gradient \( \frac{dP}{dx} \) driving the flow such that,

\[
Q = \left( \frac{\pi D^4}{128\eta} \right) \frac{dP}{dx},
\]  

Equation 1 demonstrates that hydraulic efficiency is proportional to the conduit diameter raised to the fourth power, so a modest increase in conduit diameter leads to significant gains in transport (Tyree et al., 1994; Tyree & Zimmermann, 2002). Single conduit measurements of hydraulic efficiency agree with the predictions of the Hagen–Poiseuille equation (Zwieniecki et al., 2001). Functionally, this means that all else being equal, 16 conduits with a 20-\( \mu \)m diameter are required to achieve the hydraulic efficiency of one 40-\( \mu \)m vessel (Fig. 3).

The higher area-specific conductivity of larger conduits confers several advantages, an important one being that a plant can invest less carbon in transport tissue, particularly as the conduits are dead. Secondly, the lower transport resistance associated with large conduits reduces the pressure drop that is required to move water from the roots to the leaves. Very negative water potentials can predispose the xylem to dysfunction (see Part III) and stress the leaf tissue, both of which can lead to reductions in gas exchange. Lastly, high transport efficiencies characteristic of xylem with large conduits can support increased transpiration rates, which in turn allow higher rates of photosynthesis (Brodribb & Feild, 2000; Brodribb et al., 2002; Santiago et al., 2004). Predictably, plants have exploited the relationship between diameter and efficiency such that there has been a 60-fold increase in conduit diameter since the evolution of the early 5- to 8-\( \mu \)m wide Cooksonian tracheids from the upper Silurian (416 Mya; Niklas, 1992; Fig. 4) to the nearly 500-\( \mu \)m wide vessels of extant tropical vines and lianas (Ewers, 1985).

**PART II: VASCULAR PLANTS IN THE FOSSIL RECORD**

Conducting tissue in protracheophytes and early vascular plants

The Late Silurian to the Early Devonian represent a period of remarkable terrestrial plant speciation as well as exploration of different forms of vascular structure. Geochemically based models suggest that elevated atmospheric CO\(_2\) coupled with 2–4 °C warmer sea surface temperatures characterized the climate during this initial phase of plant diversification (Berner, 2006; Came et al., 2007). Some of the extinct taxa from this period may well be the ancestors of certain bryophytic lineages or true vascular land plants, while others were early experiments in the homoihydric lifestyle. The most well-studied (though neither the oldest nor most ancestral) plants of this period are from the Early Devonian Rhynie Chert (398–396 Mya). This group has traditionally occupied the position of the simplest vascular plants but this perspective has changed as it became clear that Rhyniophytes share features of both bryophytes and vascular plants (Kenrick & Crane, 1997; Taylor et al., 2009). Kenrick & Crane (1997) provide the most widely accepted cladistic treatment of the evolution of early land plants, using among other character traits, the anatomy of vascular tissue to decide placement on the phylogeny. What emerges is a trend towards xylem comprised of progressively larger tracheids with an increasingly complex and mechanically robust wall anatomy (Kenrick & Crane, 1991, 1997; Edwards, 2003; Taylor et al., 2009) presumably reflecting selection for efficient vascular transport and conduit support under negative xylem pressures. The evolution of angiosperm vessels and...
fibres for water transport and support respectively represents the pinnacle of vascular specialization in woody plants. Informative photographs and detailed commentary of the taxa described in this section can be found in Taylor et al. (2009), Edwards (2003), Kenrick & Crane (1991), Stewart & Rothwell (1993) and Kenrick & Crane (1997).

Liverworts occupy the basal position in the land plant phylogeny so the nearest analogue to the most primitive form of vascular tissue probably resides in the gametophyte of the extant, stomatalless liverwort *Symphyogyna brasiliensis* and its relatives in the Pallaviciniineae. Non-lignified, elongate water-conducting cells with thickened secondary walls are packed together in the central regions of *Symphyogyna*’s thallus in what may ostensibly be the most primitive vascular bundle. Water travels from one cell to another via plasmodesmatal pores and perforated, primary wall regions along the length of the cell (Ligrone & Duckett, 1996; Ligrone et al., 2000). Close examination of the recently discovered fossil liverwort *Metzgeriothallus sharonae* (Mid-Devonian, 390 Mya) could confirm that vascular tissue may have been present in this extinct species, although the authors do not discuss this possibility (VanAller Hernick et al., 2008; Fig. 5). It is not unlikely however, that *M. sharonae* may represent one of the earliest experiments in the design of transport tissue as both the extant *Symphyogyna* and this fossil liverwort are ascribed to the order Metzgeriales.

The oldest macrofossil vascular land plants are the cooksonioids of the Mid-Silurian (Wenlockian, 428–422 Mya) represented by *Cooksonia* (Edwards & Feehan, 1980), and similar fossils such as *Tortilicaulis* (Edwards, 1996; Bateman et al., 1998; Boyce, 2008). Early land plants from the Silurian and Early Devonian form a diverse group with nebulous evolutionary affinities, but currently accepted hypotheses suggest that, despite its antiquity in the fossil record, *Cooksonia* is derived from the protoracheophytes, and belongs between the rhiophytes and the lycophytes (Kenrick & Crane, 1997). Efforts to confidently place *Cooksonia* on the land plant phylogeny are also hampered by limited and partially preserved...
fossil material which may or may not actually by *Cooksonia* in the strict sense (Boyce, 2008).

Cooksonioids were probably only a few centimetres tall, with erect, bifurcating axial stems that ranged in diameter from <100 μm to 1.5 mm (Boyce, 2008). Lacking roots and leaves, the shoots of these simple plants terminated in sporangia. Although *Cooksonia* is accepted as the first vascular plant (Taylor *et al.*, 2009), the actual contribution of the limited vascular tissue to water transport is questionable because the conduits are so narrow (<5 μm; Boyce, 2008). Close examination has shown that the conduits of *Cooksonia pertonii* exhibit thick annual and spiral wall rings, when compared with the thinner primary wall thickenings found in modern protoxylem (Edwards, 2003). These rings rigidify an otherwise highly perforated and what appears to be a mechanically weak conduit wall. Despite their small size, *Cooksonia*’s conduits may have adequately delivered water to axial tissues in order to maintain the necessary turgor pressure for an erect stature (Bateman *et al.*, 1998). *Cooksonia* possessed stomata, but they were low in frequency and occurred primarily in the vicinity of the sporangia, prompting the suggestion that their role was to facilitate nutrient transport via transpiration-driven water flow rather than increase CO₂ diffusion for photosynthesis (Edwards *et al.*, 1996, 1998; Boyce, 2008).

The younger, more derived flora of the famous Rhynie Chert has provided an abundance of specimens that reveal the diversity of conducting tissues in early land plants (Taylor *et al.*, 2009). The so-called G- and P-type tracheids found in eutraphytes and euphyllophytes respectively, range from 30 to 80 μm in diameter and may exhibit scalariform (ladder-like) pitting and a decay-resistant inner layer that covers the pit apertures (Kenrick & Crane, 1991, 1997; Edwards, 2003). At first glance, the P-tracheid xylem of the euphyllophyte *Psilophyton* resembles that of ferns, thereby representing the most modern conduit anatomy of Lower Devonian taxa.

Taken together, the general impression from the fossil record is that vascular tissue in the earliest land plants became progressively more efficient due to selection for larger lumen diameters. Simultaneously, tracheids became increasingly more robust as evidenced by the helical and annular thickenings in S- and G-type tracheids, relative to the smooth, thin-walled conduits of *Aglaophyton*. Such fortified conduits would have withstood the negative xylem pressures associated with water stress, rather than collapsed. Implosion-resistant, and
Vascular tissue in advanced land plants

For nearly 300 million years, tracheid-based xylem remained the most prevalent transport tissue in the plant kingdom. The ornate tracheids of the Early Devonian tracheophytes eventually led to the hydraulically efficient, large-diameter, scalariform tracheids of more derived groups such as the horsetails and ferns, and eventually to the thick-walled tracheids that provided a biomechanical and a transport function in progymnosperms, gingkos and conifers (Tyree & Zimmermann, 2002; Pittermann et al., 2006a). According to the classic scenario of Bailey & Tupper (1918) the subsequent evolution of tracheids into the fibres and vessel elements of angiosperm xylem capitalized on the functional specialization of each cell type. Fibres are short and thick-walled, providing the necessary strength for increasingly lateral canopies while the large vessel elements reduce resistance to water transport.

Not surprisingly, competition for light and the need for effective spore dispersal prompted the evolution of arborescence (Niklas, 1985, 1992; Mosbrugger, 1990), well before the appearance of secondary xylem, that is wood. In fact, the evolution of secondary xylem is not even a requirement for elevated canopies assuming that other tissues such as a hypodermal streme, thick external periderm or cortex tissue provide structural support (Niklas, 1997; Rowe & Speck, 2004, 2005). This means that during the Devonian, the tree habit was free to arise independently and more or less simultaneously in ferns, lepidophytes, horsetails and progymnosperms (Mosbrugger, 1990).

Modern and extant tree ferns lack secondary xylem so structural support comes from abundant schlerenchymatous (i.e. lignified) fibres, support roots, numerous thin axes and other plant matter that makes up the false trunk (Mosbrugger, 1990; Taylor et al., 2009). The Mid-Cretaceous fern, Tempskya is a good example of this strategy as its main axis looks disproportionately large relative to the size of the emerging fronds due to the accumulation of roots, fibres and plant debris (Taylor et al., 2009). Modern ferns, such as those in the genera Dicksonia and Cyathea rarely exceed heights of 10 m, but the reasons for this height limit have not been examined. Buckling is unlikely to occur given the compressive strength of fern trunks (Mosbrugger, 1990; Niklas, 1997). Although hydraulic limits in ferns may arise from limited conduit size and conduit density (Gibson et al., 1984) it may be that there is little to gain by growing tall in a light-limited environment already dominated by tall angiosperms with expansive canopies.

The evolution of the vascular cambium (the axial meristem that gives rise to secondary xylem) relaxed the restrictions on axial height and girth imposed by primary xylem. Fossil evidence suggests that the vascular cambium originated early in the evolution of land plants and may have evolved in response to strong selective pressure for increased plant size. The primitive progymnosperm group Aneurophytales were the first to show limited secondary xylem by the Mid-Devonian, but by the Late Devonian, secondary xylem was present in representatives of nearly all major plant groups including Lepidodendrales, horsetails, seed ferns, progymnosperms and perhaps even in the Cladoxylopsids (fern affiliates), although the vascular patterns of these fern allies are highly variable even within a specimen (Niklas, 1997; Taylor et al., 2009).

Of the three types of vascular cambia that evolved in plants, the bifacial and monocot ‘etagen’ cambia are present in mod-
ern taxa, while the unifacial vascular cambium disappeared after the Late Devonian (Esau, 1943; Niklas, 1997). The unifacial vascular cambium was present in Sphenophyllum (relatives of horsetails), and Sigillaria and Lepidodendron, both representatives of Carboniferous arborescent Lycopsods (Thomas & Watson, 1976; Taylor et al., 2009). Lepidodendron was a dominant element of the swampy Carboniferous landscape, whereas Sigillaria may have occupied drier sites (Phillips & DiMichele, 1992; Stewart & Rothwell, 1993; Taylor et al., 2009). Because the unifacial cambium was capable of only producing new xylem cells to the interior of the main axis (periclinal division), and unable to divide radially to generate new cambial initial cells (anticlinal division), the girth of these plants was thought to be determinate and established early in their development by a large number of fusiform initials (Eggert, 1961; Cichan, 1985a,b). Consequently, lateral expansion of the secondary xylem as well as the stem axis in Sphenophyllum and the tree like Lycopsods could only be achieved by the progressive, centrifugal enlargement of the cambial initials rather than an increase in the number cambial cells (Cichan, 1985a,b).

Despite the limited cambial expansion of these early trees, they often reached heights of 40 m and their basal diameters exceeded 2 m. Reconstructions suggest that Sigillaria and Lepidodendron were characterized by vertical, monopodial axes that lacked side-branches but terminated in a large, prominent meristem producing only a limited amount of xylem may have been the transport bottleneck failing to deliver enough water to both maintain turgor and support gas exchange during drought. This is consistent with the idea that arborescent lycopsods disappeared in response to a drier climate in the Late Carboniferous (Phillips & DiMichele, 1992). Concurrently, the apparent developmental inflexibility of the unifacial cambium may have limited structural diversity and branching, which also would have placed these early trees at a competitive disadvantage for resources and perhaps contributed to their demise (Rowe & Speck, 2005). Alas, this ‘nascent innovation’ was successful for only 50 million years.

By constrast, the bifacial vascular cambium first characterized by the Mid-Denovian progymnosperms Archaeopteris (Beck, 1960, 1970; Meyer-Berthaud et al., 1999) is found in all modern trees. As its name implies, the bifacial cambium produces secondary xylem towards the inside of the stem axis, and phloem to the outside. Its primary advantage lies in the capacity of the cambial initials to divide both anticlinally and periclinally such that each year, new cells are added in a manner that allows for the indeterminate expansion of the stem axis as well as the addition of branches. Of the two cambial strategies discussed thus far, selection has favoured the bifacial vascular cambium because it successfully combines water transport with mechanical support of the canopy (Rowe & Speck, 2004, 2005).

The monocot ‘etagen’ cambium is recently derived in the angiosperm monocots (Lower Cretaceous; Herendeen & Crane, 1995) and produces lignified fibres in well-ordered, discrete vascular bundles containing both xylem and phloem (Esau, 1943; Rudall, 1995; Tomlinson, 2006). There is no secondary xylem in monocots. Arborescent monocots such as palms are unusual in the plant world because most of the stem volume is occupied by primary vascular tissue that is thought to remain fully functional over the life of the plant (Tyree & Zimmermann, 2002; Tomlinson, 2006).

On the whole, the evolutionary trajectory of plant vascular systems has selected for some combination of increasing hydraulic efficiency and biomechanical support. The evaluation of Murray’s law in the context of different wood types now serves as a classic example of the support structure versus hydraulic efficiency trade-off. Murray’s law was originally developed for cardiovascular design, and states that for a fixed investment in blood and vessel volume, the optimal transport arrangement equalizes the sum of conduit radii cubed along the flow path. Interestingly, this axiom also applies to vascular plants but only if the support requirement is entirely separate from the transport function, as in the xylem of leaves, ring-porous species (see Fig. 3), vines and some ferns (McCulloh et al., 2003; McCulloh & Sperry, 2005). Significant deviation from Murray’s law occurs when vascular tissue increasingly performs a structural function, as in the case of conifers (McCulloh et al., 2004). Murray’s law has not been evaluated in extinct taxa such as the Sphenophyllum or Lepidodendron, but it is unlikely that the unifacial cambium compromised the water transport in tissue that was largely decoupled from canopy support. In other words, transport efficiency is likely to have selected for adherence to Murray’s law even in morphologically complex, although extinct taxa, but this hypothesis remains to be tested.

Because the structural and hydraulic performance of fossil taxa is impossible to examine directly, the remaining option is to uncover the biophysical principles that underlie modern plant structure and function, and to apply them to ancient taxa, or towards models of new morphologies. Indeed, the theoretical approach has yielded a vast array of spectacularly diverse, yet realistic plant ‘morphospace’ that accurately describes both modern and fossil flora (Niklas, 1997, 2004). Because the plant vascular system reflects the overall construc-
tion of a broad array of plant life forms including herbaceous, woody, lianaceous and monocot plants, we can utilize the structure–function relationships of modern xylem to generate reasonable hypotheses about the physiology and habitat of ancient plants.

PART III: THE FUNCTIONAL ANATOMY OF MODERN PLANT VASCULAR TISSUE – THE HYDRAULIC TRADE-OFFS OF RESISTANCE TO ABIOTIC STRESS

A good grasp of anatomy is useful to correctly interpret the relationships between xylem structure and function. This is because the transport characteristics of the xylem impose physical constraints on the rate at which water can be supplied to the leaves, and on the maximum transpiration rates allowed by stomata (Tyree & Sperry, 1989; Sperry et al., 1998; Brodribb, 2009). The following discussion will focus on conifers and woody angiosperms simply because most vascular structure function work has been performed on woody species. However, there is no apparent reason why the principles derived from this research should not apply equally well to non-woody plants.

The structure of conifer and angiosperm xylem

Conifer wood is homogenous, meaning that it is composed entirely of overlapping individual cells referred to as tracheids. Modern conifer tracheids range in size from about 0.5 to nearly 4 mm in length and 8–80 μm in diameter (Panshin & de Zeeuw, 1980; Pittermann & Sperry, 2003; Pittermann et al., 2006a) and their size depends on, among other factors, their location within the tree and whether or not they function in supporting the canopy. In general, root tracheids tend to be longer and wider with thinner conduit walls rather than stem tracheids (Pittermann et al., 2006b). This is because root tissue is optimized for the delivery of water to the canopy whereas stem tissue must compromise between water transport and structural support (Sperry et al., 2006; Pittermann et al., 2006a,b).

Angiosperm xylem is slightly more complex than that of conifers and exhibits a greater capacity for variation. Water transport occurs through multicellular conduits known as vessels. Vessels are composed of two or more single-celled vessel elements that are stacked one on top of the other to form an elongated tube that may be up to 0.5 mm in diameter and 2 m in length. Unlike in the tracheids, the distal regions of the individual vessel elements (known as endwalls) are digested away either entirely, or in a scalariform pattern with the end-result resembling an elongated pipe that offers a low resistance pathway for water movement from the root to the canopy (Ewers, 1985; Schulte & Castle, 1993; Ellerby & Ennos, 1998). The vessels are embedded in a matrix of short, thick-walled fibres (Fig. 3). Consequently, angiosperm xylem is thought to reflect a more optimized vascular strategy that capitalizes on the division of labour to efficiently deliver water through the vessels and while relegating storage and mechanical requirements to the fibres.

In conifers as well as in angiosperms, water moves from one conduit to another via perforations in the cell wall, also known as bordered pits (Fig. 6; Choat et al., 2008). These pits are composed of a water-permeable, cellulotic pit membrane that is surrounded on either side by the overarching secondary cell wall, the pit border. The perforated cell wall provides an aperture through which water can pass from one conduit to another through the pit membrane.

Significant progress has been made in our understanding of the evolution of xylem structure and function, and the trade-offs that accompany pit structure, conduit length and conduit diameter in conifers and angiosperms (Tyree et al., 1994; Comstock & Sperry, 2000; Sperry et al., 2006). Specifically, we now have a quantitative grasp of the functional consequences of wood bearing either vessels or tracheids, and are in better position to consider the vascular performance of fossil plants.

It is intuitive that conduit lumen diameter and conduit length highly impact conducting efficiency and that as such, selection has favoured the evolution of larger conduits (Fig. 4). Indeed, this perspective has been emphasized in the literature effectively arguing that angiosperm wood, with its longer and wider vessels, is inherently more hydraulically efficient that the homoxylous, tracheid-based wood of conifers (Tyree & Zimmermann, 2002). Broadly speaking, the hydraulic properties of both plant forms are thought to have significant implications for their resource competition, fitness and ecological dominance (Bond, 1989). However, this perspective has recently shifted because on closer inspection, the transport issue is not just one of conduit volume but of overall conduit structure: aside from length, the biggest difference between vessels and tracheids resides in the endwalls of these conduits where the interconduit pits are located.

Conifers possess intratracheid pits that are composed of two distinct regions: the outer margo region is rather porous with micron-scale openings and thus offers minimal resistance to water flow whereas the inner, thickened torus region is impermeable. By contrast, the intervessel pit membranes of angiosperms are composed of a homogenous, tightly woven mesh of microfibrils. Pore sizes range from 5 to 420 nm and the membrane lacks a central thickening (Choat et al., 2008). The functional ramifications of this differing pit structure on water transport are profound because the pit resistance to water flow is almost 60X lower in conifer pit membranes (6 ± 1 MPa s m−1) than in those of angiosperms (336 ± 81 MPa s m−1). This reduction in pit resistance is equivalent to an almost eightfold increase in tracheid length, and thus hydraulically compensates for the otherwise increased resistance that would be associated with greater number of endwall crossings due to short tracheids. By
contrast, the greater length of the angiosperm vessel counteracts the increased hydraulic resistance offered by the homogenous pit membrane. The end result is that conifer and angiosperm xylem exhibits similar hydraulic efficiency at an equivalent conduit diameter (Pittermann et al., 2005; Sperry et al., 2006).

The ancestral condition of tracheid-based wood relies on homogenous pit membranes rather than the more efficient torus–margo arrangement for the interconduit flow of water. This suggests that these ancestral tracheids may have been up to 38-fold less efficient than tracheids of equivalent size that bear the more derived torus–margo membrane arrangement (Sperry et al., 2006). Furthermore, it has been shown that across a broad sampling of conifers and angiosperms, the end-wall contribution to conduit resistance is 64 ± 4% and 56 ± 2% in conifer tracheids and eudicot vessels respectively (Wheeler et al., 2005; Pittermann et al., 2006b). Since cycads, ferns and the basal vesselless angiosperms possess tracheids without a torus–margo pit membrane, how does pit resistance scale with lumen resistance in these more primitive taxa? So far, this has only been examined across a broad sampling of vesselless angiosperms. Interestingly, vesselless angiosperms show hydraulic conductivities that are much higher than predicted, and surprisingly similar to the xylem conductivities of conifers (Hacke et al., 2007).

How can we employ these structure–function relationships in the context of plant palaeo-physiology? Xylem is preserved extremely well in the fossil record so it is possible with good sampling and sectioning techniques to re-construct hydraulic transport in extinct taxa, or at the very least get a sense of it. However, it is important to acknowledge the implicit assumption here, which is that xylem structure–function relationships have been conserved over time. This is doubtful because many extinct taxa combine suites of vascular traits that have no living analogue. *Archaeopteris*, the Devonian pro-gymnosperm is just such a case because its xylem employs tracheids with an unusual clustered arrangement of intertracheid pits that lack a torus–margo membrane (Beck, 1970; Meyer-Berthaud et al., 1999; Taylor et al., 2009). Although it is currently impossible to directly measure hydraulic efficiency in fossil xylem, it is possible to construct and test a scaled, physical model much in the way that Lancashire & Ennos (2002) used glycerine and toy truck tyres to evaluate the resistance of interconduit pits.

Perhaps the most simplistic way of retrodicting water-transport efficiency is to use the Hagen–Poiseuille relationship to calculate the lumen conductivity ($k_l$) on a xylem area ($A$) basis,

$$k_l = \frac{\pi \sum D^4}{A 128 \eta},$$

(2)

where $A$ is the area represented by the sum of $D^4$ or the total xylem area. It is important to keep in mind that scalariform

Fig. 6 A diagram describing the events of freeze–thaw and air-seeding cavitation in conifers (see text). Shaded tracheids are water filled and under negative xylem pressure ($P_X$), clear tracheids are air filled and at atmospheric pressure ($P_0$). In functional tracheids, the thickened torus region of the pit membrane is centrally located within the pit chamber. When a neighbouring tracheid is air-filled, the pit membrane deflects against the pit border and the torus is pressed against the pit aperture, thereby isolating the dysfunctional tracheid. Air seeding occurs when the torus becomes dislodged from its sealing position. In angiosperms, air seeding occurs through the largest pore in the deflected, homogenous pit membrane (see Choat et al., 2008 for details).
perforation plates in vessels, as well as interconduit pits reduce the actual conductivity by well over 40% of what the Hagen–Poiseuille relationship predicts (see discussion above, Sperry et al., 2006; Schulte & Castle, 1993). Similarly, the helical and annular thickenings found in the protoxylem of extinct plants, and the tracheids of Cooksonia and Sennicaulis can reduce the effective diameter of the conduits, and thus reduce $k_e$ (Jeje & Zimmermann, 1979).

The Hagen–Poiseuille method may be most readily applicable in a broadly comparative study of well-classified plants that fall clearly into either the woody coniferous or woody angiosperm category, where we can assume that pit membranes are likely to constitute a fraction of hydraulic resistance that is comparable to their living relatives. However, equation 2 can also be applied to taxa where pit membranes have not broadly been examined such as in the cycads and ferns, but with much less certainty. For example, Calkin et al. (1986) determined that digestion of pit membranes with cellulase increased hydraulic conductivity by 66% in Pteris vittata. Modelling fern xylem may be challenging particularly in taxa that harbour vessels in addition to the more commonly observed tracheids (Carlquist & Schneider, 2007).

However, another interesting exercise is to model fluid flow in extinct taxa with xylem anatomy that is no longer found in modern plants. Cichan (1986) was one of the first to recognize the value of this approach, and thus applied the Hagen–Poiseuille relationship to tracheids in the secondary xylem of Carboniferous pteridophytic and progymnosperm taxa in order to estimate the comparative hydraulic performance of these extinct plants. In his survey, tracheid diameters ranged from a minimum of 36 μm in the Sphenophyta (i.e. horsetail) Arthropitys communis to a maximum of 174 μm in the seed fern Medullosa noei. The tracheid size of Medullosan wood is exceptional by today’s standards because the largest conduits in extinct plants are found in the lianas, with vessels (not tracheids) commonly in excess of 150 μm (Ewers, 1985; Cichan, 1986). As expected, predicted $k_e$ was the highest in plants with the largest tracheid diameters such as M. noei and Sphenophyllum plurifoliatum, reaching or exceeding the $k_e$ of extant vines and lianas (Cichan, 1986). Clearly, these extinct plants exploited a morphospace that was quite different from today’s forms.

However, given what we know about pit resistance in woody plants, we can build upon Cichan’s method to achieve a higher level of precision in our transport models. In practice, hydraulic conductance ($K$) quantifies the efficiency of water transport through xylem, and is measured as the flow rate of liquid water ($Q$) for a given pressure gradient ($ΔΨ$) driving flow:

$$K = \frac{Q}{ΔΨ}. \quad (3)$$

However, $K$ cannot be measured directly on fossil material, so an alternative approach for estimating $K$ is to separate hydraulic resistance ($R_{CA}$, that is $1/K$) into its constituent resistances, those being the conduit lumen resistance ($R_l$) and the endwall resistance ($R_w$) not unlike resistors in series (eqn 4; for model details, see Lancashire & Ennos, 2002; Sperry & Hacke, 2004; Pittermann et al., 2006a,b; Wilson et al., 2008).

$$R_{w} = R_{L} + R_{W}, \quad (4)$$

The endwall resistance can be broken down into

$$R_{W} = \frac{2R_{pit}}{N_{pits}}, \quad (5)$$

where $N_{pits}$ represents the number of pits per endwall. Pit resistance, $R_{pit}$ is described by the addition of the pit aperture and pit membrane resistances, both of which can be separated into their constituents including the porosity of the pit membrane, the size of the pit membrane pores, the thickness of the membrane microfibrils, and the depth and width of the pit aperture (described in detail in Sperry & Hacke, 2004; Wilson et al., 2008).

Lastly, the inverse of the Hagen–Poiseuille equation estimates flow across half of a tube’s length,

$$R_{L} = \frac{64ηL}{πD^4}, \quad (6)$$

where $L$ represents tracheid length.

Wilson et al. (2008) applied a more complete version of the above model to investigate the hydraulic resistance of Medullosa, Cordaites and a Pinus species. This choice of living and extinct plants allowed the authors to compare the structure and function of tracheids with and without torus–margo pitting. Interestingly, Medullosa and Cordaites have tracheids that have angiosperm-like, homogenous pit membranes, presumably a suboptimal combination of conduit size and pitting that is expected to produce a substantial drop in hydraulic efficiency (on a sapwood area basis) relative to equivalently sized tracheids with torus–margo pitting (Pittermann et al., 2005). However, the conduit-specific conductivity of Medullosa was calculated to be over two magnitudes higher than that of extant conifers, a reflection of the unusually wide and long tracheids that constitute this species’ xylem (Wilson et al., 2008). The authors suggest that the vasculature and hydraulic performance of these fossil plants is similar to angiosperm lianas, whose climbing habit eliminates any need for the xylem to perform canopy support function. This is consistent with the climbing hooks and tendrils present on the branches and pinnae of Medullosa specimens (Krings et al., 2003).

Because water transport sets the upper limit for transpiration, the relatively high hydraulic efficiency of Medullosan xylem is in line with its large leaves and high leaf stomatal densities (Wilson et al., 2008). This plant probably required large amounts of water to support photosynthesis, which may have narrowed its habitat to humid tropical floodplains (DiMichele et al., 2006).
The approach to partitioning resistances in fossil angiosperm xylem can be accomplished in manner similar to that of Wilson et al. (2008) but it could prove to be more challenging because vessels may be longer than tracheids by several orders of magnitude and the vessel network may be tortuous, with complex pitting patterns (Tyree & Zimmermann, 2002; Kitin et al., 2004). We can however turn to the structure–function relationships derived from extant angiosperms to find simple, anatomically based correlates of hydraulic efficiency. Again, vessel diameter may prove to be the most useful metric because it scales well with length and the pitted endwall area, and thus correlates strongly with xylem transport capacity (Wheeler et al., 2005; Sperry et al., 2006).

Lastly, the \( R_{CA} \) of conifer tracheids with torus–margo pitting can be inferred from the following summary equation,

\[
R_{CA} = \left[ \frac{128\pi}{\pi D^2} + \frac{r_T D}{F_T L^2} \right] (1 + C)^2, \tag{7}
\]

where \( R_L \) and \( R_W \) are described by \( [128\pi/(\pi D^2)] \) and \( [r_T D/(F_T L^2)] \) respectively (Pittermann et al., 2006b). Essentially, mean \( R_{CA} \) is determined by conduit lumen diameter \( (D) \), the length of the conduit \( (L) \), intertracheid pit resistance on an area basis \( (r_T) \), the fraction of the tracheid wall area occupied by pits \( (F_T) \) and the ratio of the tracheid double wall thickness to lumen diameter \( (C) \) (Pittermann et al., 2006b; Sperry et al., 2006). The average intertracheid \( r_T \) is \( 6 \pm 1 \text{ MPa} \text{ s}^{-1} \) but some degree of variation exists in the north temperate conifer clades that may reflect tracheid location within the plant as well as species’ drought resistance and habitat preference (Mayer et al., 2002; Burgess et al., 2006; Pittermann et al., 2006b; Domec et al., 2008).

**Relationships between xylem anatomy, and drought and freeze–thaw induced cavitation**

Not only can wood anatomy be used to describe species’ hydraulic efficiency but it can also help identify species’ limits to water transport. This is because the vascular features that allow us to model conductivity can also capture the susceptibility of xylem to hydraulic failure caused by drought or freeze–thaw induced cavitation. In this way, wood anatomy can potentially serve as an additional indicator of a fossil plant’s access to water and/or palaeoclimate.

Water transport by the C–T mechanism works remarkably well but it is not without its limitations. When leaf transpiration exceeds the transport capacity of the xylem such as during a drought, the pressure in the water column can become negative enough to allow the phase change of water from a liquid to a vapour state. This transition is known as cavitation or air-seeding, and it is caused by the aspiration of air into the transpiration stream through the pit membrane. If the pull of the transpiration stream creates sufficient tension in the sap, the air bubble expands and the conduit is simultaneously drained of water. Ultimately, a mixture of air and vapour fills the entire conduit to create an embolism, which blocks water transport in that cell (Fig. 6). Because embolism reduces the number of functional conduits, water must subsequently travel from the root to the leaf at progressively more negative xylem pressures through a higher resistance pathway. Under increasing drought conditions, these higher tensions predispose the xylem to further cavitation events that can potentially lead to the dangerous situation of runaway embolism and plant death (Tyree & Sperry, 1988; Hacke & Sperry, 2001; Tyree & Zimmermann, 2002; Choat et al., 2008).

Species’ cavitation resistance is typically presented in the form of vulnerability curves, which express the per cent loss of hydraulic conductivity due to embolism in response to more negative water potentials (Fig. 7; Hacke & Sperry, 2001). Vulnerability curves can be generated from almost any portion of a plant such as roots, stems and leaves. The xylem pressure at which a segment exhibits 50% loss of hydraulic conductivity is referred to as the \( P_{50} \), or the ‘cavitation pressure’, and can be used to compare cavitation resistance between plant organs or species.

Despite the looming threat of a hydraulic catastrophe, plants are only as cavitation resistant as their environment requires them to be. This is because there are several costs associated with embolism resistance, namely carbon investment and reduced transport efficiency. Progressively negative water potentials can threaten xylem conduits with implosion because the tension exerted on the water column exposes the conduit walls to a combination of hoop and bending stresses (Hacke et al., 2001). Hence, xylem conduits must be sufficiently, but not overly reinforced to withstand wall collapse. Anatomically, this translates to a tight, positive correlation between the ratio of conduit double wall thickness \( (t) \) to hydraulic mean lumen diameter, that is the empty space confined by the inner walls of the conduit \( [b \cdot (t/b)^2] \) and species’ cavitation pressure (Fig. 8; Kolb & Sperry, 1999; Hacke 2010 Blackwell Publishing Ltd
Hence, conduit (sampling is required to achieve adequate resolution (Fig. 9). Diameter is also coupled with species’ cavitation pressure, Erwin).

To achieve improved cavitation resistance, an increase in tracheid (as well as a support role. This means that on average, stem tracheids exhibit greater ($t/b_h^2$) than vessels at a given cavitation pressure (Fig. 8; Sperry et al., 2006; Hacke et al., 2001). To achieve improved cavitation resistance, an increase in tracheid ($t/b_h^2$) is accomplished by shrinking the tracheid lumen diameter rather than increasing tracheid wall thickness. Consequently, conifer xylem exhibits a 46-fold range in hydraulic conductivity – a significant strength versus transport efficiency trade-off (Pittermann et al., 2006a; Sperry et al., 2006).

A similar cavitation safety versus efficiency trade-off is evident in angiosperms but unlike in the conifers, it may be more tightly coupled to the process of air seeding at the pit level. The recently proposed ‘pit area hypothesis’ suggests that vulnerability to air seeding is related to the intervessel pit area on a conduit. As air seeding in angiosperms is thought to occur through the largest pore on a pit membrane, the likelihood of an air seeding event rises with increasing pit area (Wheeler et al., 2005; Sperry et al., 2006; Choat et al., 2008).

Intervessel pit area scales with vessel diameter, thus providing an explanation for the weak, although consistent relationship between vessel diameter and vulnerability to cavitation. In conifers, air seeding is also thought to occur through the pit membrane but no relationship exists between the pit area and cavitation pressure (Pittermann et al., 2006b). In water filled tracheids, water moves from one tracheid to another through the porous margo region of the pit membrane. Should one tracheid become embolized, surface tension in the margo deflects the pit membrane such that the torus is appressed against the pit aperture and air is prevented from entering the neighbouring, functional tracheid. Air seeding occurs when the xylem pressure is negative enough to dislodge the torus from its sealing position (Fig. 6). Consequently, it is the ratio of the torus diameter to the pit aperture diameter that is thought to control air seeding in conifers, while the progressive reductions in pit aperture with $P_{50}$ may represent the pit-level hydraulic cost of cavitation resistance (Burgess et al., 2006; Domec et al., 2008; Hacke & Jansen, 2009).

Lastly, hydraulic dysfunction may also be caused by freeze–thaw cavitation, a common phenomenon in plants inhabiting seasonal or high elevation climates. When xylem sap freezes, dissolved gases are coalesced into bubbles during ice formation. This is because air is insoluble in ice. The bubbles remain inert while the sap is frozen, but may expand as it melts and tensions are re-introduced into the xylem. If xylem pressures are sufficiently negative during thawing, the largest of these bubbles may expand and nucleate an embolism (Figs 6 and 7). The likelihood of freeze–thaw induced embolism is described by a modification of the capillary equation, such that the xylem pressure ($P_x$) must be more negative than the critical freeze–thaw cavitation pressure ($P_{50}$) in order for the bubble to expand and embolism to occur.
where \( T \) is the surface tension of water and \( r_b \) is the radius of the largest bubble. Hence, the larger the bubble, the less negative the \( P_{Xb} \) required for bubble expansion (Davis et al., 1999; Pittermann & Sperry, 2003).

In contrast to drought-induced embolism, conduit diameter is explicitly linked to species’ vulnerability to freezing induced embolism. This is because \( r_b \) is a function of radial air content, which is directly proportional to conduit diameter (Pittermann & Sperry, 2003, 2005). Practically, this means that species with an average conduit diameter >30 \( \mu \)m are highly susceptible to freeze–thaw cavitation, even under mildly negative xylem tensions. By contrast, plants with narrow conduit diameters are largely protected from this form of stress because the smaller bubbles that are produced upon freezing have a greater tendency to shrink and redissolve during the thaw (Pittermann & Sperry, 2005). Hence, taxa with narrow conduits such as alpine conifers are freeze–thaw cavitation resistant but at the cost of reduced hydraulic conductance, while the opposite is true of plants with large-diameter conduits.

The interdependence between xylem pressure and conduit diameter was examined in conifer wood, and used to roughly approximate the freeze–thaw vulnerability curves from a tracheid diameter distribution according to,

\[
P_{Xb} = 120D_t^{1.53},
\]

where \( D_t \) is the critical cavitation diameter, which is the conduit diameter that will embolize due to freezing and thawing at a pressure \( P_{Xb} \) (Pittermann & Sperry, 2005). Whether equation 9 applies to angiosperm wood is currently unknown.

Although the relationships between wood anatomy and resistance to drought and freeze-thaw stress are well developed and could potentially be applied to fossil material, it is important that any inferences about species’ habitat or microhabitat based on xylem anatomy be approached with caution. If possible, conclusions should consider the evolutionary lineage, the overall plant form, and leaf venation patterns, which are coupled to species hydraulic and photosynthetic capacity (Sack & Frola, 2006; Brodribb et al., 2007; Brodribb, 2009), and may also be related to habitat (Royer et al., 2008). For example, inferences about species’ resistance to drought-induced cavitation based on diameter alone may be erroneous, because conduit diameter can be weakly linked to cavitation in angiosperms (Wheeler et al., 2005; Sperry et al., 2006) but may be influenced by evolutionary lineage in both angiosperms and conifers (Cavender-Bares et al., 2001; Pittermann et al., 2006a,b). We know that despite having xylem with large diameter conduits, ring-porous species such as oaks, are actually resistant to drought-induced cavitation and exhibit low water potentials \textit{in situ} (Cochard et al., 1992) and may thus deviate from the seemingly simple relationship that should exist between conduit diameter and \( P_{Xb} \). However, the relationship between vessel diameter and vulnerability to freezing-induced cavitation in oaks is generally consistent with theory (Sperry & Sullivan, 1992; Davis et al., 1999; Cavender-Bares et al., 2005).

The relationship between stomatal conductance and the structure and function of xylem

The guiding principle of effective water transport is that the hydraulic supply of water must meet the transpirational demands of the leaves (Sperry et al., 1998; Sperry, 2000). Not surprisingly, the stomata play a key role because should the water supply become compromised, or transpiration exceed the plant’s hydraulic capacity, stomatal closure is the first line of defence against desiccation. However, stomatal conductance \( (g_s) \) must also be well regulated because an appropriately low \( g_s \) will impose a carbon limitation on photosynthesis. Hence, photosynthesis and stomatal conductance are closely coupled in what was probably the first of an integrated sequence of adaptations that transformed the gas-exchange characteristics of plants during their colonization of land (Hetherington & Woodward, 2003; Franks & Brodribb, 2005; Franks & Beerling, 2009a,b). Stomatal distributions have been used to infer palaeoclimates from fossil leaves but can leaf stomatal distributions lead us to an improved understanding of plant palaeophysiology?

The close coupling between stomatal conductance and water transport is well documented and several studies have shown that alterations in stem water transport can cause either stomatal closure or stomatal opening. Stomatal behaviour is governed by the vapour pressure deficit between the leaf interior and ambient atmosphere, water status, the leaf boundary layer, soil-to-leaf water transport and chemical signals such as abscisic acid. However, it is the leaf water status that encapsulates the holistic effect of these factors on stomatal conductance because leaf water potential regulates the turgor pressure of both stomatal guard cells as well as the subsidiary cells surrounding the stomata (Meinzer & Grantz, 1990; Saliendra et al., 1995; Buckley, 2005). Not surprisingly, the leaf water potential is to a large extent, controlled by the transport capacity of the xylem and its ability to recharge water lost by transpiration. Indeed, an artificial reduction of xylem transport rapidly induces stomatal closure and reduces transpiration, whereas root pressurization restores leaf water potential and induces stomatal opening (Saliendra et al., 1995; Hubbard et al., 2001). Alternatively, partial de-foliation of the plant canopy increases the supply of water relative to the demand, thereby inducing an increase in stomatal conductance (Oren et al., 2001).

A strong relationship exists between stem hydraulic conductance and photosynthesis suggesting that leaf gas exchange is proportional to, or constrained by vascular supply (Brodribb & Feild, 2000; Hubbard et al., 2001; Santi-
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ago et al., 2004; Maherali et al., 2008; Brodribb, 2009). These findings are corroborated by extensive work documenting the coupling between leaf vasculature, stomatal conductance and gas exchange across a variety of plant life forms (Brodribb et al., 2003, 2005). Because at least 25% of the total resistance to plant water flow resides in the leaves, leaves may exert a disproportionately high degree of control over plant water relations (Sack et al., 2003). It may also be that the leaf stomatal and vascular performance may affect the structure and function of ‘upstream’ stem xylem, although that possibility has not previously been discussed. Certainly, such a coordinated response would require some degree of phenotypic plasticity in both the leaves and the xylem in the short term, but it may also have significant long-term or even deep-time evolutionary implications on the structure and function of xylem.

Atmospheric CO2 levels may have constrained the structure and function relationships of soil-to-leaf hydraulic transport in just such a manner. There is much geological, biological and theoretical evidence to suggest that atmospheric CO2 levels have fluctuated dramatically over time (Zachos et al., 2001; Beerling, 2002; Berner, 2006; Fletcher et al., 2003). Indeed, CO2 levels are thought to have reached highs well over 4000 ppm during the Devonian and lows below 500 ppm during the Carboniferous (Fig. 1). Interestingly, much of the evidence for deep-time atmospheric CO2 fluctuations has either been derived from or corroborated by measures of the leaf stomatal response. Specifically, it has been shown that across a broad sampling of plants from the angiosperms and conifers, stomatal density increases in response to low atmospheric CO2 whereas the opposite is true under elevated levels of CO2 (Woodward, 1987; McElwain et al., 1999; Royer et al., 2001; Retallack, 2001; Beerling, 2002; Kouwenberg et al., 2003). Data derived from the stomatal index, a more refined measure of the stomatal response that controls for leaf expansion, also indicate that in some taxa, CO2 is a developmental driver of leaf stomatal distributions (Lake et al., 2002). The discovery that stomatal abundance is related to atmospheric CO2 augmented our understanding of palaeoclimates because CO2 could be retrodicted from leaves much older than ice cores. In this way, stomatal density in combination with isotope and palaeosol signals serves as a reasonable CO2 proxy with which to evaluate the accuracy of climate models.

We know, however, that stomata play a dual role in regulating water loss as much as CO2 uptake, yet the effect of varying CO2 levels on the palaeo-physiology, especially water relations of the sampled plants has attracted less attention. The effect that increasing stomatal densities may have on leaf water loss was apparent to Woodward (1987) who showed that the stomatal conductance of Acer pseudoplatanus seedlings grown under 225 ppm of CO2 was twice as high as that of seedlings grown under 340 ppm. Functionally, this meant that the water-use efficiency of the 225 ppm-grown seedlings was well over 50% lower than that of the 340 ppm grown plants. A similar response was observed across eight species of conifers grown at 280 and 800 ppm (J. Pittermann, unpublished data, Fig. 10). These results imply that plants may have been predisposed to a high degree of water stress during periods of low CO2 such as the Pleistocene glacial periods. Increased susceptibility to drought stress combined with reduced water use efficiency may have been a contributing factor to the reduced vegetation cover during the Pleistocene (Cowling & Sykes, 1999).

If we accept that the apparent changes in stomatal densities are consistent with the fluctuating CO2 levels of the Mid-to-Late Phanerozoic, then atmospheric CO2 may have had a profound effect on the whole plant conductance to water. For example, the ‘topdown’ stomatal response to CO2 may have at various times selected for plant hydraulic architecture that was better equipped to mine water via deeper and more complex root systems. Such plants may have also been more efficient at transporting water by allocating a greater proportion of resources to xylem, or by increasing the transport efficiency of their xylem tissue. Hence, under periods of good water availability and low atmospheric CO2, it is tempting to speculate that selection may have favoured the presence of more efficient, large-diameter conduits or a greater fraction of tissue devoted to water transport, in order to meet the increased transpiration demands inherent to leaves with high stomatal densities.

The alternative hypothesis is that reduced atmospheric CO2 levels may have imposed a carbon limitation on growth in a manner that reduced leaf area, such that whole-plant transpiration scaled with reduced plant size. Consequently, xylem area/leaf area, leaf-specific hydraulic efficiency and conduit diameter would remain unchanged. Indeed, several studies have shown smaller leaf areas in plants grown under subambient CO2 (Dippery et al., 1995; Cowling & Sage, 1998). However, the data do indicate that transpiration rates continue to be higher in these plants than those grown at ambient CO2 levels (Ward et al., 1999), suggesting that on a whole-plant scale, the increased water loss may also impose a

Fig. 10 The stomatal conductance of Cupressaceaeous and Pinaceaeous conifer seedlings grown at 280 and 800 ppm in growth cabinets (mean ± SD; J. Pittermann, unpublished data).
significant physiological limitation. Lastly, an increase or
decrease in the pressure gradients within the xylem may alter
the flow rates such that transpirational demands are effectively
met without a need for anatomical changes in the xylem tissue
(Fig. 11). This response, would however predispose the plants
to a greater possibility of cavitation caused by air seeding.

We can begin to explore the impact of atmospheric CO$_2$ on
plant water relations by returning to some basic relationships
between xylem transport, anatomy and stomatal conductance.

The relationship between $g_s$ and leaf-specific hydraulic
conductance ($K_l$) can be described such that,

$$g_s = K_l \left( \frac{\Delta \Psi}{VPD} \right),$$  (10)

where $\Delta \Psi$ is the water potential difference between the soil
and the leaves ($\Psi_{soil} - \Psi_{leaf}$) driving water movement and
$VPD$ represents the vapour pressure deficit between the leaf
interior and the ambient air (Bond & Kavanagh, 1999;
Hubbard et al., 2001). $K_l$ is a measure of hydraulic efficiency
on a whole-plant basis (Bond & Kavanagh, 1999; Sperry,
2000; Hubbard et al., 2001). If we assume that $k_s$ scales with
$K_l$ (certainly $k_s$ scales with leaf specific conductivity, $K_l$; J. Pit-
termann, unpublished data), then substituting equation 2 for
$K_l$ in equation 10 yields the final relationship,

$$g_s \propto \frac{(\Delta \Psi) \pi \sigma D^4}{128 \eta VPD}.$$  (11)

Equation 11 expresses a theoretical correlation between
stomatal conductance and the theoretical maximal transport
capacity of the xylem, as constrained by conduit diameter.
Simplistically, equation 11 implies that an overall rise in $g_s$
requires an increase in conduit diameter, for a given xylem
area. Such a transport response would require the co-ordina-
tion between the vascular cambium and stomatal patterning
during leaf development, and there is evidence to suggest that
in some species, stomatal development is labile and thus
responsive to atmospheric CO$_2$ (Lake et al., 2002; Beerling,
2005) but whether this couples to vascular development
remains unknown. Alternatively, xylem anatomy may exhibit
limited phenotypic plasticity, and thus impose fundamental
limits on leaf gas exchange as appears to be the case in diffuse
and ring porous angiosperm trees (Bush et al., 2008).

If a link does indeed exist between atmospheric CO$_2$, stomata-
cal conductance, stomatal density and vascular design, it is
tempting to speculate that the 70 million years during the
Mid-Devonian to Early Carboniferous provided atmospheric
conditions that supported the evolution of secondary xylem
(wood). Climate models suggest that this period of the Phan-
erozoic was characterized by CO$_2$ levels that are thought to
have been at least as low or lower than those of today, and
which may have stimulated not only an increase in stomatal
densities but also the appearance of large leaves (Beerling
et al., 2001; Osborne et al., 2004). Furthermore, it is at this
time that elevated atmospheric oxygen levels are thought to
have supported an increase in xylem lignification and possibly
selection for woody taxa (Graham et al., 1995; Berner, 2005;
Berner et al., 2007). A concurrent argument is that low CO$_2$
selected for high stomatal densities, which in turn increased
evaporative cooling thereby releasing leaves from the con-
straints of overheating. This is also consistent with survey
results from the fossil record showing a 25-fold enlargement of
leaf areas during this time (Osborne et al., 2004). In any case,
if atmospheric CO$_2$ precipitated changes in leaf size and stoma-
tal density, then we might expect a co-ordinated response from
the vascular system designed to increase transport efficiency.

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**Fig. 11** Some theoretical relationships between
xylem structure and function in response to low and
high ambient CO$_2$.
PART IV: CONCLUDING REMARKS

Plant water transport and climate

Plant water transport irrevocably changed our planet. Even the earliest transpiring plants would have subtly modified their local water regime, whereas the Devonian forests were probably key players in the formation of soils, carbon sequestration and climate change (Retallack, 1997; Algeo & Scheckler, 1998; Berner, 2005; Berner et al., 2007). Xylem function certainly impacts atmospheric moisture, and the Amazon rainforest is a dramatic, modern day example of how large-scale water transport controls continental climates as nearly 50% of global precipitation is recycled from forests (Malhi et al., 2008). Deep roots extract and potentially re-distribute the soil water that is ultimately transpired back to the atmosphere, a phenomenon that is critical to maintaining hydration when water transport is a key element of large-scale ecosystem stability (Betts et al., 2004). Regional meteorological phenomena will also be affected by alteration of the forest canopy boundary effects so, taken together, there is indisputable evidence that plant water transport is a key element of large-scale ecosystem stability (Betts et al., 2005).

Evapotranspiration is a common metric of area-based terrestrial water loss but recent work suggests that transpiration alone can modify continental hydrology. Extensive field studies have shown that elevated CO$_2$ reduces stomatal conductance and transpiration, an effect that generally improves the water balance of terrestrial vegetation (Körner, 2003). However, the suppression of transpiration leads to greater water retention in soils and ultimately higher water discharge into rivers, a phenomenon referred to as ‘physiological forcing’ (Betts et al., 2007). How would this scenario play out under low CO$_2$ conditions, which typically give rise to increased stomatal conductance? Given the uncertainties of climate modelling, a simple answer may not exist but all things being equal, a reasonable guess may be that increased transpiration would lower soil moisture, and thus impact terrestrial water levels.

Future directions

Plant vascular tissue is one of potential drivers of plant diversification (Donoghue, 2005), so much insight can be gained into the evolution of plants by applying a neontological and quantitative approach to fossil wood. Although the Hagen–Poiseuille method of computing theoretical hydraulic efficiencies is useful, incorporating pit resistance into fossil plant transport models will allow us to broaden our understanding of xylem function in extinct plants, as well as the significance of evolutionary transitions from one conduit type to another such as in the case of the Rhyniophytic plants. As we do this however, comparative studies of xylem structure and function in modern taxa need to develop within a broader evolutionary framework. For example, we know little about the relationships between cavitation resistance, pit resistance and hydraulic efficiency in monocots and Gnetales.

Aside from the extensive descriptive work documenting the anatomy of fern xylem, not much is currently known about the comparative hydraulic systems of ferns, and the homogenous fern intertracheid pit membrane in particular (Carlquist & Schneider, 2007). This is important information because tracheids with homogenous pit membranes represent the ancestral state of xylem in leafy tracheophytes (Euphyllophytes; Kenrick & Crane, 1997). However, new data collected from across the fern lineage show that fern xylem is as efficient on an area basis as that of angiosperms and conifers (J. Pittermann & E. Limm, unpublished data), suggesting that the large tracheid size in combination with what appears to be a highly permeable pit membrane (Carlquist & Schneider, 2007) combine to ramp up water transport in an otherwise seemingly suboptimal combination of vascular traits (Pittermann et al., 2005).

We can also exploit our current understanding of the relationship between pit area and pit structure in angiosperms and conifers respectively, to gain more insight into the drought resistance of early land plants. The pit area hypothesis holds across angiosperms of different evolutionary lineages, although membrane porosity may also variably affect resistance to cavitation (Jarbeau et al., 1995; Hacke et al., 2006, 2007; see also Choat et al., 2008). Assuming that pit membranes are well preserved in fossil specimens, perhaps the best approach may be to examine the pit membrane size, scaling and porosity in a comparative manner, sampling taxa across the land plant phylogeny.

Understanding the structure and function of xylem can inform us not only about the physiological limits of plant water transport and the constraints it places on photosynthesis, but this information also has potential to augment models of palaeocommunity assembly. For instance, the Devonian and Carboniferous are characterized by significantly different climatic patterns than those of today, as well as changes in the relative positions of land masses and tremendous diversification of the terrestrial flora (DiMichele & Gastaldo, 2008). Plant distributions in these ancient and unfamiliar landscapes will, to some extent, be determined by xylem efficiencies and drought resistance. Altogether, this is an excellent time to fruitfully study the evolution of plant water transport across a multitude of scales, ranging from the molecular, organismal and up to the ecosystem level. Certainly, the intersection of plant physiology and palaeobotany offer much opportunity for truly novel discoveries.
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REFERENCES


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