Hydraulics of *Asteroxylon mackei*, an early Devonian vascular plant, and the early evolution of water transport tissue in terrestrial plants

J. P. WILSON AND W. W. FISCHER

Division of Geological and Planetary Sciences, California Institute of Technology, Pasadena, CA, USA

ABSTRACT

The core of plant physiology is a set of functional solutions to a tradeoff between CO_2 acquisition and water loss. To provide an important evolutionary perspective on how the earliest land plants met this tradeoff, we constructed a mathematical model (constrained geometrically with measurements of fossils) of the hydraulic resistance of *Asteroxylon*, an Early Devonian plant. The model results illuminate the water transport physiology of one of the earliest vascular plants. Results show that *Asteroxylon*'s vascular system contains cells with low hydraulic resistances; these resistances are low because cells were covered by scalariform pits, elliptical structures that permit individual cells to have large areas for water to pass from one cell to another. *Asteroxylon* could move a large amount of water quickly given its large pit areas; however, this would have left these plants particularly vulnerable to damage from excessive evapotranspiration. These results highlight a repeated pattern in plant evolution, wherein the evolution of highly conductive vascular tissue precedes the appearance of adaptations to increase water transport safety. Quantitative insight into the vascular transport of *Asteroxylon* also allows us to reflect on the quality of CO_2 proxy estimates based on early land plant fossils. Because *Asteroxylon*'s vascular tissue lacked any safety features to prevent permanent damage, it probably used stomatal abundance and behavior to prevent desiccation. If correct, low stomatal frequencies in *Asteroxylon* reflect the need to limit evapotranspiration, rather than adaptation to high CO_2 concentrations in the atmosphere. More broadly, methods to reveal and understand water transport in extinct plants have a clear use in testing and bolstering fossil plant-based paleoclimate proxies.

Received 27 September 2010; accepted 01 December 2010

Corresponding author: Jonathan P. Wilson. Tel.: 626-395-3752; fax: 626-568-0935; e-mail: jpwilson@caltech.edu

INTRODUCTION

The evolutionary transition from green algae to terrestrial plants presented numerous physiological challenges. Terrestrial plants needed to employ a suite of biochemical, developmental, and anatomical adaptations to resist desiccation, including cuticular waxes, tissue-producing meristems, and dedicated water transport tissue (Graham & Gray, 2001). Fossils of many of these early plants demonstrate unfamiliar combinations of anatomical features, resembling leafless, dichotomously branched, photosynthetic cylinders, and efforts to understand their physiology have relied on mathematical models (Konrad *et al.*, 2000; Roth-Nebelsick *et al.*, 2000; Roth-Nebelsick, 2001; Roth-Nebelsick & Konrad, 2003; Boyce, 2008). This theoretical work suggested that early terrestrial plants had unusual functional restrictions, including photosynthetic capability that was limited by the

capacity to absorb water from the environment (Konrad *et al.*, 2000; Roth-Nebelsick & Konrad, 2003). More derived plants, on the other hand, are amenable to experimental analysis through greenhouse studies of nearest living relatives or ecological analogs because living representatives contain anatomical structures identical to those seen in the fossil record (Beerling *et al.*, 1998; Llorens *et al.*, 2009). Because many Early Devonian plants lack close living relatives or anatomical analogs, there is a substantial gap in what can be learned by experimental analysis alone. We bridge this gap by taking a different approach. Using models of water transport constrained by the geometry and dimensions of vascular anatomy preserved in fossils, we can reconstruct the physiology of one of the earliest plants with vascular tissue preserved, the Early Devonian plant *Asteroxylon mackei*.

Analysis of the physiology of *Asteroxylon* yields two benefits for the study of the interaction between the biosphere and the

Earth. First, because of its age and phylogenetic relationships, we gain a critical early data point in the evolutionary trajectory of plant physiology. Substantial previous work has shown that disparate lineages of plants repeatedly evolve functionally similar solutions to the same physiological constraints, including leaves (Boyce & Knoll, 2002; Galtier, 2010), the vascular cambium (Spicer & Groover, 2010), and conducting tissue (Bailey, 1953; Carlquist, 1975; Tyree & Zimmermann, 2002; Wilson et al., 2008; Wilson & Knoll, 2010). Patterns of physiological convergence demonstrate that although anatomical features may differ morphologically, they can yield comparable functional results. For example, the water transport tissue of several Paleozoic seed plants closely resembles, in terms of hydraulic yield, the transport tissue of angiosperms, despite preceding them by at least 150 Myr, which suggests that the evolutionary advantage of angiosperm wood was not simply high conductivity, but both increased safety and the evolution of a vascular cambium that produces high-throughput vessels in addition to fibers for structural support (Wilson et al., 2008; Wilson & Knoll, 2010). Analysis of *Asteroxylon*, one of the earliest plants with anatomy preserved in three dimensions, can shed light on the functional adaptations, and tradeoffs, that characterized the earliest vascular plants.

Second, there is a critical need to understand the evolution of global climate on geological timescales, and a central goal is understanding the history of atmospheric carbon dioxide concentrations. A profitable approach has been developing proxies based on plant physiology: through physiology, plants respond to, and record, through fossilization, environmental and biophysical information in their tissues. Much effort has been spent deriving CO_2 proxies from land plants (McElwain & Chaloner, 1995; Beerling et al., 1998; Beerling & Royer, 2002a,b; Roth-Nebelsick & Konrad, 2003; Roth-Nebelsick, 2005). Carbon dioxide enters into plant tissues through dynamic apertures on leaves, called stomata. This forms the logic for a plant-based CO_2 proxy. All else equal, at high atmospheric CO_2 concentrations, fewer stomata are required on photosynthetic surfaces to satisfy carbon demand.

One fundamental challenge for paleoclimate methods based on stomatal frequency is calibrating the proxy such that it is valuable in deep time when applied to fossil plants. Most approaches rely on controlled greenhouse experiments using closest environmental or evolutionary relatives for calibration (McElwain & Chaloner, 1995; Beerling et al., 1998; Beerling & Royer, 2002b). When these calibrations have been applied, reconstructions of the history of CO_2 generally display a mid-to-late Mesozoic high (3–5× PAL), a Carboniferous-to-Permian low (1–2× PAL) and higher levels in the Devonian atmosphere (McElwain & Chaloner, 1995; Beerling & Royer, 2002a; Royer, 2006). A complication arises, however, because of known tradeoffs in plant physiology; the acquisition of CO_2 results in water loss. More (and/or larger) stomata not only permit more carbon dioxide to be assimilated into leaves, but also further expose plants to the drying power of the

atmosphere. Plants need carbon dioxide as a substrate for photosynthesis, but lose water from their tissues to acquire it. This fundamental tradeoff is true for all living and ancient photosynthetic plants. Stomatal density and behavior is part and parcel of water transport in plants. With careful calibration, some of the complications introduced by water transport can be mitigated. However, a significant problem is presented by the earliest land plants, which sit far outside ecological and evolutionary calibration. Ultimately, to understand the data and quality of interpretations behind plant-based CO_2 estimates, we need to develop physiological models of vascular hydraulics in ancient plants.

BACKGROUND

Hydraulic models are based on an analogy from the flow of current in an electrical circuit to how water passes through living plants (Van den Honert, 1948). Water moves from the soil to the atmosphere through plant tissues under tension. Evaporation of water from cell walls inside leaves generates forces that pull water to the site of evaporation; this creates a water potential gradient from the leaves to the soil, allowing water to move against gravity. The magnitude of this gradient is a function of the vapor pressure gradient between the atmosphere and the site of evaporation and the hydraulic resistance of vascular tissue. Steep water potential gradients incur osmotic and structural costs; consequently, plants have evolved many features to respond quickly to environmental change, and modified tissues to reduce hydraulic resistance. Adaptations that reduce hydraulic resistance, however, are in functional conflict with morphological adaptations to increase water transport safety. Plants have explored a wide range of strategies to solve this tradeoff (Bailey, 1953; Tyree & Zimmermann, 2002).

Plants mitigate damage from excessive evapotranspiration through a variety of methods, but two end points of an ecophysiological spectrum can be pointed out. One end-member operates by localizing the safety margin within the morphology of vascular tissues, whereas the other achieves safety by closely managing the upstream end of the transpiration stream, through stomatal behavior and density. It has been hypothesized that the earliest terrestrial plants were closer to the first position on this spectrum than the second (Edwards, 1998; Edwards et al., 1998), and this characterization is supported by comparative physiology of living plants. For example, when the ecophysiological traits of extant plants from different clades have been compared under the same environmental conditions, more derived groups (e.g. angiosperms) have faster-responding stomatal guard cells, and are able to close leaf apertures in response to rapid changes in temperature, irradiance, and relative humidity (Brodribb & Holbrook, 2004; Brodribb et al., 2005). On the other hand, more primitive plant groups (e.g. ferns and lycophytes) do not respond as quickly to rapid environ-

mental changes and must close stomata before runaway evapotranspiration completely desiccates their tissues, even at the expense of photosynthesis (Brodribb & Holbrook, 2004).

Recent work has shown that it is possible to develop quantitative models and hypotheses of the physiology of extinct plants from preserved vegetative anatomy (Wilson *et al.*, 2008; Wilson & Knoll, 2010) and this work revealed that extinct seed plants displayed a wider range of water transport strategies than discovered from extant plants using comparative biology. In principle, this approach can be applied to early land plants, but the dramatic morphological differences shown in primitive plant conducting cells require substantial modification of existing mathematical models.

The Early Devonian vascular plant *Asteroxylon mackei* is one of the earliest vascular plants that is both stably placed in morphological phylogenetic trees and has fossil material with internal and external anatomy preserved in three dimensions (Fig. 1A–C). *Asteroxylon* is widely found throughout sandy strata within the Rhynie Chert, dated to 404 ± 2 Ma (Mark *et al.*, 2011). The preserved vegetative and reproductive anatomy suggests that it was a stem group lycophyte (Kenrick & Crane, 1997). *Asteroxylon* (Edwards, 1998; Edwards *et al.*, 1998), along with other Early Devonian protracheophytes (McElwain & Chaloner, 1995) and tracheophytes (McElwain & Chaloner, 1995; Franks & Beerling, 2009), had large stomata but low stomatal densities. *Asteroxylon*, in particular, has stomata that are 20 times larger, in terms of area, than most extant angiosperm stomata; its stomatal frequency is lower than that of extant angiosperms by a factor of approximately 20, as well (Edwards, 1998; Edwards *et al.*, 1998; Franks & Beerling, 2009). Other plants found in the Rhynie Chert contain even lower stomatal frequencies: between 4 and 10 stomata per mm^2 [in contrast to Asteroxylon, which contains 10–30 per mm^2 (McElwain & Chaloner, 1995; Edwards, 1998)]. A reasonable hypothesis is that these low frequencies reflect high concentrations of CO_2 in the Early Devonian atmosphere (McElwain & Chaloner, 1995; Beerling & Royer, 2002b; Royer, 2006); another possibility is that the low stomatal densities in these fossils reflects early evolved iterations of vascular tissue that required few stomata to avoid catastrophic drying of the vasculature (Edwards, 1998; Edwards *et al.*, 1998). To evaluate these different hypotheses, we reconstruct water transport in *Asteroxylon* using a new mathematical hydraulic model constrained by anatomical measurements of water conducting tissues made from three-dimensionally preserved fossils.

METHODS

Our approach begins with a model of hydraulic resistance in plant tracheids (Hacke *et al.*, 2004; Sperry & Hacke, 2004; Wilson *et al.*, 2008; Wilson & Knoll, 2010). Previous work examined fluid flow through tracheid morphologies character-
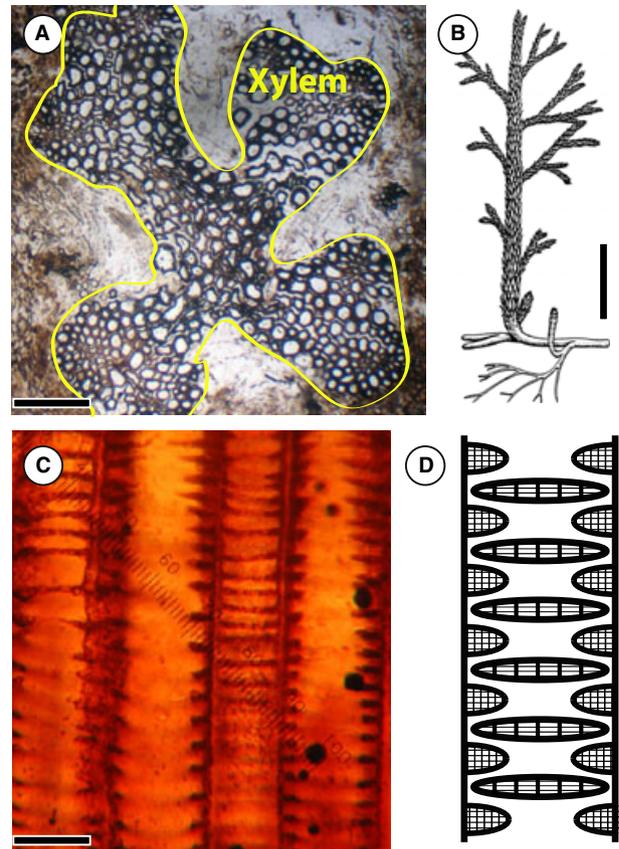
Fig. 1 Morphology and anatomy of *Asteroxylon mackei*, an Early Devonian vascular plant. (A) Cross-section of permineralized *A. mackei* stem, vascular tissue outlined in yellow (Harvard University Paleobotanical Collection #29288). Scale bar is 200 μm. Specimen is from the Rhynie Chert deposit. (B) Reconstruction of *A. mackei*, based on permineralized material from the Rhynie Chert, from Kidston & Lang (1920); note the small leaves (microphylls). Scale bar is 10 cm. (C) Longitudinal section through *A. mackei* vascular tissue, showing several xylem cells next to each other. Scale bar is 25 μm. (D) Schematic showing scalariform-type pitting used to quantify conductivity through *A. mackei* tracheids. Elliptically shaped porous areas (ovals with internal crossed lines) are spirally arranged to represent pitted area within tracheids.

istic of seed plants; this study focuses on details of plant water transport cells that are characteristic of ferns, lycophytes, and early terrestrial plants. The salient details of the tracheid model approach are included here; a detailed description can be found in Wilson *et al.* (2008). Additional information about the justification, construction, and parameter sensitivities of tracheid-based hydraulic models can be found in (Hacke *et al.*, 2004; Sperry & Hacke, 2004; Wilson *et al.*, 2008; Wilson & Knoll, 2010). Total hydraulic resistance has a component from drag through the conduit center (quantified as flow through a circular pipe) combined with flow from one cell to another through small openings called pits. Pits consist of two short openings separated by a porous membrane, and each tracheid contains numerous pits. More pits lower the hydraulic resistance of a cell; we model them as resistors in parallel. The input parameters for this type of model are tracheid diameter,

Table 1 List of critical parameters for the hydraulic model: measured parameters (average tracheid diameter, average tracheid length, minimum length, average thickness-to-span ratio) and model parameters (fraction of tracheid surface area covered by pit membrane, pit membrane pore diameter). Tracheid lengths for points in Figs 2 and 3 are randomly selected from a Gaussian distribution with a mean of 1.56 and standard deviation of 0.39, as described in the text

	Diameter (µm)	SD (n = 40)	Length (mm)	SD (n = 3)	Minimum length (mm)	SD (n = 35)	Thickness-to-span ratio $(t/s)^2$	SD (n = 66)	Membrane pore size (nm)	Surface area covered by scalariform pits (%)	Surface area covered by circular-bordered pits (%)
Asteroxylon mackei 0299	24.45	8.3	1.56	0.39	1.21	0.15	0.097	0.077	40	45	35
Asteroxylon mackei 0289	22.34	5.6	n/a*				0.048	0.009	40	45	35

*No longitudinal sections available; used length from 0299.

tracheid length, number of pits, size of pits, and membrane porosity (Table 1). Each of these parameters can be constrained and measured using well-preserved plant fossils, or constrained using estimates based on extant material.

Previous models quantified the hydraulic resistance of pits that are largely circular, which are straightforward to quantify using the Hagen–Poiseuille equation (Tyree & Ewers, 1991), but ferns, lycopsids, and early terrestrial plants contain elliptically shaped scalariform pits. To calculate the hydraulic resistance across these elliptical openings, we consider the aperture as a short elliptical cylinder and find an exact solution for the Navier–Stokes equations for such a topology. (See Appendix for details.)

As described in previous work (Tyree & Ewers, 1991; Tyree & Zimmermann, 2002; Hacke *et al.*, 2004; Sperry & Hacke, 2004; Wilson *et al.*, 2008; Wilson & Knoll, 2010), flow through tracheids can be quantified by using a series of equations representing flow through the lumen of a cell combined with flow through two sets of pits (one set into a cell, another set on the way out):

$$R_{\text{total}} = R_{\text{lumen}} + 2\frac{R_{\text{pit}}}{N_{\text{pits}}}.$$

Flow through the lumen of a cell is simply flow through a circular tube of the given diameter and length, based on the Hagen–Poiseuille equation, a special case of the Navier–Stokes equation, describing laminar flow through a cylinder.

Pits contain apertures and membranes, which can be considered short, circular cylinders (or elliptical cylinders in the case of scalariform pits: see Appendix) and infinitely thin sheets, respectively:

$$R_{\text{pit}} = 2(R_{\text{aperture}}) + R_{\text{membrane}}.$$

A molecule of water can only flow through one set of pits, so they are modeled as resistors in parallel; increasing the number of pits decreases the total resistance of a cell.

We measured tracheid dimensions from two specimens of *Asteroxylon mackei* in the Harvard University Paleobotanical Collections (#22998 and 22989). Tracheid diameter, length, and pitted area was measured and calculated from images

(obtained by light microscopy) using IMAGEJ software (found at: http://rsbweb.nih.gov/ij/). Tracheid lengths are difficult to measure in fossils because both end walls need to appear in a single thin section; we were only able to measure tracheid lengths in specimen #22998. To solve this problem, we generated a Gaussian distribution of tracheid lengths with a mean and standard deviation measured from specimen #22998. Simulated tracheid lengths were drawn from this distribution. To assess whether this distribution reflects actual tracheid lengths, we measured the length of 35 tracheids that had one end wall preserved, but not both, because of poor preservation. This provides a lower bound to tracheid length – they cannot be any shorter, and are likely to be significantly longer. Minimum lengths fall comfortably within a cumulative distribution function plot of our estimated distribution (Fig. 2). Because length is a linear parameter in tracheid hydraulic models, sensitivity analyses show that values from extremes of the estimated distribution change total conductivity by <20%.

Asteroxylon tracheids have been described as containing helical thickenings, analogous to the secondary wall of more advanced conducting cells, separated by areas that are essentially primary cell wall (Edwards, 2003). Water passes from one cell to another through the area between these helical or elliptical thickenings. *Asteroxylon*'s tracheids are therefore most hydraulically similar to cells that contain scalariform pits; no model for water transport through scalariform pits exists because of their elliptical shape. We take an approach that replicates the geometry of tracheids with helical thickenings using elliptical apertures (Appendix). We represent the resistance of an elliptically shaped pit membrane aperture as a short ellipse:

$$R_{\text{aperture}} = \frac{(4\mu a^2 + b^2)}{\pi a^3 b^3} t_{ap}.$$

To quantify the hydraulic resistance of these wall areas, which would otherwise be intractable given their morphology, we mapped these areas as spirally or alternately arranged scalariform pits that occupy much of the conducting cell's circumference (Fig. 1D). This method allows us to simulate 'pitted
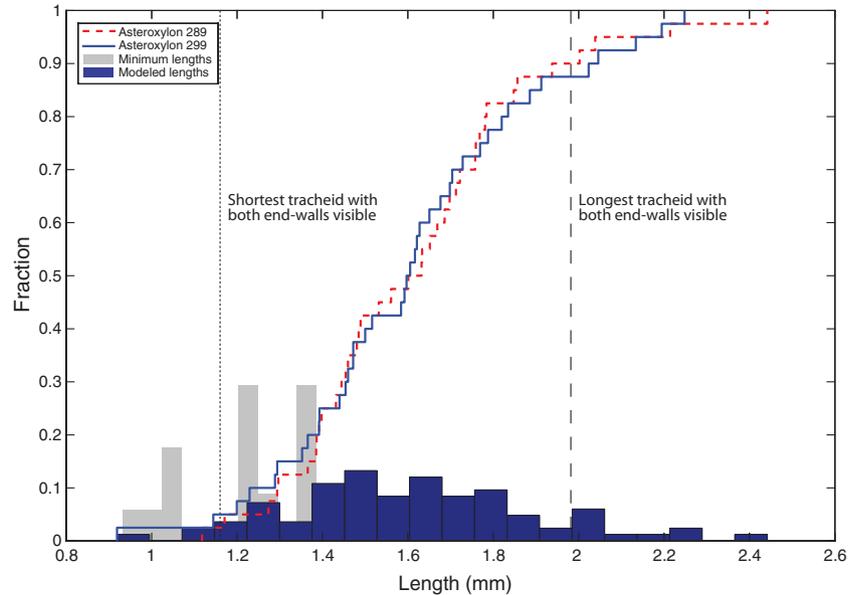
Fig. 2 Statistical distribution of tracheid lengths in *Asteroxylon mackei*. Lines are cumulative distribution functions of modeled lengths for two specimens. Dark histogram is distribution of modeled values. Because thin sections rarely preserve both ends of a single tracheid, we measured minimum lengths of 43 tracheids that only had a single wall preserved (gray histogram). These tracheids cannot be any smaller than the amount we measured, and fall comfortably within the distribution of modeled lengths.

areas' that approximate values measured in fossils. In this regard, our estimates are conservative; actual pit area probably exceeds values we measured, and pit membrane porosity may, if anything, exceed our estimates. The resulting total conductivity of *Asteroxylon* tracheids is therefore a minimum estimate.

To view *Asteroxylon* within the hydraulic context of other plants, we chose to compare its conductivity with that of *Cordaites*, an extinct coniferophyte known from Late Paleozoic coal swamps (Taylor *et al.*, 2008). We chose *Cordaites* for two reasons: first, it is one of the few fossil plants with published (modeled) conductivity data, which also contains tracheids that overlap with the size range of tracheids found in *Asteroxylon* (Wilson & Knoll, 2010). This provides a reference frame for our analysis of *Asteroxylon*, at least in terms of conductivity for a given cell size. Second, *Cordaites* is found within well-watered environments. This provides control for other aspects of plant physiology (not modeled here) that could arise from comparing plants from dramatically different environments. *Cordaites*, however, is a seed plant and contains tracheids with circular-bordered pits, not the scalariform pit morphology found in *Asteroxylon*.

Conductivity is only one half of the story: structures to increase conductivity (e.g. thinner walls that increase lumen area, wider cells, more porous membranes, and more pit area) place cells at risk of damage from water stress (e.g. cavitation and implosion). Narrower tracheids with thick walls are more effective at resisting implosion, whereas wider tracheids are more vulnerable (Hacke *et al.*, 2001; Pittermann *et al.*, 2006). To quantify the ability of *Asteroxylon*'s tracheids to resist implosion, we measured the thickness-to-span ratio of 66 tracheids (Table 1).

Another method for estimating cavitation thresholds comes from measuring pit area per tracheid; although this statistic does not work universally well for all groups (Christman *et al.*, 2009), data for many taxa show an inverse relationship between cavitation pressure (P_{50}) and pit area per xylem cell (Wheeler *et al.*, 2005). No data exist on this relationship in basal land plants; we therefore measured pit area in *Asteroxylon* and used relationships from angiosperms (Wheeler *et al.*, 2005; Hacke *et al.*, 2007; Jansen *et al.*, 2009) to estimate the conditions under which *Asteroxylon*'s tracheids would cavitate. Cavitation in plants is often expressed as P_{50}, or the pressure at which whole-plant conductivity is reduced to 50% compared to that of a well-watered control; in our analysis this should be viewed as the pressure at which enough tracheids have cavitated to reduce conductivity by half.

RESULTS

Ranging from 12 to 35 μm in diameter, and averaging <2 mm in length, tracheids from *Asteroxylon mackei* are of comparable width, but short in comparison with the vasculature of most modern tracheid-based plants. Their short size would predict low conductivity, except that virtually the entire conduit surface is covered by pit membrane, or its equivalent. This proportion of membrane area allows scalariform pits to function with remarkably low hydraulic resistance. Tracheids with scalariform pits have higher conductivity than tracheids with similar dimensions and pit fractions, but with circular-bordered pits. This appears to be a counterintuitive result, but it is entirely a function of the amount of pit area per tracheid. In order to confirm that scalariform pits cause the increased conductivity of *Asteroxylon* tracheids, we simulated tracheids with the same dimensions and membrane porosity, but with circular-bordered pits (resulting in decreased pit area) (Fig. 3A,B). Scalariform pits increase single-cell conductivity by nearly an order of magnitude.
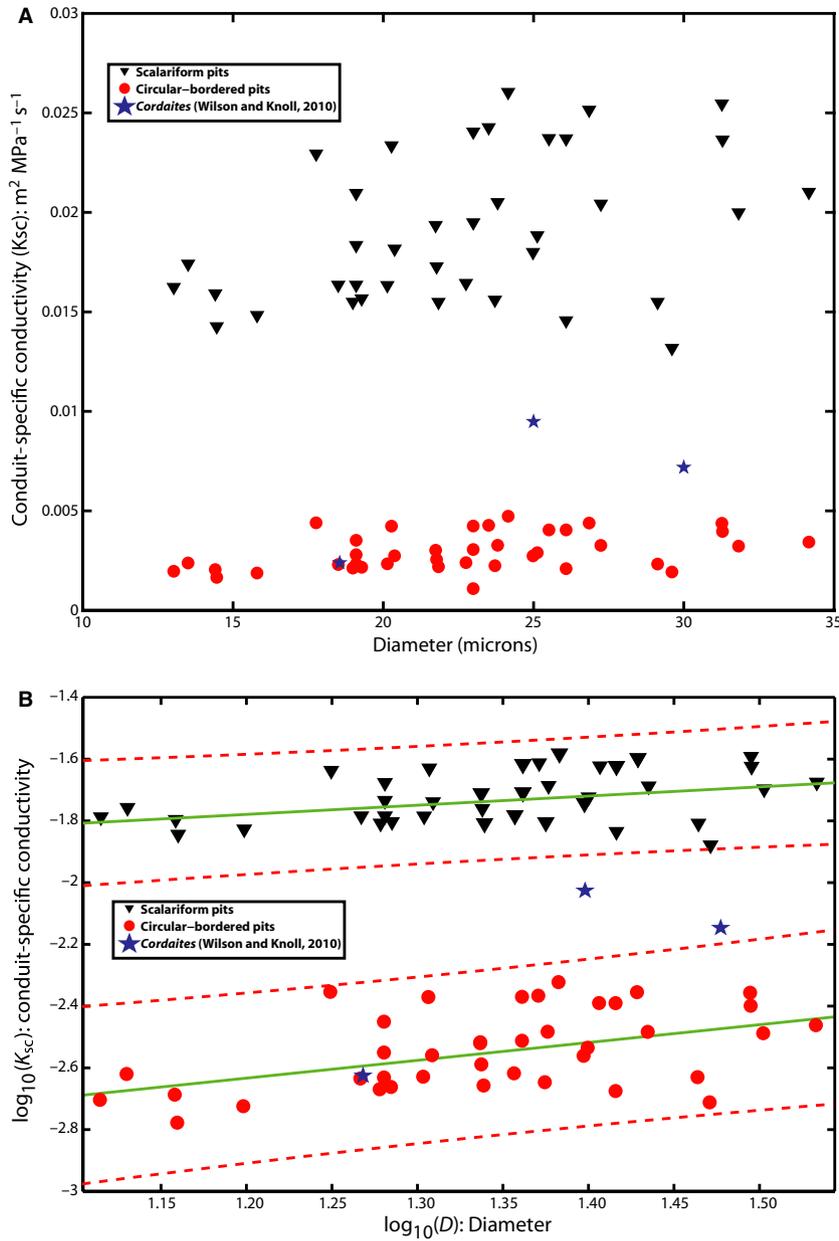
Fig. 3 Conductivity of *Asteroxylon mackei* tracheids as a function of diameter: scalariform pits increase conductivity over circular-bordered pits. (A) Conduit-specific conductivity (conductivity normalized to cross-sectional wall area; m^2 MPa^{-1} s^{-1}) vs. diameter (μm). (B) Log-transformed conduit-specific conductivity vs. diameter. Green lines show least-squares linear regressions through datasets, red lines are 95% confidence intervals on regressions. Equation for circular-bordered pit data: $\log_{10}(K_{sc}) = 0.5768*\log_{10}(D) - 3.3288$. Equation for scalariform pit data: $\log_{10}(K_{sc}) = 0.2972*\log_{10}(D) - 2.1357$. Blue stars are modeled conductivities through tracheids of an extinct conifer, *Cordaites* (Wilson & Knoll, 2010). Black triangles are *A. mackei* tracheids with scalariform pits, red dots are tracheids with circular-bordered pits with approximately the same pitted area.

The upper conductivity range of *Asteroxylon* tracheids is comparable with the lower end of model estimates of conductivity of a Paleozoic conifer genus, *Cordaites*. Although the sample size is limited, this result suggests that one of the earliest evolved vascular plants could have occupied a portion of the conductivity space that remained occupied by more derived plants. This suggests that a large amount of the solution space for water transport was explored early in the history of plant evolution.

Asteroxylon could transport water with low resistance, but at a cost (Fig. 4A,B). These tracheids had a reduced safety margin when confronted with water-stress-induced damage: increased pit area leads to increased probability of embolism. When *Asteroxylon*-pitted areas are compared with vesselless an-

giosperms, which also contain tracheids with scalariform pits, they have low cavitation thresholds (Fig. 4B). When compared directly to the performance of vessels, which tend to have higher cavitation resistances, *Asteroxylon*'s tracheids have slightly higher cavitation thresholds. In both cases, however, the increased pit area because of *Asteroxylon*'s tracheid morphology leads to cavitation under modest levels of water stress.

Further costs accrue because of the method of structural reinforcement of *Asteroxylon* tracheids. Recent work has shown that thicker tracheid walls allow conduits to resist permanent damage (e.g. implosion) from large-magnitude tensile stresses (Hacke *et al.*, 2001). Figure 4(A) shows measured conduit thickness-to-span ratios from *Asteroxylon*. The distribution has a long tail, but the mean of the distribution
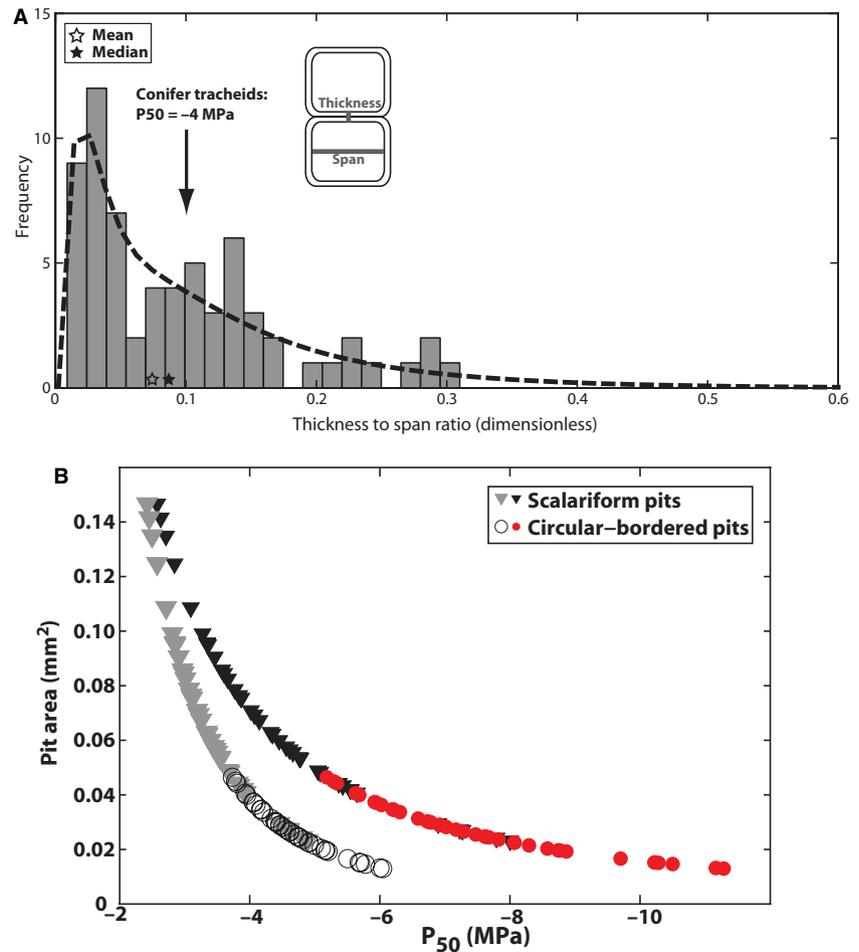
Fig. 4 Measures of safety margins in *Asteroxylon mackei* show tracheids that lack structural reinforcement to prevent cavitation, embolism and implosion by excessive transpiration. (A) Frequency distribution of thickness-to-span ratio of *Asteroxylon* tracheids; higher thickness-to-span ratios are more effective at resisting damage from tensile forces, whereas lower ratios cavitate and implode at lower-magnitude tensions (Hacke *et al.*, 2001). Dashed line is an estimate of the frequency distribution using a normal kernel function across 100 equally spaced points of thickness-to-span values. (B) Two estimates of *A. mackei* cavitation pressures, based on comparative analyses of vesselless angiosperms with scalariform pits [left curve (Hacke *et al.*, 2007)] and comparative analyses of vessel-bearing angiosperms [Rosaceae; right curve (Wheeler *et al.*, 2005)]. Circles (open and closed) represent estimates from circular-bordered pit areas whereas triangles (gray and black) represent estimates from scalariform pit areas. Smaller tracheids have lower pit areas and are inferred to cavitate at higher water demands. Scalariform pits have higher pit areas that leave tracheids more vulnerable to cavitation.

lies close to angiosperm vessels (with measured P_{50} between −2 and −4 MPa). These data provide further support for the view that *Asteroxylon*'s vascular tissue had low safety margins. Considering the vascular system of *Asteroxylon* as a whole, the tradeoff for high conductivity (increasing pit area) appears to be low hydraulic safety margins (decreasing the amount of tracheid surface area that is reinforced by secondary wall).

These low safety margins provide insight into the environments where *Asteroxylon* could grow: *Asteroxylon*'s leaves are small, and located near its vascular tissue, suggesting that tracheids were subject to tensions that were quantitatively controlled by external relative humidity. This, in turn, implies that even if *Asteroxylon* were found in a wet environment, midday sun, exacerbated by the lack of any form of canopy, would be enough to generate tensions of −2 to −3 MPa within its tracheids. These tensions exceed our predicted cavitation pressure for *Asteroxylon*; drought from midday sun would be sufficient to induce cavitation and limit growth of *Asteroxylon*.

Analysis of water transport in *Asteroxylon* reveals a plant that probably grew seasonally and opportunistically, rather than throughout the year. This is supported by inferences from the whole-plant morphology of *Asteroxylon*, with multiple aerial shoots connected through a subterranean rhizome (Kidston & Lang, 1920). In many living lycophytes, the subterranean portion of a plant persists over multiple seasons, but the aerial portion grows in response to humid seasons or water availability and subsequently dies back (Raven *et al.*, 1999). This might also have been true of *Asteroxylon*.

DISCUSSION

The vascular system of *Asteroxylon* is surprisingly conductive. This result contrasts with the prediction that low transpiration rates characterized the more primitive plants from the Rhynie Chert, particularly the early vascular plants *Rhynia* and *Nothia* and the non-vascular plant *Aglaophyton* (Roth-Nebelsick & Konrad, 2003). These estimates were made in a different fashion, by considering diffusion of water through plant cells, rather than laminar flow through xylem. This discrepancy could be real, however; the large and porous cells within *Asteroxylon*'s vascular system, relative to other plants found in the Rhynie Chert, would have provided a substantial hydraulic advantage over coexisting plants.

Asteroxylon's anatomy may have demanded a highly conductive vascular system. Even in a plant with simple leaves, the increased surface area of the plant's microphylls must have increased the water transport demand (Fig. 1B). Passive water loss through cuticle could add to water demand such that a low-resistance vascular system becomes necessary to maintain hydration throughout the entire plant. Viewed in this light, the evolution of scalariform pitting occurred in step with, and may have permitted, the evolution of the first leaves.

The conducting cells in *Asteroxylon* are morphologically and functionally similar to the primary xylem of many living plants, including seed plants and angiosperms (Bailey, 1953). Today, the use of highly conductive tissue with low safety margins early in a plant's development, is balanced by producing secondary xylem (wood) with higher safety margins. The development of secondary xylem did not occur in *Asteroxylon*, which raises the question: if safety from environmental stress is not found within the vascular system of *Asteroxylon*, where might it be located?

It is likely that any safety features were located in *Asteroxylon*'s leaves. For example, we would expect this plant's stomata to shut early, if at all possible (Brodribb & Holbrook, 2004) and, failing that, stomata should be few in number. Furthermore, given the large size of *Asteroxylon*'s stomata (Franks & Beerling, 2009), guard cells would be expected to shut slowly (Brodribb & Holbrook, 2004). This behavior would exacerbate the physiological effects from rapid changes in temperature, irradiance and humidity. Cavitation vulnerability and stomatal size both suggest that a large component of stomatal frequency was controlled by plant hydraulics.

The results from our hydraulic model of *Asteroxylon* vascular tissue support the hypotheses of Edwards (1998) and Edwards *et al.* (1998), and suggest that the low abundance of stomata on early land plants' surfaces played a major role in reinforcing water transport needs by contributing to resistance to cavitation, rather than solely reflecting high atmospheric CO_2 concentrations. If correct, this would preclude the simple interpretation of low stomatal density in early land plants as indicating high CO_2 based on comparing their stomatal frequencies with those of living plants (McElwain & Chaloner, 1995; McElwain, 1998). In addition to the documented top-down effect of CO_2 on stomatal density and conductance, it is likely that there is a bottom-up effect from plant hydraulics that must be taken into account. And given the phylogenetic and ecophysiological proximity of *Asteroxylon* to other rhyniophytes used in current CO_2 reconstructions, we hypothesize that the pattern of low stomatal densities seen in these Early Devonian plants reflects tradeoffs in plant hydraulics, rather than high $p CO_2$. We do not conclude that CO_2 was low; rather, there are a number of reasons to expect high concentrations of CO_2 in the atmosphere in the Paleozoic, most importantly because solar luminosity is estimated to be approximately 3% lower than the present day (Sackmann & Boothroyd, 2003). Higher CO_2 levels are also consistent with biogeochemical model estimates (Bergman *et al.*, 2004; Berner, 2006). However, factoring in the tolerances of *Asteroxylon*'s vascular system would revise any proxy estimates based on *Asteroxylon*'s stomatal frequencies downward, but by an unknown amount. Future work on gas exchange and hydraulics in living lycophytes and bryophytes, such as *Huperzia* and *Anthoceros*, which probes the tradeoff between assimilation and transpiration, could shed light on this understudied component of the stomatal density proxy in early vascular plants and put these estimates in physiological and environmental context.

More importantly, however, combining measurements of past stomatal densities with computational models of plant hydraulics provides direct paleoecological information based on individual plants. In the case of the Rhynie Chert, where plants are preserved in place, this allows direct simulation of Early Devonian environments, permitting estimates of paleotemperatures and relative humidity.

Early Devonian terrestrial ecosystems were likely quite different from those of the Cenozoic. Our analysis of *Asteroxylon* from the Rhynie Chert paints the picture of an environment with early land plants operating close to their safety margin. This perspective makes it clear why plants have evolved many different solutions to increasing water transport efficiency through time. A large body of recent work on the evolution of terrestrial plant physiology reveals a common theme of increasing selective pressure for water transport safety, and efficiency, through time (Sperry *et al.*, 2006, 2008; Pittermann, 2010). Analysis of *Asteroxylon* reveals a recurring pattern in plant vascular evolution, that is, the appearance of tissues for increased conductivity precedes adaptations for increased safety. This relationship is also seen in early seed plants (Wilson *et al.*, 2008), early conifers (Wilson & Knoll, 2010) and the earliest vessel-bearing angiosperms (Sperry, 2003; Sperry *et al.*, 2007).

The hydraulic design of *Asteroxylon*'s water transport cells supports hypotheses that strong selective pressures have yielded multiple solutions to the CO_2–water tradeoff over the evolutionary history of land plants; these include low-resistance valves in conifers (Pittermann *et al.*, 2005, 2006; Choat & Pittermann, 2009; Pittermann, 2010), multicellular vessels in angiosperms (Bailey, 1953; Sperry *et al.*, 2006, 2007; Pittermann, 2010) and high-conductivity tracheids in early seed plants (Wilson *et al.*, 2008; Wilson & Knoll, 2010). *Asteroxylon* provides a critical early vantage point from which to view these evolutionary paths, revealing that early land plants functioned using comparatively risky ecophysiological strategies.

ACKNOWLEDGMENTS

REFERENCES

Bailey IW (1953) Evolution of the tracheary tissue of land plants. *American Journal of Botany* **40**, 4–8.

Beerling DJ, Royer DL (2002a) Fossil plants as indicators of the phanerozoic global carbon cycle. *Annual Review of Earth and Planetary Sciences* **30**, 527–556.

Beerling DJ, Royer DL (2002b) Reading a CO_2 signal from fossil stomata. *New Phytologist* **153**, 387–397.

Beerling DJ, McElwain JC, Osborne CP (1998) Stomatal responses of the 'living fossil' Ginkgo biloba L. to changes in atmospheric CO_2 concentrations. *Journal of Experimental Botany* **49**, 1603–1607.

Bergman NM, Lenton TM, Watson AJ (2004) COPSE: a new model of biogeochemical cycling over Phanerozoic time. *American Journal of Science* **304**, 397–437.

Berner RA (2006) GEOCARBSULF: a combined model for Phanerozoic atmospheric O_2 and CO_2. *Geochimica et Cosmochimica Acta* **70**, 5653–5664.

Boyce CK (2008) How green was *Cooksonia*? The importance of size in understanding the early evolution of physiology in the vascular plant lineage. *Paleobiology* **34**, 179–194.

Boyce CK, Knoll AH (2002) Evolution of developmental potential and the multiple independent origins of leaves in Paleozoic vascular plants. *Paleobiology* **28**, 70–100.

Brodribb TJ, Holbrook NM (2004) Stomatal protection against hydraulic failure: a comparison of coexisting ferns and angiosperms. *New Phytologist* **162**, 663–670.

Brodribb TJ, Holbrook NM, Zwieniecki MA, Palma B (2005) Leaf hydraulic capacity in ferns, conifers and angiosperms: impacts on photosynthetic maxima. *New Phytologist* **165**, 839–846.

Carlquist S (1975) *Ecological Strategies of Xylem Evolution*. University of California Press, Berkeley, CA.

Choat B, Pittermann J (2009) New insights into bordered pit structure and cavitation resistance in angiosperms and conifers. *New Phytologist* **182**, 557–560.

Christman MA, Sperry JS, Adler FR (2009) Testing the 'rare pit' hypothesis for xylem cavitation resistance in three species of *Acer*. *New Phytologist* **182**, 664–674.

Edwards D (1998) Climate signals in Paleozoic land plants. *Philosophical Transactions of the Royal Society of London, Series B: Biological Sciences* **353**, 141–156.

Edwards D (2003) Xylem in early tracheophytes. *Plant, Cell & Environment* **26**, 57–72.

Edwards D, Kerp H, Hass H (1998) Stomata in early land plants: an anatomical and ecophysiological approach. *Journal of Experimental Botany* **49**, 255–278.

Franks PJ, Beerling DJ (2009) Maximum leaf conductance driven by CO_2 effects on stomatal size and density over geologic time. *Proceedings of the National Academy of Sciences of the USA* **106**, 10343–10347.

Galtier J (2010) The origins and early evolution of the megaphyllous leaf. *International Journal of Plant Sciences* **171**, 641–661.

Graham LE, Gray J (2001) The origin, morphology, and ecophysiology of early embryophytes: neontological and paleontological perspectives. In *Plants Invade the Land: Evolutionary and Environmental Perspectives* (eds Gensel PG, Edwards D). Columbia University Press, New York, pp. 140–159.

Hacke UG, Sperry JS, Pockman WT, Davis SD, Mcculloch KA (2001) Trends in wood density and structure are linked to prevention of xylem implosion by negative pressure. *Oecologia* **126**, 457–461.

Hacke UG, Sperry JS, Pittermann J (2004) Analysis of circular bordered pit function – II. Gymnosperm tracheids with torus-margo pit membranes. *American Journal of Botany* **91**, 386–400.

Hacke UG, Sperry JS, Feild TS, Sano Y, Sikkema EH, Pittermann J (2007) Water transport in vesselless angiosperms: conducting efficiency and cavitation safety. *International Journal of Plant Sciences* **168**, 1113–1126.

Jansen S, Choat B, Pletsers A (2009) Morphological variation of intervessel pit membranes and implications to xylem function in angiosperms. *American Journal of Botany* **96**, 409–419.

Kenrick P, Crane PR (1997) *The Origin and Early Diversification of Land Plants: A Cladistic Study*. Smithsonian Institution Press, Washington, DC.

Kidston R, Lang WH (1920) On Old Red Sandstone plants showing structure, from the Rhynie Chert bed, Aberdeenshire. Part III. *Asteroxylon mackei*, Kidston and Lang. *Transactions of the Royal Society of Edinburgh* **52**, 643–680.

Konrad W, Roth-Nebelsick A, Kerp H, Hass H (2000) Transpiration and assimilation of early Devonian land plants with axially symmetric telomes – simulations on the tissue level. *Journal of Theoretical Biology* **206**, 91–107.

Lekner J (2007) Viscous flow through pipes of various cross-sections. *European Journal of Physics* **28**, 521–527.

Llorens L, Osborne CP, Beerling DJ (2009) Water-use responses of 'living fossil' conifers to CO_2 enrichment in a simulated Cretaceous polar environment. *Annals of Botany* **104**, 179–188.

Mark DF, Rice CM, Fallick AE, Trewin NH, Lee MR, Boyce A, Lee JKW (2011) 40Ar/39Ar dating of hydrothermal activity, biota and gold mineralization in the Rhynie hot-spring system, Aberdeenshire, Scotland. *Geochimica Et Cosmochimica Acta* **75**, 555–569.

McElwain JC (1998) Do fossil plants signal palaeoatmospheric CO_2 concentration in the geological past? *Philosophical Transactions of the Royal Society of London, Series B: Biological Sciences* **353**, 83–95.

McElwain JC, Chaloner WG (1995) Stomatal density and index of fossil plants track atmospheric carbon dioxide in the Palaeozoic. *Annals of Botany* **76**, 389–395.

Pittermann J (2010) The evolution of water transport in plants: an integrated approach. *Geobiology* **8**, 112–139.

Pittermann J, Sperry JS, Hacke UG, Wheeler JK, Sikkema EH (2005) Torus-margo pits help conifers compete with angiosperms. *Science* **310**, 1924–1924.

Pittermann J, Sperry JS, Wheeler JK, Hacke UG, Sikkema EH (2006) Mechanical reinforcement of tracheids compromises the hydraulic efficiency of conifer xylem. *Plant, Cell & Environment* **29**, 1618–1628.

Raven PH, Evert RF, Eichhorn SE (1999) *Biology of Plants*. W.H. Freeman, New York, NY, USA.

Roth-Nebelsick A (2001) Heat transfer of rhyniophytic plant axes. *Review of Palaeobotany and Palynology* **116**, 109–122.

Roth-Nebelsick A (2005) Reconstructing atmospheric carbon dioxide with stomata: possibilities and limitations of a botanical pCO_2-sensor. *Trees – Structure and Function* **19**, 251–265.

Roth-Nebelsick A, Konrad W (2003) Assimilation and transpiration capabilities of rhyniophytic plants from the Lower Devonian and their implications for paleoatmospheric CO_2 concentration. *Palaeogeography, Palaeoclimatology, Palaeoecology* **202**, 153–178.

Roth-Nebelsick A, Grimm G, Mosbrugger V, Hass H, Kerp H (2000) Morphometric analysis of *Rhynia* and *Asteroxylon*: testing functional aspects of early land plant evolution. *Paleobiology* **26**, 405–418.

Royer DL (2006) CO_2-forced climate thresholds during the Phanerozoic. *Geochimica et Cosmochimica Acta* **70**, 5665–5675.

Sackmann IJ, Boothroyd AI (2003) Our Sun. V. A bright young Sun consistent with helioseismology and warm temperatures on ancient Earth and Mars. *Astrophysical Journal* **583**, 1024–1039.

Sperry JS (2003) Evolution of water transport and xylem structure. *International Journal of Plant Sciences* **164**, S115–S127.

Sperry JS, Hacke UG (2004) Analysis of circular bordered pit function – I. Angiosperm vessels with homogenous pit membranes. *American Journal of Botany* **91**, 369–385.

Sperry JS, Hacke UG, Pittermann J (2006) Size and function in conifer tracheids and angiosperm vessels. *American Journal of Botany* **93**, 1490–1500.

Sperry JS, Hacke UG, Feild TS, Sano Y, Sikkema EH (2007) Hydraulic consequences of vessel evolution in angiosperms. *International Journal of Plant Sciences* **168**, 1127–1139.

Sperry JS, Meinzer FC, Mcculloh KA (2008) Safety and efficiency conflicts in hydraulic architecture: scaling from tissues to trees. *Plant, Cell & Environment* **31**, 632–645.

Spicer R, Groover A (2010) Evolution of development of vascular cambia and secondary growth. *New Phytologist* **186**, 577–592.

Taylor TN, Taylor EL, Krings M (2008) *Paleobotany: The Biology and Evolution of Fossil Plants*. Academic Press, New York, NY, USA.

Trewin NH, Rice CM (1992) Stratigraphy and sedimentology of the Devonian Rhynie chert locality. *Scottish Journal of Geology* **28**, 37–47.

Tyree MT, Ewers FW (1991) Tansley Review No. 34. The hydraulic architecture of trees and other woody plants. *New Phytologist* **119**, 345–360.

Tyree MT, Zimmermann MH (2002) *Xylem Structure and the Ascent of Sap*. Springer-Verlag, Berlin.

Van den Honert TH (1948) Water transport in plants as a catenary process. *Discussions of the Faraday Society* **3**, 146–153.

Wheeler JK, Sperry JS, Hacke UG, Hoang N (2005) Inter-vessel pitting and cavitation in woody Rosaceae and other vesselled plants: a basis for a safety versus efficiency trade-off in xylem transport. *Plant, Cell & Environment* **28**, 800–812.

Wilson JP, Knoll AH (2010) A physiologically explicit morphospace for tracheid-based water transport in modern and extinct seed plants. *Paleobiology* **36**, 335–355.

Wilson JP, Knoll AH, Holbrook NM, Marshall CR (2008) Modeling fluid flow in Medullosa, an anatomically unusual Carboniferous seed plant. *Paleobiology* **34**, 472–493.

APPENDIX

Flow through elliptical pits

Flow through a cylinder can be described by the Hagen–Poiseuille equation, but flow through pipes with asymmetrical cross-sections must be described using different solutions to the nonlinear Navier–Stokes equations (eqns 1 and 2). The approach taken here for an elliptical cylinder was lucidly described by Lekner (2007).

$$\partial_x v_x + \partial_y v_y + \partial_z v_z = 0. \tag{1}$$

$$\rho(v_x\partial_x + v_y\partial_y + v_z\partial_z)v_x = -\partial_x p + \eta(\partial_x^2 + \partial_y^2 + \partial_z^2)v_x$$
$$\rho(v_x\partial_x + v_y\partial_y + v_z\partial_z)v_y = -\partial_y p + \eta(\partial_x^2 + \partial_y^2 + \partial_z^2)v_y \tag{2}$$
$$\rho(v_x\partial_x + v_y\partial_y + v_z\partial_z)v_z = -\partial_z p + \eta(\partial_x^2 + \partial_y^2 + \partial_z^2)v_z.$$

Given a pipe with an elliptical cross-section with semimajor axis a and semiminor axis b, the area can be expressed as:

$$\frac{x^2}{a^2} + \frac{y^2}{b^2} = 1. \tag{3}$$

Flow through an elliptical aperture is proportional to the velocity through ellipse (4):

$$v_z = u\left(1 - \frac{x^2}{a^2} - \frac{y^2}{b^2}\right). \tag{4}$$

Integrating along the cylinder yields the expression (5):

$$Q = 4u \int_0^a dx \int_0^{b\sqrt{1-\frac{x^2}{a^2}}} dy \left(1 - \frac{x^2}{a^2} - \frac{y^2}{b^2}\right), \tag{5}$$

which simplifies to:

$$Q = \frac{\pi}{4\mu}\frac{\partial p}{\partial z}\frac{a^3 b^3}{a^2 + b^2}, \tag{6}$$

where μ is viscosity and $\partial p/\partial z$ is the pressure drop along the length, which drives the flow. We rearrange eqn (6) to express in form of Ohm's Law:

$$V = IR; \quad \frac{\partial P}{\partial z} = QR. \tag{7}$$

This allows us to solve for resistance of an elliptical aperture, which is proportional to aperture thickness:

$$R_{\text{aperture}} = \frac{(4\mu a^2 + b^2)}{\pi a^3 b^3} t_{ap}. \tag{8}$$