BIOPHYSICAL PERSPECTIVES OF XYLEM EVOLUTION: IS THERE A TRADEOFF OF HYDRAULIC EFFICIENCY FOR VULNERABILITY TO DYSFUNCTION?

by

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Summary

In this review, we discuss the evolution of xylem structure in the context of our current understanding of the biophysics of water transport in plants. Water transport in land plants occurs while water is under negative pressure and is thus in a metastable state. Vessels filled with metastable water are prone to dysfunction by cavitation whenever gas-filled voids appear in the vessel lumen. Cavitated vessels fill with air and are incapable of water transport until air bubbles dissolve. We know much more about how cavitations occur and the conditions under which air bubbles (embolisms) dissolve. This gives us an improved understanding of the relationship between xylem structure and function.

We argue that the efficiency of water transport increases in vessels with increasing diameter. The vulnerability of large diameter vessels to frost-induced embolism is dramatically increased. Thus there is a selection for small diameter vessels in cold climates. The relationship between vessel diameter and vulnerability of large diameter vessels to drought-induced embolism is much weaker. The correlation is too weak to permit comparative physiologists to predict vulnerability based on vessel diameter, but the correlation is strong enough to be of some evolutionary significance.

Key words: Xylem evolution, cavitation, xylem dysfunction, frost, drought.

Symbols

D = conduit diameter, D_{95} = mean value of diameter of all conduits that account for 95% of the hydraulic conductivity of a stem, E = Young's modulus, K_i = initial hydraulic conductivity, K_m = maximum hydraulic conductivity, P_c = probability of cavitation, P_w = pressure in water, P_v = pressure in a void (embolism), PLC = percent loss hydraulic conductivity, r_m = radius of curvature of meniscus, r_p = radius of pore where meniscus is located, V = volume, VC = vulnerability curve, η = viscosity, Ψ = water potential, Ψ_i = initial water potential, Ψ_{xp} = xylem pressure potential, Ψ_{50} = Ψ_{xp} which induces 50 PLC, ρ = stem density, σ = breaking stress of a stem, τ = surface tension.

Introduction

Comparative physiologists view physiological and biophysical characteristics of plants in terms of how they contribute to fitness in a particular environment. A well adapted plant is presumed to have a more optimal suite of characteristics than a less adapted plant. Not all characteristics need to be optimal for species survival because some suboptimal characters may be compensated by others that are more optimal. One heuristic viewpoint for the interpretation of evolution is that plants are tremendously tolerant in their capacity to cope with the environment structurally or physiologically. Provided a

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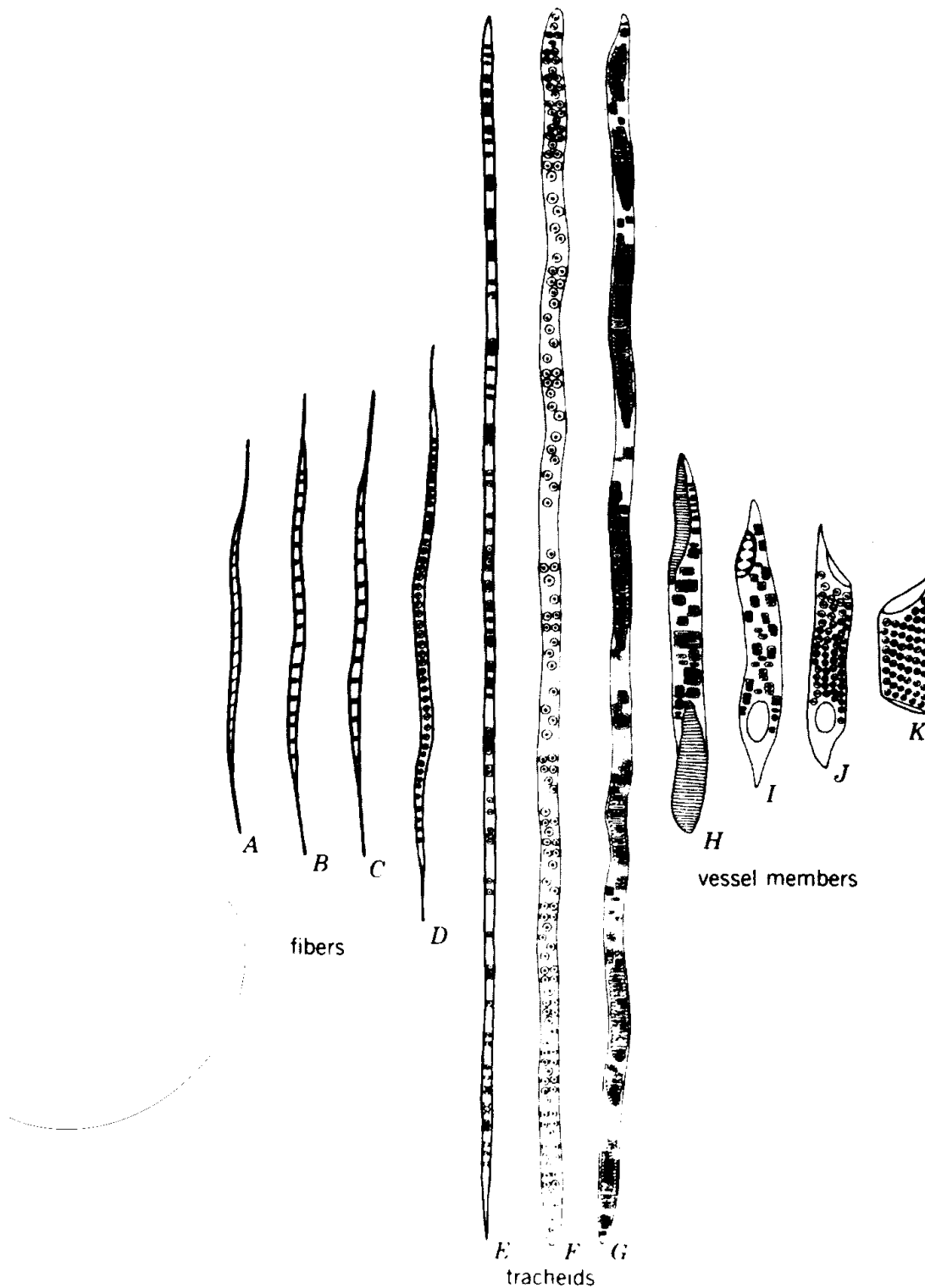
Fig. 1. Diagrammatic illustration of average size and structure of tracheary elements in the mature wood of some conifers and dicotyledons (Bailey & Tupper 1918). – E–G: Long tracheids from primitive woods (G showing *Trochodendron* or *Dioon*, axially foreshortened). – D–A: Evolution of fibres, showing decrease in length and reduction in size of pit borders. – H–K: Evolution of vessel elements, decrease in length, replacement of scalariform perforations and pits by simple perforations and alternate pits.

plant structure meets minimal requirements and performance levels, its shape, size, and structure can vary widely within boundary conditions set by biophysical and biological laws. Adaptive characteristics are viewed as benefiting the plant, but the benefits generally have an associated cost (Givnish 1986) and sometimes become irrelevant depending on changes in the physical environment or competing organisms (Grace & Tilman 1990). For example, increased height will benefit a plant in terms of competition for light, but at a cost of increased carbon investment in the construction and maintenance of stems for mechanical support. Or a chemical defence against an herbivore may be countered by increased chemical tolerance in the herbivore.

The evolution of land plants started approximately 420 million years ago in the Silurian period. In the new land environment, where water was much less abundant, the principal functions that had to be perfected included photosynthesis (light interception and gas exchange with minimal water loss), reproduction (spore release, pollen capture, seed and fruit release), mechanics (the need to provide support for static and dynamic loading of organs no longer supported by the buoyancy of water) and hydraulics (absorption, transport of water to keep pace with evaporation). Much of the evolution of land plants can be interpreted in terms of what structures were needed to provide the above functions (Niklas 1992).

Unlignified cellulose provides excellent tensile strength, so this permitted aquatic plants to live in fresh water and sustain the positive turgor pressures in cells without exploding. But cellulose is poor at providing stability against compressive and bending forces; plants without lignified walls are mechanically limited to heights of about 0.5 m (Niklas 1993a & b). Once plants left the relatively buoyant environment of water to reside in much less buoyant air, the need to support their own mass against the force of gravity was met by the evolution of lignification (Barghoorn 1964) which made large upright land plants possible. Lignification provided strength against compressive and bending forces permitting both tall plants and plants with crowns extending over long horizontal distances. Lignification also stabilises cells

against collapse under negative pressure which permitted a novel water-transport system (see below). Lignification has the added benefit of making cellulose less digestible for herbivores. Lignification appears in some primitive algae and may have evolved first as a defense against herbivory (Niklas 1992).

One characteristic that has fascinated wood anatomists for nearly a hundred years is the evolution of xylem conduit size, i.e., the length and diameter of tracheids or vessels, and the specialisation of cell types between support and water transport functions (Bailey & Tupper 1918; Baas 1986; Carlquist 1988). A continuous record of upright land plants dates back to the upper Silurian era about 400 million years ago (Andrews 1961; Banks 1964). For the first 300 million years, tracheids appear to have been the only highly specialised water-conducting elements in existence. At the end of this time, when flowering plants arose, woody xylem became more specialised by a separation of water conduction from mechanical support (Bailey & Tupper 1918). The classical illustration in Figure 1 shows how xylem tracheids may have evolutionarily diverged to become specialised for support (fibres) and for efficient transport (vessel elements). There has been a general evolutionary trend from small diameter tracheids to large diameter vessel elements. It is presumed that part of the selection for large conduit diameter has been driven by the benefit of increased efficiency in hydraulic conductance.

Wide conduits are much more efficient water conductors than narrow conduits so evolution has driven plants towards wider conduits. But it is strange that the upper limit on useful vessel diameter is approximately 0.5 mm (Putz & Mooney 1991). This limit has been reached in parallel in many diverse taxa in trees, vines and monocots. The range of conduit diameters is from 0.01 to 0.5 mm (a factor of 50 from minimum to maximum) whereas the range of functional unit lengths is much wider, i.e., a factor of 50,000 from 1 mm for tracheids to 50 m for vessels (Zimmermann 1983). A vessel, as a functional unit, is defined here as a catena of vessel elements closed at all surfaces by pit membranes so that vessel to vessel transport of water

requires passage through pit membranes of simple or bordered pits in the same way that tracheid to tracheid transport is through pit membranes of bordered pits. However, the pit membrane of most conifers is specialised having a central torus and a fibril or margo (Zimmermann 1983).

The reason for the upper limit of conduit diameter cannot be traced to a loss in mechanical strength. The strength of large plants (which determines their maximum possible mechanical height) is determined by density-specific stiffness and density specific strength. The former is the ratio of Young's modulus to density (E/ρ) and the latter is the tissue breaking stress to density (σ/ρ). The ideal mechanical tissue with which to construct a vertical stem is one for which both of these ratios are maximised because this maximises the extent to which a stem can grow vertically before it bends or breaks under its own weight (Niklas 1993a & b). It is always possible to enhance hydraulic conductance and maintain strength by surrounding a few very large conduits (which decrease local strength but increase conductance) with a large number of small lignified cells such as fibres (to preserve a suitable σ and E).
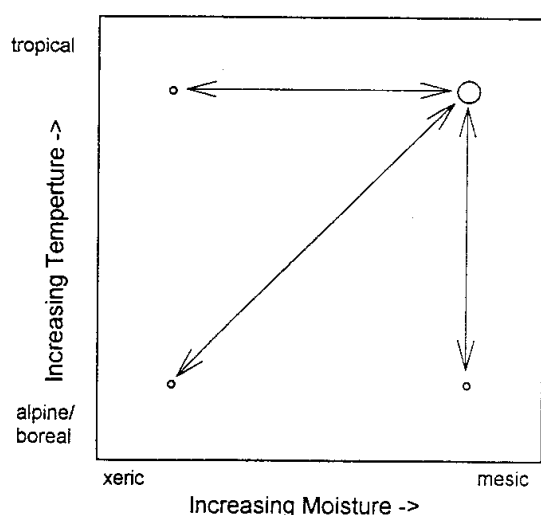

Fig. 2. Diagram showing ecological gradients in temperature and moisture and the direction of evolution of vessel diameter in response to these gradients. The double-headed arrows indicate that plants evolve in both directions along the indicated gradients.

It is presumed by many that there is some kind of tradeoff between large conduits with high hydraulic efficiency and the vulnerability of xylem to dysfunction (Zimmermann 1983). This presumption is borne out of many anatomical surveys using both systematic and floristic approaches (see for example Carlquist 1975, 1977a & b, 1988, 1989; Baas 1976, 1982; Carlquist & Hoekman 1985). These studies have shown a general trend in conduit diameter shown in Figure 2. Wet-warm environments tend to favour species with wide conduits whereas cold or dry environments tend to favour species with narrow conduits. The purpose of this review is to discuss the possible benefits and costs of large conduits from a biophysical perspective.

Biophysical benefits of wide conduits

Wide conduits are of benefit because they are more efficient conductors of water. The rate of water flow, w $(m^3 \ s^{-1})$, through a pipe of diameter D (m) is proportional to the pressure gradient, dP/dx $(Pa \ m^{-1})$. The Hagen-Poiseuille law gives the proportionality constant between w and dP/dx in terms of D and the viscosity of water, η $(= 1.0 \times 10^{-3}$ Pa s at 20 °C), i.e.,

$$w = \pi D^4 / 128\eta \ dP/dx \qquad (1)$$

From Equation 1 it is clear that the efficiency of transport in a pipe increases with the fourth power of its diameter. This means, for example, that one pipe of 40 μm diameter can carry as much water as 16 pipes of 20 μm (Fig. 3) or, alternatively, one pipe of 40 μm diameter is as efficient as 256 pipes of 10 μm.

Xylem conduits (vessels or tracheids) are not continuous from roots to leaves, so the Hagen-Poiseuille law does not account for all the pressure drop needed in xylem to maintain flow. On its path from the soil to the leaf, water must pass between many pairs of conduits via pit membranes. The pit membranes are quite important to the safety of water transport (more about that later), but an extra pressure drop is needed to drive water from conduit to conduit via the pit pairs. Tracheids are 1 to 3 mm long so water must pass through an average of 300 to 1000 tracheids per metre of wood. About half the pressure drop is in the tracheid lumina and half in the pits (Zim-

A
40
0
0   40

B
80
40
0
0   40   80

C
160
120
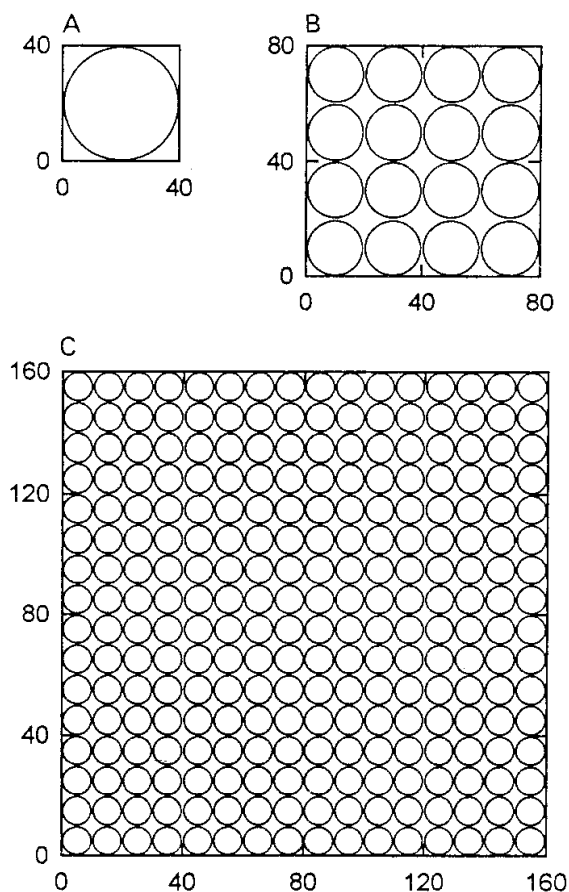80
40
0
0   40   80   120   160

Fig. 3. Hagen-Poiseuille law prediction of relation between conduit diameter and conductance. The vessel blocks in A, B, and C have equal conductance, i.e., one vessel 40 μm diameter (A) is as conductive as 16 vessels 20 μm diameter (B), or as conductive as 256 vessels 10 μm diameter (C). Notice that more wood cross section is needed to contain many small vessels having a combined conductance of a few large vessels.

mermann 1983). In contrast, vessels are typically 0.1 to 10 m long so the pressure drop in the vessel lumina is presumably a larger fraction of the total pressure drop needed to move water in the xylem.

It is important to realise that there is a cost associated with the pressure drop required to transport water from roots to leaves. This is because water is drawn up under tension by leaves, i.e., the fluid pressure in leaves must fall below atmospheric pressure to draw water up from below. This reduced fluid pressure in leaves imposes an important stress on living tissues in leaves. If this stress becomes too large it causes a reduction in gas exchange, photosynthesis and can cause xylem dysfunction (Tyree & Sperry 1988).

Although the Hagen-Poiseuille law does not give the total pressure drop for water flow in conduits, it accounts for enough to provide some useful insights. The Hagen-Poiseuille law allows us to understand why there is an effective lower limit on conduit diameter in trees of about 10 μm for tracheids. Besides storage, xylem serves two equally important functions: mechanical support and water transport. Let us imagine that a conifer (taxon A) had 90% of its wood volume composed of tracheids and that it had just enough wood to provide adequate water transport and mechanical support. Taxon A is unlikely to evolve an identical wood structure but with tracheids half as wide (5 μm, taxon B) since it would then require 16 times more tracheids to provide the same hydraulic supply and would require 4 times more wood to contain the 16 times more tracheids. Although taxon B would have 4 times more wood and likely to be much stronger, such a growth habit would require too much investment of carbon in stems and their subsequent maintenance. Taxon A might well evolve an alternative structure with wider conduits if this could be done without sacrificing strength because such a structure would transport water with less cost in leaf water stress. If wide conduits are mechanically weaker than small conduits, then an evolutionary strategy selecting for a mixture of large and small conduits would work and this seems to be the strategy taken by vessel-bearing trees.

To understand why an upper limit of vessel diameter is a few hundred microns, we must first discuss the mechanism of water transport in trees so we can understand the kind of dysfunction that might interrupt water transport.

Mechanism of xylem sap ascent, cavitation and risks

The evolution of cell walls allowed the plant kingdom to solve the problem of osmoregulation in freshwater environments; confining protoplasm inside a rigid exoskeleton prevented cell rupture as a result of osmotic

inflow of water. The cost of cell walls was a loss of motility. In contrast, in the animal kingdom osmoregulation involved the evolution of a vascular system that bathed most cells in iso-osmotic blood plasma; this avoided rigid walls and permitted cell and organismal motility. Cell walls also placed constraints on the evolution of long-distance transport systems. Tissues were too rigid to evolve a heart pump mechanism. Instead plants evolved two novel transport systems. One is a positive pressure system that moves concentrated, sugar-rich sap in the phloem from leaves to growing meristems or storage areas. Phloem transport uses a standing-gradient osmotic flow mechanism similar to that found in some animal excretory organs, but it is unique in that it occurs at very high pressure (up to 3 MPa) and requires two standing-gradient systems in tandem. One loads sugar and pushes phloem sap and the other unloads sugar and pulls phloem sap (Van Bel 1993).

Mechanism of xylem sap ascent

The other transport system for sap ascent rarely works under positive pressure, i.e., fluid pressure above atmospheric. It does happen in certain large woody plants (e.g., *Acer, Betula, Vitis*) during early spring when roots are active but leaves have not unfolded. If at this time the xylem is cut, it bleeds sap. In certain herbs, especially grasses, xylem pressure becomes positive at night when transpiration is suppressed, and even without injury water can drip (guttate) from leaves via hydathodes which are normally located at leaf margins (Zimmermann 1983; Mauseth 1988).

During the growing season, water is lifted up into the leaves by less-than-atmospheric pressure created in the leaf xylem by transpiration. Our current understanding of the mechanism of sap ascent is based on the cohesion theory usually ascribed to H.H. Dixon (1914), and a useful biophysical review of the theory can be found in Pickard (1981) and Tyree & Sperry (1989). Briefly, the process can be described as follows. Evaporation from cell wall surfaces inside the leaf causes the air-water interface to retreat into the finely porous spaces between cellulose microfibrils in the wall (Carpita 1982). Capillarity (a conse-

quence of surface tension) tends to draw the interface back up to the surface of the pores and places the mass of water behind it under negative pressure. This negative pressure is physically equivalent to a tension (a pulling force) that is transmitted to soil water via a continuous water column (Van den Honert 1948). The system is vulnerable to dysfunction because any break in the column in a conduit drains the conduit of water and necessarily disrupts further water flow.

Xylem transport can occur by this mechanism because of the special properties of water and the structure of xylem. Hydrogen bonding promotes cohesion between water molecules and allows water to remain liquid under tension. Calculations of the theoretical tensile strength of water indicate that tensions in excess of 100 MPa would be needed to induce cavitations in the bulk phase of water (Apfel 1972; Oertli 1971; Pickard 1981; Borghetti et al. 1993). The maximum pulling force attainable is a function of the pore size in the cell walls of mesophyll parenchyma, the microsites of evaporation in leaves. The theoretical pull through pores of 5 nm diameter, based on the capillary equation, would be 60 MPa.

When the tensile strength of water is exceeded or when a bubble of sufficient diameter is introduced into a conduit, the conduit cavitates, i.e., the void in the water column rapidly expands to fill the entire conduit. Conduits are of a finite length (shorter than the plant) and interconnected by pits pairs. The pits do not permit air to pass freely from one conduit to another because of surface tension in the water filled pores of pit membranes (see below) and this is an important part of the defence mechanism for preventing cavitation dysfunctions from propagating through the entire plant. A void or gas bubble is prevented from moving through pits from one vessel to another by the primary cell wall (pit membrane) located at the median plane of the pits (Sperry & Tyree 1988). In conifer tracheids, the passage of air is prevented by the aspiration of the torus in bordered pits (Sperry & Tyree 1990), i.e., the torus is displaced by differential pressure to seal the hole in the border of one side of the pit pair.

Cavitation and stability of gas-filled voids (emboli)

A cavitation event in xylem conduits ultimately results in dysfunction. A cavitation occurs when a void of sufficient radius forms in water under tension. The void is gas filled (water vapour and some air) and is inherently unstable, i.e., surface tension forces will make it spontaneously collapse unless the water is under sufficient tension (negative pressure) to make it expand. We must take a necessary diversion to explain why this is true.

The chemical force driving the collapse is the energy stored in hydrogen bounds, the intermolecular force between adjacent water molecules. In ice, water is bound to adjacent water molecules by 4 hydrogen bonds. In the liquid state, each water molecule is bound by an average of 3.8 hydrogen bonds at room temperature. In the liquid state hydrogen bonds are forming and breaking all the time permitting more motion of molecules than in ice (Slatyer 1968). But when an interface between water and air is formed some of those hydrogen bounds are broken and the water molecules at the surface are at a higher energy state because of the broken bonds. The force (N = Newtons) exerted at the interface as hydrogen bonds break and reform can be expressed in pressure units (Pa) because pressure is dimensionally equal to energy (J = Joules) per unit volume of molecules, i.e., $J\ m^{-3} = N*m\ m^{-3} = N\ m^{-2} = Pa$. Stable voids in water tend to form spheres because spheres have the least surface area per unit volume and thus a spherical void has the minimum number of broken hydrogen bonds per unit volume of void. The pressure tending to make a void collapse is given by $2\tau/r$, where r is the radius of the spherical void and τ is the surface tension of water (= 0.072 Pa m at 25 °C).

For a void to be stable, its collapse pressure $(2\tau/r)$ must be balanced by a pressure difference across its surface or meniscus = $P_V - P_W$, where P_W is the pressure of the water and P_V is the pressure of the void.

$$P_V - P_W = 2\tau/r \qquad (2)$$

P_V is usually above absolute zero pressure (= perfect vacuum) since the void is usually

filled with water vapour and some air. Relatively stable voids are commonplace in daily life, e.g., the air bubbles that form in a cold glass of water freshly drawn from a tap. An entrapped air bubble is temporarily stable in a glass of water because P_W is a relatively constant 0.1 MPa and P_V is determined by the ideal gas law, $P_V = nRT/V$ where n = the number of moles of air in the bubble, R = gas constant, T = absolute temperature, and V = the volume of the bubble. So the tendency of the void to collapse $(2\tau/r)$ makes V decrease which causes P_V to increase according to the ideal gas law because P_V is inversely proportional to V. The rise in P_V provides the restoring force across the meniscus needed for stability. But an air bubble in a glass of water is only temporarily stable because according to Henry's law, the solubility of a gas in water increases with the pressure of the gas. So the increased pressure exerted by $2\tau/r$ makes the gas in the bubble more soluble in water and it slowly collapses as the air dissolves, i.e., as n decreases.

Air bubbles are rarely stable in xylem conduits, because transpiration can draw P_W to values < 0. As P_W falls towards 0 the bubble expands according to the ideal gas law, but V can never grow larger than the volume of the conduit, so P_V can never fall to or below zero to balance $2\tau/r$. Once the bubble has expanded to fill the lumen, the conduit is dysfunctional and no longer capable of transporting water. Fortunately for the plant, a dynamic balance at the meniscus is ultimately achieved. This stability will be discussed first in the context of a vessel and its pit membranes.

As the air bubble is drawn up to the surface of the pit membrane (Fig. 4), the pores in the pit membrane break the meniscus into many small menisci at the opening of each pore. As the meniscus is drawn through the pores the radius of curvature of the meniscus, r_m, falls towards the radius of the pores, r_p. As long as r_m exceeds r_p, the necessary conditions for stability are again achieved, i.e.,

$$P_V - P_W = 2\tau/r_m \qquad (3)$$

Usually a dysfunctional conduit will eventually fill with air at atmospheric pressure (as demanded by Henry's law) so P_V eventually
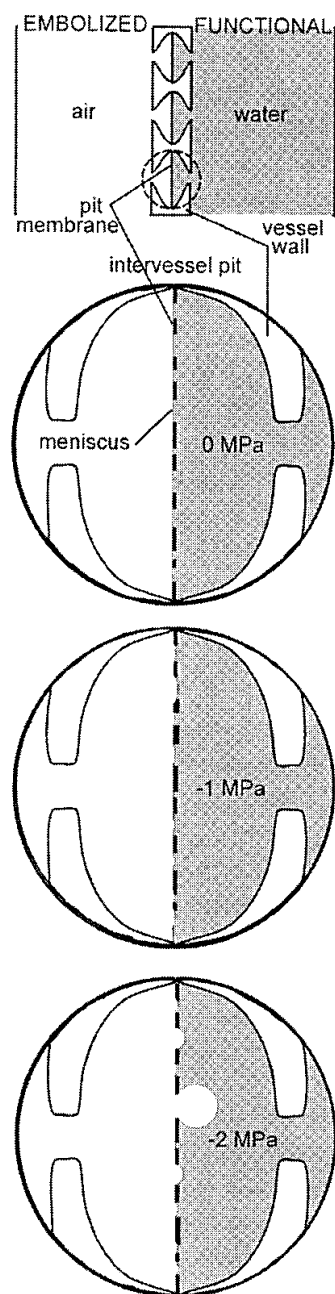
Fig. 4. Diagram of wall structure between adjacent xylem vessels showing intervessel pit structure. The porous pit membrane develops from primary cell walls of the two vessels and middle lamella. It is overarched by thick secondary walls to form a pit chamber that opens to the vessel lumen via a pit aperture. When a vessel is embolised, air is prevented from spreading to adjacent functional vessels by the capillary force of the air-water meniscus spanning pit membrane pores. As the pressure difference increases across the pit membrane pores, the meniscus is gradually pulled through.

approaches 0.1 MPa as gas diffuses through water to the lumen and comes out of solution When P_v equals 0.1 MPa the conduit is said to be fully embolised. As P_w rises and falls as dictated by the demands of transpiration r_m adjusts at the pit-membrane pores to achieve stability. When the conduit is fully embolised, both sides of Equation (3) can be expressed in terms of xylem pressure potential,

$$\Psi_{xp} = -(P_v - P_w) = -2\tau/r_m \qquad (4)$$

The minimum Ψ_{xp} that can be balanced by the meniscus is given when r_m equals the radius of the biggest pit-membrane pore bordering the embolised conduit. If the biggest pore is 0.1 or 0.05 μm, then the minimum stable Ψ_{xp} is -1.44 or -2.88 MPa, respectively. So the porosity of the pit-membrane is critical to preventing dysfunction of vessels adjacent to embolised vessels (Sperry & Tyree 1988). When Ψ_{xp} falls below the critical value then the air bubble is sucked into an adjacent vessel seeding a new cavitation.

Consequently, the genetics that determines pit morphology and pit-membrane porosity must be under strong selective pressure. A safe pit-membrane will be one with very narrow pores and one thick enough and thus strong enough to sustain substantial pressure differences without rupturing. But the cost is that narrow pores and long pores (in thick pit-membranes) do not conduct water efficiently (see the Hagen-Poiseuille law above). So a balance must be achieved between safety and efficiency of water transport. Hydraulic efficiency could be increased by increasing the surface area of pit membranes but this would weaken the conduit and make it more liable to collapse under negative pressure.

The situation for tracheids of conifers is different because air movement from an embolised tracheid to an adjacent tracheid is prevented by the sealing (aspiration) of the torus against the overarching border of the pit (Fig. 5). The porosity of the margo that supports the torus is too large to prevent meniscus passage at pressure differences exceeding 0.1 MPa in most cases (Sperry & Tyree 1990). But the margo is quite elastic, a pressure difference of just 0.03 MPa is sufficient to deflect the torus into the sealed position (Fig. 5).
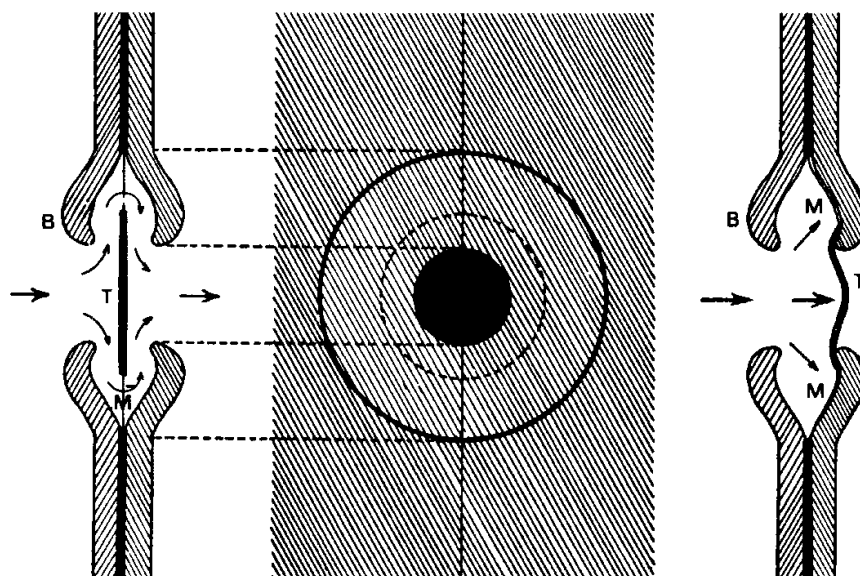
Fig. 5. Centre: Surface view of the radial wall of a coniferous tracheid, showing a bordered pit. – Left: The same pit in section, arrows indicating the path of water from one tracheid into the next. – Right: Section showing the valve-like action of the torus. T = torus; M = pit membrane; B = pit border (Bailey 1913).

Air bubbles pass between tracheids when the pressure difference becomes large enough to rip the torus out of its sealed position (Sperry & Tyree 1990).

It is not clear when first land plants solved the developmental problem of how to construct cell walls having pits with a strong pit membrane containing fine pores. Nor is it clear why vesselless structures of conifers persist given that it is less efficient (measured in terms of hydraulic conductance per unit cross section). The reasons deserve further study. One possible reason is that conifer wood has greater conduit redundancy (= safety in numbers) investing less genetic code for varied cell types than vessel bearing angiosperms. The greater genetic information required to code for woody cell types of angiosperms would presumably require more evolutionary time to arise.

The mechanism of seeding cavitations

We now have a biophysical understanding of the stability of emboli and how emboli can be sucked into a water-filled conduit from a neighbouring embolised conduit. Plants will always have some embolised conduits to seed embolism into other conduits. Embolisms are the natural consequence of foliar abscission, herbivory, wind damage, and other mechanical fates that might befall a plant. It is now appropriate to ask if all emboli are seeded from adjacent conduits or if some other mechanism occurs in some or most of the cases. The mechanism must be understood if we are to predict the tradeoff (if any) between conduit size and safety, i.e., is a large efficient conduit more likely to cavitate than a small one?

Four mechanisms for the nucleation of cavitations in plants have been proposed and these are illustrated in Figure 6, which show for each mechanism the sequence of events that might occur as Ψ_{xp} declines in the lumen of a conduit.

Of these four, homogeneous nucleation is the most studied mechanism in the physical sciences (Pickard 1981). Homogeneous nucleation can be quantitatively predicted from the statistical mechanics of molecular motion in water. The calculation is done to predict the probability of a void spontaneously occurring in the 'centre' of a water container, i.e., far enough away from the container walls that the molecular bonds between the wall and water can be ignored. The factors
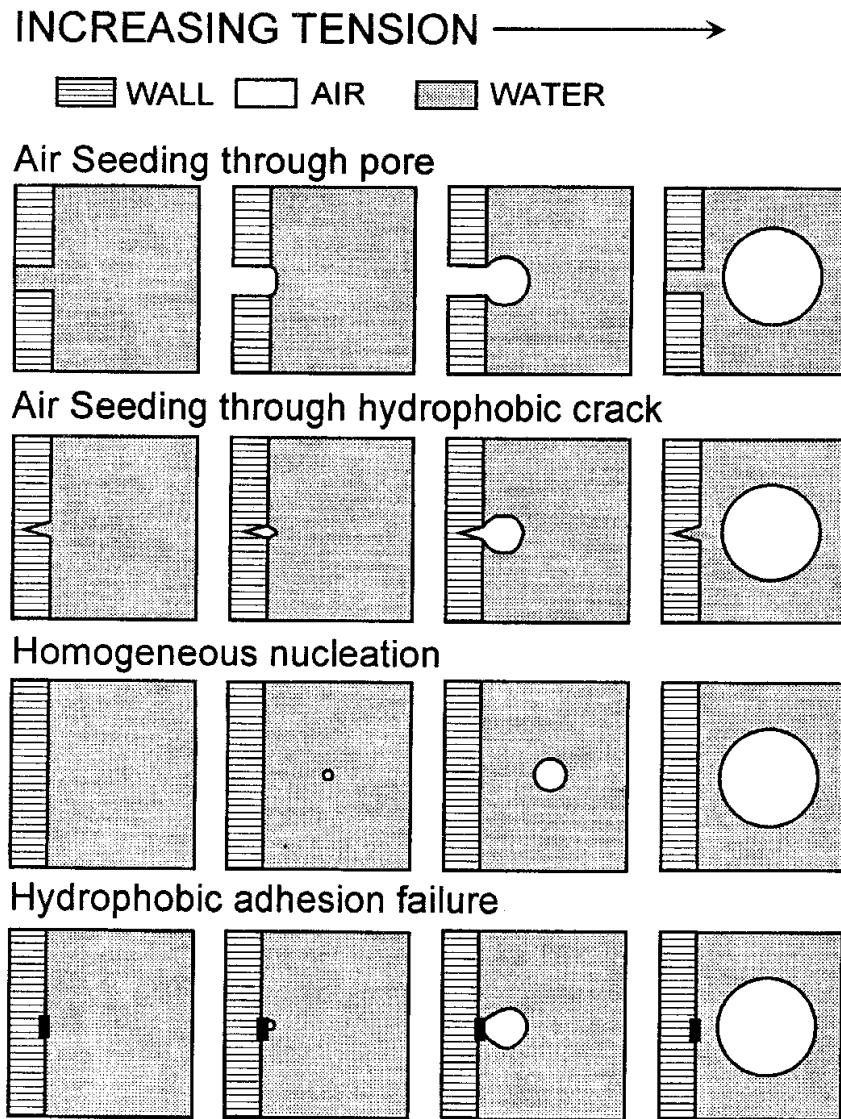
344 344

Fig. 6. Diagram illustrating four mechanisms of cavitation nucleation in xylem conduits.
See text for details.

that have to be taken into account is the kinetic energy of thermal motion of water molecules and the probability that several water molecules will be moving away from a common locus with sufficient kinetic energy to break the hydrogen bonds holding them together. If the water is under tension (negative pressure) the hydrogen bonds will already be stretched and thus weakened making the statistical probability of a void forming more likely. The size of the void that must form to be unstable and continue to expand is given by Equation (2) and is also a function of Ψ_{xp}. Readers may consult Pickard (1981)

for details and other reference for the statistical formulations, but all the reader has to know is that the probability, P_c, of a cavitation occurring will increase with: a) the volume of the water in the container (conduit volume), b) tension applied to the volume, and c) with the time (duration) the tension is applied. In short P_c increases with the time-tension-volume domain.

Heterogeneous nucleation occurs when water pulls away from the container wall (top two and last example in Fig. 6). The nature of the forces binding water to wall-material differs from that holding water to water. Hetero-

geneous nucleation is less well understood because it depends on the nature of wall defects. The best understood mechanisms are nucleation at a hydrophobic crack and nucleation by air seeding. Pickard (1981) discusses at length nucleation at hydrophobic cracks. Briefly, air bubbles can remain stable in hydrophobic cracks without dissolving because at the hydrophobic interface the angle of contact at the wall-water-air interface is altered forming a reversed meniscus. Both the angle of contact and the size of the crack determine the radius of curvature of the meniscus, but an equation similar to Equation (4) can be used to predict the Ψ_{xp} needed to pull a bubble out of a crack based on its radius of curvature. A second kind of heterogeneous nucleation might occur at the interface of water and hydrophobic patches without the presence of air bubbles. No predictive equations are available for this case. The third kind of heterogeneous nucleation (the air seeding hypothesis of Zimmermann 1983) has already been discussed but not named. Air seeding occurs when an air bubble is sucked into a water-filled lumen via a pore from an adjacent air space. One interesting side question is: are there pathways for air entry that communicate directly between the outside atmosphere and a xylem conduit? Or do all air-seeding events originate from adjacent air-filled conduits? An experimental answer to this question will be presented near the end of this paper.

Risk versus size – the original hypothesis

We can use what is known about the mechanisms listed above to provide predictions about a possible relationship between conduit size and the probability for a cavitation event. Such predictions provide an underlying mechanism for thinking that both hydraulic efficiency and vulnerability to cavitation might increase with conduit size and that there might be a tradeoff between efficiency and vulnerability. What follows is basically the argument made for many decades *before experimental protocols were available to test the predictions.*

Consider two identical stems with conduits of equal length supplying water to the same leaf area. Stem A has 100 conduits 40 µm in

diameter; stem B has 1600 conduits 20 µm in diameter. Both stems will have the same hydraulic conductivity, according to the Hagen-Poiseuille law. If the conduits cavitate by homogeneous nucleation we have to make predictions based on a time-tension-volume domain. Let us say that for the volume of the 100 conduits in stem A the probability is that 50 cavitations will occur in a given time-tension domain. This same volume would equal 400 conduits for stem B. Thus in stem A, 50 vessels out of 100 will cavitate with *perhaps* a 50% loss of conductivity, whereas the same 50 cavitations (out of 400 conduits) will result in a 12.5 % loss of conductivity. Thus stem B is *perhaps* 4 times safer (due to greater redundancy = safety in numbers).

Applying the same argument to heterogeneous nucleation is a little more complex because of some unknown factors, i.e., the nature of imperfections in walls (hydrophobic cracks, hydrophobic patches, or pores for air-seeding). Generally the way around the unknown is to assume that the probability of an imperfection increases with the surface area. Let us assume that in the given surface area of 100 conduits in stem A there are enough imperfections to cause cavitation in 50 of the conduits by the time Ψ_{xp} has fallen from 0 to –3 MPa. Because the conduits in stem B are half the diameter of conduits in stem A it takes 200 conduits to have the same surface area as 50 conduits in stem A. Probability would predict 50 cavitations in those 200 conduits (or 400 cavitations in 1600 conduits). Thus when stem A suffers 50% loss of conductance stem B suffers only 25% loss and stem B is *perhaps* 2 times safer.

Pathway redundancy: a cost or benefit?

The above argument needs to be generalised somewhat to take into account the built in pathway redundancy of xylem. This generalisation will be at the sacrifice of predictive power, but it is important for conceptual understanding.

The concept of redundancy is illustrated in Figure 7. Redundancy is provided by the sharing of walls (and pits) in common between adjacent conduits. In Figure 7A are 6 conduit catenas with each catena comprised of 6 con-
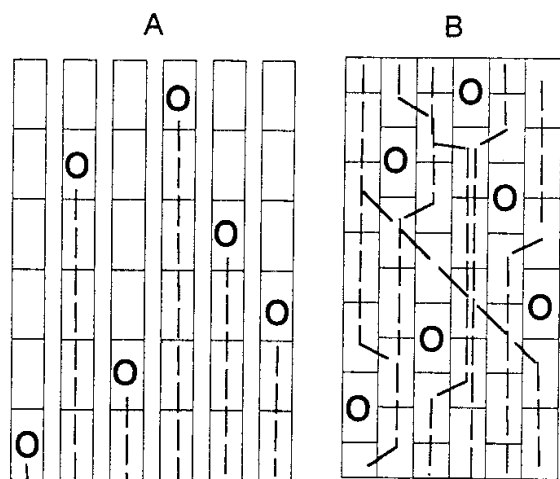
A   B

Fig. 7. Diagram illustrating pathway redundancy. – A: No redundancy; 6 files of 6 conduits connected end-to-end. Water flow can occur only through the ends but not through the sides. – B: Pathway redundancy; 6 files of 6 conduits connected end-to-end and through side walls. Circles indicate placement of embolisms and dashed lines indicate some of the possible pathways of water movement.

duits with no redundancy, i.e., each catena is a linear assemblage of conduits connected at the apical and basal ends. The open circles signify 6 conduits that have been blocked by cavitations. Although only 16.7% (1/6) of the conduits are cavitated, all lines of water transport are blocked. Contrast this to the 36 conduits in 6 adjoining catenas in Figure 7B. This example has more redundancy in pathways. The same 6 conduits are shown embolised, but if all adjacent walls conduct water through pits then there are many redundant pathways to permit water transport, some of which are indicated by dashed lines.

In Figure 7A the stem has suffered a 100% loss of conductance with only 16.7% of the conduits cavitated, whereas the loss of conductivity is much less (perhaps 20 or 30%, see below) in Figure 7B. The percent loss in conductance in Figure 7B cannot be predicted without knowing the relative resistance to water flow in lumina versus the resistance for passage in adjacent walls, but it will be significantly less than 100%.
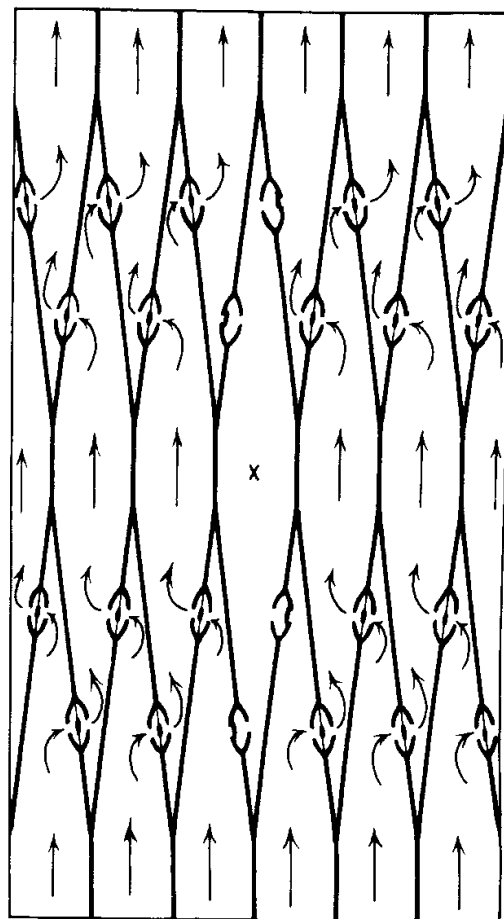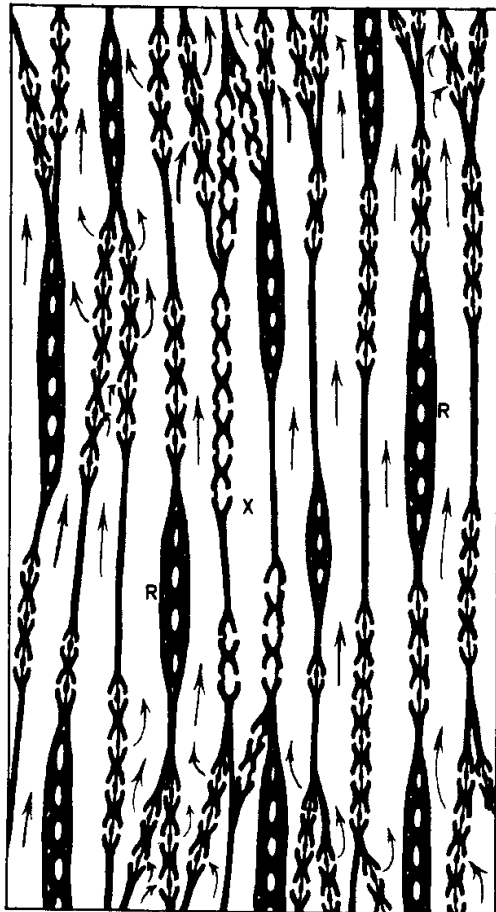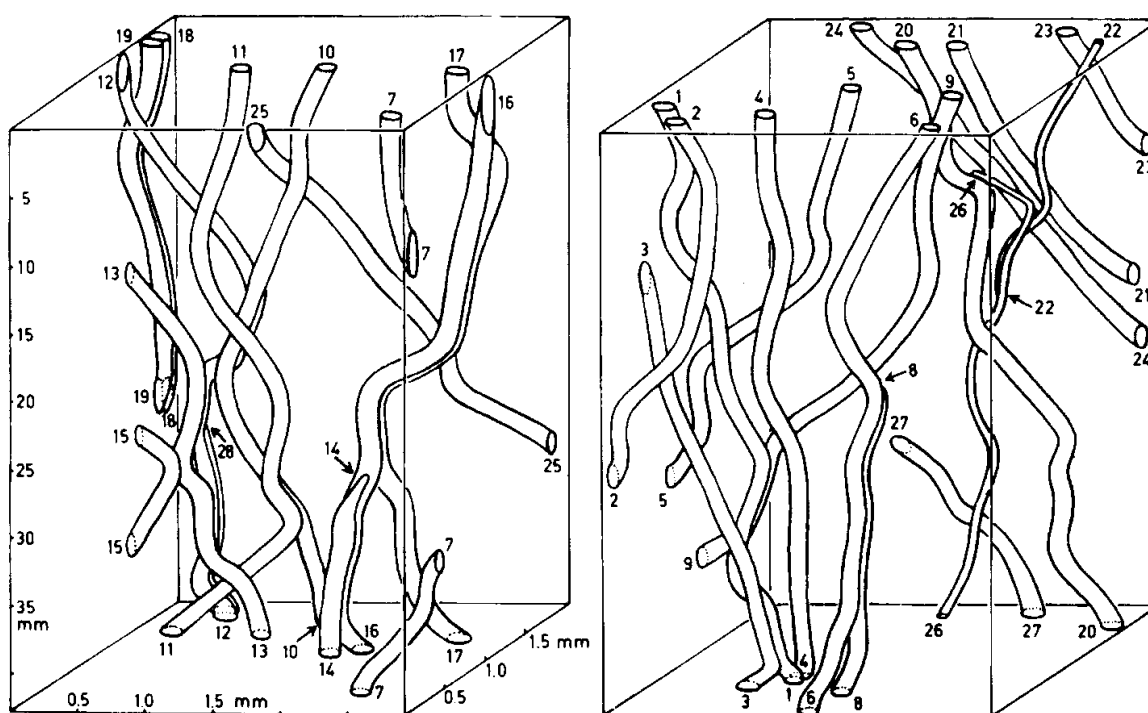
Fig. 9. Course of vessels in a piece of wood of *Cedrela fissilis*. Individual vessels have been arbitrarily separated into two blocks for clarity. Vessels are numbered where they exit from the block. Arrows show vessel ends. Note that the axial scale is foreshortened ten times (Zimmermann & Brown 1971).

Redundancy (defined as the percentage of wall surface in common) is highest in conifers (Fig. 8) but is still substantial in vessel-bearing trees. In vessel-bearing plants, vessels in any given cross section are either solitary or in vessel groups of 2 to 4 or rarely more vessels (Carlquist 1988). The grouping occurs because vessels wander around in the wood making contact with first one then another vessel along its path up a stem (Fig. 9). Redundancy defined in terms of the hydraulic efficiency afforded by the contacts may be as great in vessels as in tracheids, but no studies to assess this question have been made.

Let us now return to the predictions of safety versus conduit diameter taking into ac-count redundancy. If we allow stems A and B to have conduits of different length, but always 100 versus 1600 vessels in a given cross section, then the quantitative argument changes somewhat. But since conduit width and length generally are positively correlated (Baas 1986; Ewers & Fisher 1989; Ewers et al. 1990), stem B would have more than 1600 conduits for the same volume or surface area and would thus be even safer than the previous prediction based on conduit diameter alone.

The question of safety versus conduit size could be answered if there were some way to actually count the number of cavitated conduits in stems after applying a controlled ten-

←

Fig. 8. Left: Diagrammatic view of a tangential section of coniferous wood. The tracheid in the centre (marked X) is embolised and does not conduct water. Water flows around it. The negative pressure in the xylem has pulled the pit membranes away from the embolised cell to seal it off. This diagram is not to scale; in reality tracheids are much longer in relation to diameter. – Right: The same situation is shown in a more diagrammatic manner (Zimmermann 1983). – R = ray.

sion (= $-\Psi_{xp}$). Unfortunately this cannot be done in three dimensional wood although it has been attempted in stem cross sections (Hargrave et al. 1994). Our only probe of the question is to examine how loss of hydraulic conductivity versus tension correlates with conduit size. The relationship between percent loss of conductivity due to air embolism (PLC) and percent cavitated conduits awaits a more thorough analysis of redundancy.

Recently, Cochard has addressed the issue through a theoretical model of what would happen in imaginary stems having conduits with predefined resistance to water flow in lumina and common walls. His model approximated the structure of a conifer, i.e., stems made out of identical conduits with different placement of conduits relative to each other. Four structural models were used. The first had files of conduits with end walls all aligned in a plane (as in Figure 7A), each conduit was connected to two others (one above and one below) with flow only down the axis of each file of cells. The second had each conduit connected to four others as in Figure 8; this permitted water to flow around embolised conduits. The third had 6 walls in common per conduit as in Figure 7B. The fourth had 8 contacts per cell (not shown).

In theory, we could describe flow in such structural models with three-dimensional resistance networks with thousands of resistors in series and parallel. We could then model for the impact of embolisms on conductance by setting lumen resistance values to infinity to represent an embolism. In practice, it is impractical to solve such complex resistor networks using analytical solutions. A more practical approach is to use computer iteration of a resistance and capacitance network similar to that used to model the dynamics of water flow through whole trees (Tyree 1988). The capacitors represent the elastic modulus of lumina and describe how much they change in volume as pressure changes. The value used for the capacitors is arbitrary if the aim is to model only for steady-state flow characteristics. Cochard modeled for three-dimensional arrays 12 conduits long, 12 conduits wide, and 12 conduits deep. Even this small model stem segment had up to 8,640 resistors and 1,728 capacitors.

The approach was to start with no flow, i.e., no pressure gradient along the axis of the stem and then impose a pressure gradient and compute the transient of flow until capacitors had completely charged to their steady-state pressure values. The steady-state value of flow was used to compute maximum conductance. At this point some of the conduits were randomly 'cavitated'; lumina were selected at random using a random number generator. No flow was allowed in and out of these conduits. The program was iterated more until a new steady state was established with a lower conductance. The process was repeated until 99 PLC was achieved. The objective was to relate the percent cavitated lumina to the PLC and to see how the four structural models, differing in redundancy, behaved.

The model predicted that the stem conductance of the model stems with different numbers of walls in common did not differ in hydraulic conductance when there was no embolism. The reason for this was that all conduits had the same pressure potential in any given plane perpendicular to the axis of flow so there was no pressure drop to cause flow in the lateral direction. Once some lumina were embolised, the models diverged in behaviour (Fig. 10A). In all cases the PLC exceed the percent cavitated conduits, but models with more walls in common had more redundancy, i.e., the least PLC for any given percent cavitated conduits.

Recently, Tyree and Alexander have addressed redundancy experimentally in *Thuja canadenis* stems. This species has a distinct transition in tracheid diameter between wood formed in spring versus summer. The smaller summer-wood tracheids contribute little to hydraulic conductance and lumen volume. Since spring-wood tracheid volume was fairly uniform in our samples, the number of tracheids embolised herein should be approximately proportional to the change in wood density as lumina go from being water-filled to air-filled. A plot of PLC versus change in density reveals a nearly linear relationship (Fig. 10B); note that decline in density is plotted as positive numbers on the x-axis. For what follows, we will presume a similar linear relationship between PLC and percent embolised conduits applies to vessel-bearing stems.
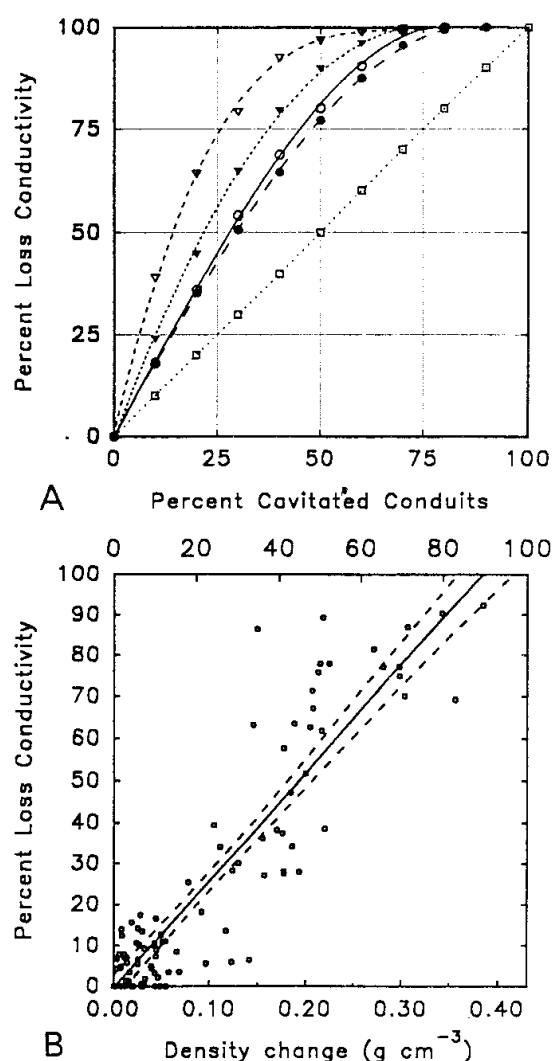
Fig. 10. A: Results of Cochard's redundancy model. Plotted are predicted percent loss hydraulic conductivity versus percent embolised conduits for different levels of redundancy measured by the number of walls in common through which water can flow in adjacent conduits. Open triangles = 2 walls in common per conduit, closed triangles = 4 walls in common per conduit, open circles = 6 walls in common, closed circles = 8 walls in common. Open squares indicate 1:1 relationship. – B: Experimental relationship between percent loss hydraulic conductivity and change in stem density (= lower x-axis) and percent of lumen volume embolised (= upper x-axis). Solid line is a linear regression of the data, dashed line is 95% confidence interval. Data are consistent with ≥ 10 walls in common per conduit. Unpublished data collected for *Thuja occidentalis* stem segments.

Experimental evidence for tradeoffs of size versus safety

Vulnerability curves

A vulnerability curve (VC) is a plot of PLC versus Ψ_{xp} that induced the PLC. PLC is measured by collecting a shoot and excising a segment from it under water. The initial hydraulic conductivity K_i is measured in a conductivity apparatus. The initial conductivity is usually less than the maximum possible because of some air embolism. The air emboli are dissolved or displaced by flushing the stem segment with degassed water at a pressure of 0.1 to 0.2 MPa. After each flush the conductance is measured until a maximum value = K_m is reached. PLC is calculated from $100(1-K_i/K_m)$. A VC is constructed by dehydrating replicate plants to different known Ψ_{xp} values and then measuring PLC (Sperry, Donnelly & Tyree 1988). Dehydration is usually done on excised branches. Consequently air emboli are prone to be sucked into conduits from the cut base via pit membranes. It is generally presumed that this procedure does not bias the VC and this has been confirmed in some instances by comparing the VC from dehydrating whole rooted plants versus excised branches of the same species (Tyree et al. 1992). Some variations on the method are needed for conifers because tori often remain aspirated after dehydration (Sperry & Tyree 1990).

The VC for a number of species is reproduced in Figure 11. These species represent the range of vulnerabilities observed so far, i.e., 50% loss conductivity occurring at Ψ_{xp} values ranging from –0.7 to –11 MPa. We have access to VC data for about 60 species representing plants from many different climates (temperate, Mediterranean, moist tropical, desert) and several growth forms (grasses, vines, trees, and shrubs). In order to access the relationship between vulnerability to cavitation and conduit size we tabulated the Ψ_{50} = the Ψ_{xp} at the 50 PLC point shown in Figure 11.

Hydraulic diameters

Ideally Ψ_{50} data should be compared to measures of conduit volume or surface area, unfortunately such data are rarely available.
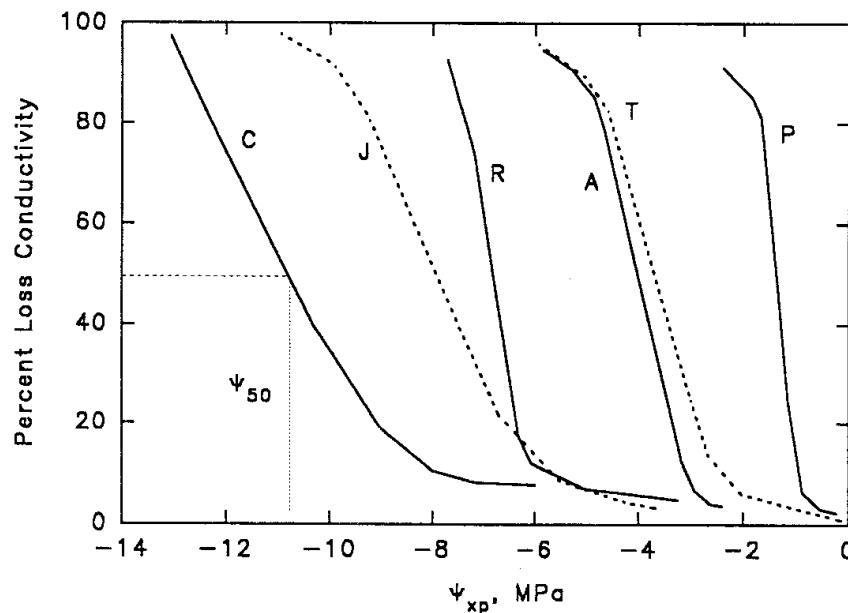
Fig. 11. Vulnerability curves for various species. Y-axis is percent loss of hydraulic conductivity induced by the xylem pressure potential, Ψ_{xp}, shown on the x-axis. C = *Ceanothus megacarpus*, J = *Juniperus virginiana*, R = *Rhizophora mangle*, A = *Acer saccharum*, T = *Thuja occidentalis*, P = *Populus deltoides*.

Later some examples of this kind of data will be given. In order to include all available taxa we took conduit diameter as a measure of conduit size. In many cases people equate wide conduits with highly vulnerable conduits, so this restricted analysis will at least address one common hypothesis.

The problem is to find an appropriate measure of diameter for all taxa. A few large conduits contribute much more to conductivity values than do many small ones as discussed above. One way around the problem is to measure 200 to 400 conduit diameters (D) at random and tabulate a column of D^4 ranked from largest to smallest value. The D^4 powers are summed for all conduits and then summed again until the sum equals 95% of the total stem conductance. This approximates the number of conduits likely to be responsible for about 95% of the total stem conductance. The mean diameter, D_{95}, is computed for these conduits. When D_{95} values were compared to means of all, D_{100}, the D_{95} values were generally 5 or 10% more than D_{100} for diffuse-porous trees. This is because D values varied over a fairly narrow range. In ring-porous trees, where there are

many small vessels and only a few large vessels D_{95} was 50% to 100% more than D_{100}.

Another possible estimator is to take the mean hydraulic diameter D_H which is defined $((\Sigma D^4)/N)^{1/4}$ where N is the number of conduits. D_H is the mean diameter that vessels in a stem would have to be if it is to have the same number of conduits as the sampled stem all of diameter D_H and have the same total conductivity. This estimator was discarded because it produced a D_H between D_{100} and D_{95}. Another diameter that could be used was recently defined by Sperry et al. (1994) as $D_S = \Sigma D^5 / \Sigma D^4$. D_S values were close to D_{95} values for ring-porous species, but were deemed no less arbitrary than D_{95} values.

Vulnerability versus hydraulic diameter

A plot of Ψ_{50} versus D_{95} is shown in Figure 12. Different species are represented by different letters (upper and lower case). A key to the species and literature sources is given in Table 1. A log-log transform was used to look for both linear and non-linear correlations, e.g., if Ψ_{50} should increase with D^x then the log-log transform would lin-

$LOG(-\Psi_{50}) = 1.154 - 0.452 LOG(D_{95})$ $R^2 = 0.22$ $P = 0.007$
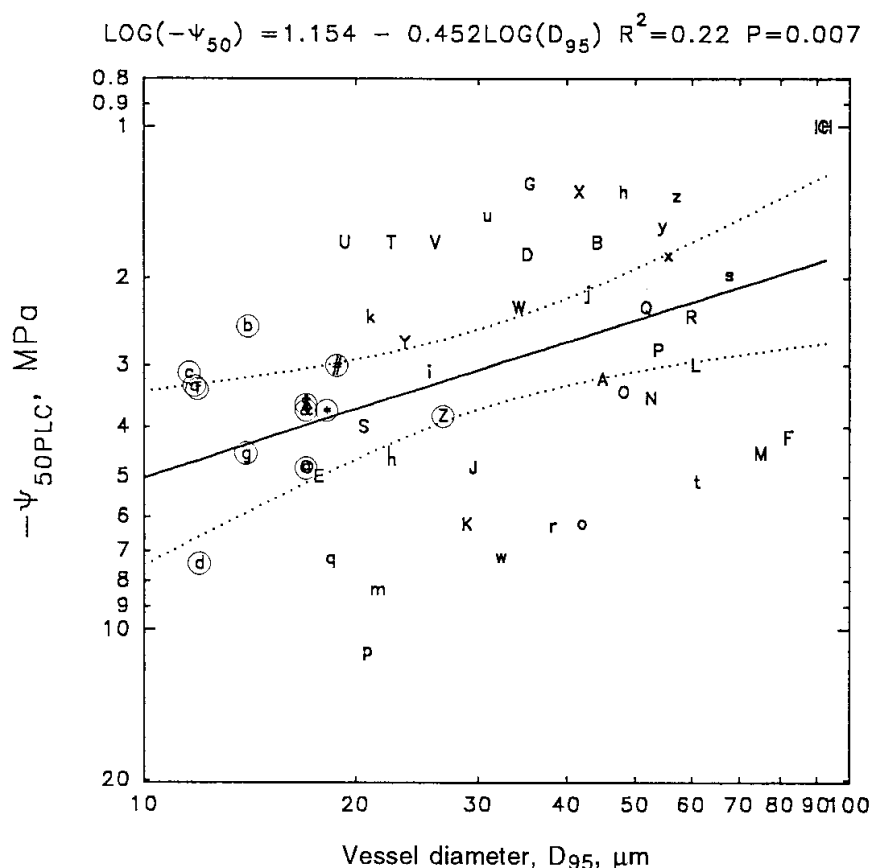

Fig. 12. Log-log plot of xylem tension causing 50 percent loss hydraulic conductivity ($-\Psi_{50PLC}$) and mean diameter of the vessels that account for 95% of the hydraulic conductance (D_{95}). Each symbol is a different species (conifers are circled); see Table 1 for species list. The solid line is the linear regression of the log-transformed data. The dotted line is the 99% confidence interval for the regression. See text for more details.

earise it. The regression line is shown in bold in Figure 12. The dotted lines are 95% confidence intervals on the regression. A linear regression (not shown) had a slightly smaller $R^2 = 0.18$. The weak, but statistically significant, regression accounts for only 21% of the variation. The weak correlation may be of use to evolutionary biologists but is not of sufficient accuracy to be of predictive value to a comparative physiologist, i.e., a physiologist cannot predict the vulnerability of a species by measuring the mean conduit diameter. It is still true that large conduits cavitate at a lower water stress than small conduits within a given stem (Tyree & Sperry 1989; Hargrave et al. 1994).

Figure 12 does not clearly answer the question of whether Ψ_{50} is proportional to

volume ($= \pi D^2 L / 4$) or surface area ($= \pi D L$), since the conduit lengths, L, were not generally reported. Conduit length varied from 1 mm for tracheids to 1.4 m for *Quercus agrifolia*. (Large conduit lengths were not represented since vulnerability curves were generally measured on young stems where conduit lengths are less than in tree trunks.)

We must rely on smaller subsets of data to examine dependency of Ψ_{50} on volume or surface area. All the conifers in this study had tracheid lengths in the range of 1.1 to 1.3 mm. The conifer species are circled in Figure 12. No significant correlation was found between Ψ_{50} and volume or surface area for the conifer subset. Sperry and Sullivan (1992) recently sought a correlation between Ψ_{50} and conduit volume in 5 diverse species (2 conifers,

Table 1. List of species and habitat of species in Figure 12.

The species are preceded by a letter and number. The letter is the symbol used in Figure 12 an the number refers to the following references:

1	Cochard & Tyree (unpublished)	11	Sperry & Sullivan 1992
2	Machado & Tyree 1994	12	Kolb & Davis 1994
3	Cochard, Ewers & Tyree 1994	13	Sperry & Tyree 1990
4	Zotz, Tyree & Cochard 1994	14	Sperry, Donnelly & Tyree 1988
5	Tyree et al. 1991	15	Tyree, Alexander & Machado 1992
6	Sperry, Tyree & Donnelly 1988	16	Cochard & Tyree 1990
7	Cochard et al. 1992	17	Tyree & Dixon 1986
8	Tognetti & Borghetti 1994	18	Jarbeau, Ewers & Davis 1994
9	Salleo & LoGullo 1993	19	S.D. Davis (unpublished)
10	Sperry et al. 1994	19	Cochard 1992

Species (Family)			Comments
TROPICAL			
A	1	*Cordia alliadora* (R.& P.) Cham. (Boraginaceae)	Tree
B	1	*Ficus citrifolia* P.Mill. (Moraceae)	Tree
C	2	*Ochroma pyramidale* (Cav. ex Lam.) Urban (Bombacaceae)	Tree
D	1	*Ouratea lutcens* (H.B.K.) Engler (Ochnaceae)	Shrub, understory
E	1	*Psychotria horizontalis* Sw. (Rubiaceae)	Shrub, understory
F	3	*Rhipidocladum racemiflorum* (Steud.) McClure (Gramineae)	Bamboo, vinelike
G	4	*Clusia uvitana* Pittier (Clusiaceae)	Hemiepiphyte, CAM/C
H	5	*Schefflera morototoni* (Aubl.) Maquire (Araliaceae)	Tree
I	2	*Pseudobombax septenatum* (Jacq.) Dug. (Bombacaceae)	Tree
J	6	*Cassipourea elliptica* (Sw.) Poir. (Rhizophoraceae)	Tree
K	6	*Rhizophora mangle* L. (Rhizophoraceae)	Tree, mangrove
TEMPERATE			
L	16	*Quercus alba* L.	Tree, N. America
M	11	*Quercus gambellii* Nutt.	Tree, N. America
N	7	*Quercus petraea* (Matt.) Liebl.	Tree, Europe
O	7	*Quercus pubescens* Willd.	Tree, Europe
P	7	*Quercus robur* L.	Tree, Europe
Q	7	*Quercus rubra* L.	Tree, N. America
R	16	*Quercus rubra* L.	Tree, N. America
S	14	*Acer saccharum* Marsh.	Tree, N. America
T	10	*Alnus incana* (L.) Moench	Tree, N. America
U	10	*Alnus crispa* (Ait.) Pursh	Tree, N. America
V	10	*Betula occidentalis* Hook.	Tree, N. America
W	10	*Betula papyrifera* var *humilis* (Reg.) Fern & Raup	Tree, N. America
X	15	*Populus deltoides* Bartr.	Tree, N. America

(Table 1 continued)

Species (Family)	Comments
TEMPERATE (continued)	
Y 10 *Populus tremuloides* Michx.	Tree, N. America
Z 10 *Picea glauca* (Moench) Voss	Tree, N. America
a xx *Picea rubrens* Sarg.	Tree, N. America
b 13 *Abies balsamea* (L.) Mill.	Tree, N. America
c 11 *Abies lasiocarpa* (Hook.) Nutt.	Tree, N. America
d 11 *Juniperus scopulorum* Sarg.	Tree, N. America
e 13 *Juniperus virginiana* L.	Tree, N. America
f 17 *Thuja occidentalis* L.	Tree, N. America
g 17 *Tsuga canadensis* (L.) Carr.	Tree, N. America
@ 19 *Cedrus atlantica* (Endl.) Carr.	Tree, Europe
# 19 *Pinus sylvestris* L.	Tree, Europe
$ 19 *Picea abies* (L.) Karst.	Tree, Europe
& 19 *Pseudotsuga menziesii* (Mirb.) Franco	Tree, N. America
* 19 *Abies alba* Mill.	Tree, Europe
MEDITERRANEAN (European)	
h 8 *Alnus cordata* Loisel.	Tree, Europe
i 9 *Quercus ilex* L.	Tree, Europe
j 9 *Quercus cerris* L.	Tree, Europe
k 9 *Laurus nobilis* L.	Tree, Europe
MEDITERRANEAN (California chaparral)	
l 12 *Salvia mellifera* Greene	Shrub
m 19 *Adenostoma fasiculatum* Hook. & Arn.	Shrub
n 19 *Adenostoma sparsifolium* Torrey	Shrub
o 19 *Ceanothus oliganthus* Nutt.	Shrub
p 12 *Ceanothus megacarpus* Nutt.	Shrub
q 19 *Ceanothus spinosus* Torrey & A.Gray	Shrub
r 18 *Heteromeles arbutifolia* (Lindley) Roem.	Shrub
s 19 *Quercus agrifolia* Nee.	Small tree
t 19 *Quercus dumosa* Nutt.	Small tree
u 19 *Rhamnus californica* Eschsch.	Shrub
v 19 *Rhamnus crocea* Nutt.	Shrub
w 19 *Rhamnus ilicifolia* Kellogg	Shrub
x 19 *Rhus integrifolia* (Nutt.) Brewer & S.Watson	Shrub
y 18 *Malosma laurina* (= *Rhus laurina*) (Nutt.) Abrams	Shrub
z 19 *Rhus ovata* S.Watson	Shrub

2 diffuse-porous and 1 ring-porous species). Among the species, diameters ranged from 10 to 100 μm and L from 1 to 400 mm. No correlation was found between Ψ_{50} and volume.

Mechanism of cavitations (why no tradeoff?)

There may be one or more reasons for a weak correlation between Ψ_{50} and measures of conduit size among taxa. One possibility is that the mechanism for cavitation is unrelated to conduit size. Alternatively, the 'cost' to make 'safe' conduits may be so slight that there is little selective tradeoff between size and safety. Reduced vulnerability to cavitation will still be strongly selected against in xeric or cold environments, but not in warm, mesic environments. In mesic environments we would expect genetic mistakes for high vulnerability to be tolerated and would thus eventually come to represent a large fraction of the gene pool.

It is unlikely that Ψ_{50} would ever be found to correlate with volume since this depends on a homogeneous nucleation mechanism that can be discounted on purely physical grounds. Pickard (1981) has shown that the probability of homogeneous nucleation is vanishingly small at biological pressure potentials.

We are left with only heterogeneous mechanisms, and the question comes down to whether the wall defects causing nucleation are random defects or under genetic control. Pit membrane porosity might be under genetic control because this involves the orderly synthesis of cellulose microfibrils and the regulation of secondary wall growth and lignification around the pit followed by partial enzymatic hydrolysis of the pit membrane (Butterfield & Meylan 1982). It is harder to imagine how the creation of hydrophobic cracks or patches might be genetically controlled, it is easier to imagine that these are random mistakes.

Fortunately, we can resolve the issue, because experiments can discriminate between the air-seeding mechanism and the other three. All mechanisms predict cavitation when xylem fluid is under tension, but the air-seeding mechanism predicts that air can be blown into vessels while the fluid is under positive pressure. The air-seeding mechanism requires only a pressure differential (P_a-P_w) where

P_a is the air pressure outside and P_w is the fluid pressure inside (Fig. 6). It makes no difference if P_a is 0.1 and P_w is –3.0 or if P_a is 3.1 and P_w is 0.1. Experiments have been performed to prove that the same vulnerability curve results whether P_w is reduced by air dehydration or P_a is increased in a pressure bomb (Cochard, Cruiziat & Tyree 1992). The results of this experiment are illustrated in Figure 13 (see also Jarbeau et al. 1994).

Willow stem segments with leaves were enclosed in a pressure chamber with cut ends protruding into the open air. Water was passed continually through the xylem under positive pressure. While stem conductance was being monitored, the gas pressure, P_a, in the pressure chamber was gradually increased. Initially the hydraulic conductance of the stem segment did not decrease until a critical pressure of 1 MPa was applied. (Each solid circle in Figure 13 represents the application of pressure of 30 to 40 minutes.) When P_a was gradually increased beyond the critical value the stem conductance began to fall (increased PLC). When P_a was gradually decreased, the PLC stopped decreasing. The VC from this experiment was identical to that found for similar branches dehydrated in the air.

This is the strongest evidence presented so far that the air-seeding mechanism explains how cavitations occur, although there is other circumstantial evidence (Crombie et al. 1985; Sperry & Tyree 1988). If the air seeding hypothesis is correct, there is no reason to believe there ought to be a strong correlation between pit membrane porosity and conduit size.

Freezing-induced dysfunction and conduit diameter

The above discussion of drought-induced xylem dysfunction still does not answer the question of why conifers with small conduits tend to dominate in boreal and alpine habitats. Do small conduits confer some advantage in cold environments? There is a growing body of evidence that small conduits are less vulnerable than large conduits to freezing-induced embolism.

Hammel (1967) and Sucoff (1969) have shown that conifer stem segments can be frozen and thawed while the rest of the shoot is warm and transpiring without permanent
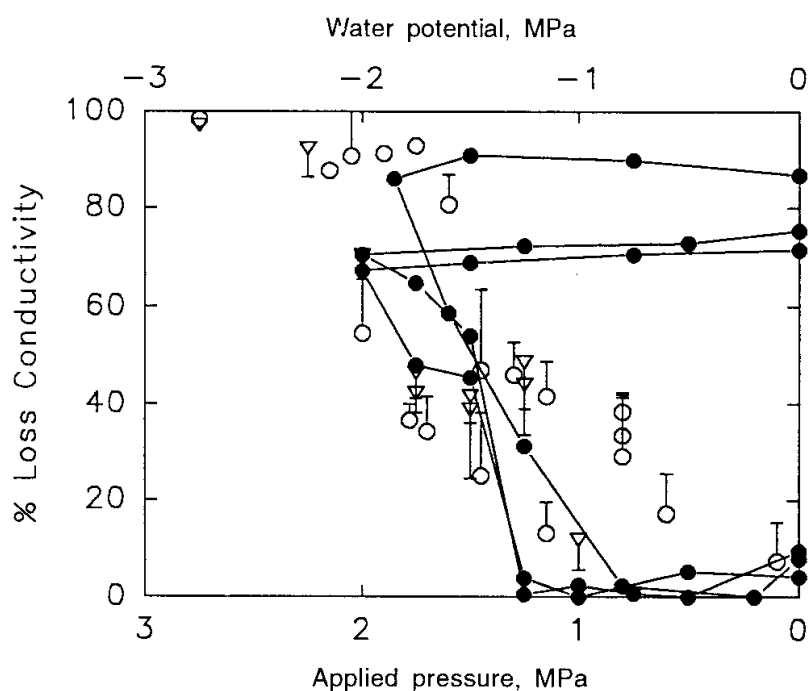
Water potential, MPa

Fig. 13. Vulnerability curves of willow obtained by three different methods. The open circles are vulnerability data obtained by air dehydration of excised branches causing xylem tension (negative Ψ_{xp}). The open triangles were obtained from shoots dehydrated in a pressure chamber with xylem tension following pressure release. The solid circles connected by straight lines were obtained by dehydration in a pressure chamber without tension in the xylem, i.e., conductance was continually measured by perfusing stems with water above atmospheric pressure while simultaneously applying air pressure to the leaves and stem surfaces. The sequence of pressure changes was from 0 to 2 MPa in steps of 0.3 to 0.6 MPa held for 1 h at each pressure. The applied pressure caused a loss of conductivity without xylem tension and the loss of conductivity was the same as when there was xylem tension of equal magnitude. These results are consistent with the air-seeding hypothesis (replotted from Cochard, Cruiziat & Tyree 1991).

loss of hydraulic conductivity. In contrast, the first frost event is known to induce > 90 PLC in oaks (Cochard & Tyree 1990; Sperry & Sullivan 1992) and some wide vessel chaparral shrubs (Langan & Davis 1994).

Freezing should induce embolisms, because air is not soluble in ice. So when water freezes, air comes out of solution. If water is saturated with air at 0 °C when it freezes then approximately 2.8 ml of air will come out of solution for every 100 mls of water frozen. What happens to this air when the ice melts? If the ice melts slowly and no tension develops in the tissue, then the air will dissolve. But if tensions develop beyond the critical values in Equation (4), then the bubbles will expand to make the conduit fully embolised and dysfunctional. Apparently this happens

in oak with vessels 100 μm in diameter, it happens less in maple with vessels 30 μm in diameter and least in conifers with tracheids 10 μm in diameter. Sperry and Sullivan have demonstrated a strong correlation with vulnerability to freezing-induced embolism and conduit volume. Figure 14 compares the Ψ_{50} values (open circles) of 5 species versus conduit volume or specific conductivity; it can be seen that there is no tradeoff between conduit size and drought-induced dysfunction (as stated previously); nor is there a tradeoff between increased stem hydraulic efficiency and drought-induced dysfunction.

The solid circle (Ψ_{50F}) values were obtained in the following way: shoots were dehydrated to various initial water potentials, Ψ_i, and then wrapped in a bag, frozen to -20 °C
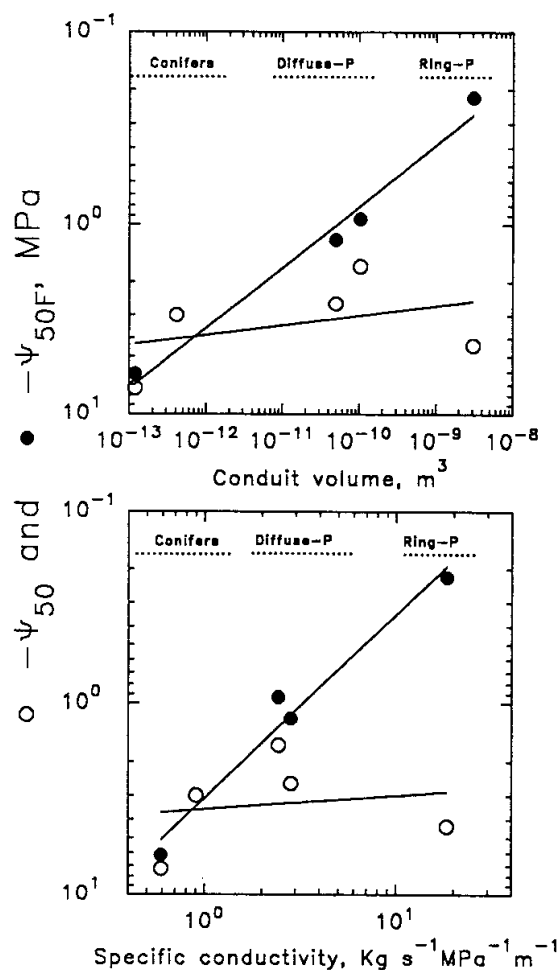
Fig. 14. Relationship between xylem tension and loss of conductivity. Open circles are tension (negative Ψ_{xp}) needed to induce 50 PLC = Ψ_{50}, closed circles are tension before a freeze, required to induce 50 PLC = Ψ_{50F}. The upper plot shows the relationship with conduit volume and the lower plot shows the relationship with specific conductivity. The lower curve suggests a tradeoff between vulnerability to freezing-induced loss of conductance and specific conductivity of wood. (Replotted from Sperry & Sullivan 1992.)

overnight, and then thawed to > 20 °C. This procedure induced a certain PLC. What is plotted is the Ψ_i that induced 50 PLC after the freeze-thaw cycle. While there is a strong correlation, it is not clear why Ψ_i measured before a freeze should influence PLC after a freeze-thaw cycle.

The Ψ_i value could not influence the amount of air coming out of solution upon freezing,

the same amount of air will come out regardless of the initial conditions because the solubility of air in ice is very low compared to water at 0 °C. Since the samples frozen were not frost tolerant, many living cells must have died and this would alter Ψ_i an unknown amount, but certainly the value of Ψ_{xp} will have risen from a value initially close to Ψ_i to something much less negative. It is the magnitude of Ψ_{xp} after the freeze and the capacity of the previously frozen tissue to take up water from conduits after the freeze that will determine how much the embolisms expand and how much PLC results. If no air dissolved immediately after the thaw, only 2.8% of the tissue volume would be occupied by air (= the volume fraction of air in solution at the time of the freeze). Only if these bubbles are expanded to fill the entire conduits would we expect values of 50 PLC or more.

It is difficult to know if PLC measured by tension-freeze experiments will induce the same level of PLC as in the field because the additional tendency of transpiration to cause bubble expansion is not duplicated. But trees certainly freeze under tension and some tension remains after the thaw. These pioneering experiments should stimulate other clever experiments that will mimic what happens in nature. Some work of this kind is underway (Langan & Davis 1994).

Why should large conduits be more prone to freeze-induced dysfunction? It probably has something to do with how long it takes air bubbles to dissolve rather than the tension when the ice first forms. This is because bubbles have to dissolve before the onset of a critical tension causing them to expand. The physics of air bubble dissolution is now well understood (Pickard 1989; Yang & Tyree 1992; Tyree & Yang 1992). An analysis of the kinetics of bubble dissolution reveals that the time it would take for a bubble to dissolve increases approximately with the square of its initial diameter. If many small bubbles were formed when ice melted and if the bubbles were the same size regardless of size of the conduit, then conduit size may not influence freezing-induced dysfunction. But Ewers (1985) studied bubble formation while freezing and thawing water in small glass capillary tubes and observed that bigger bubbles formed

in large diameter tubes than in small tubes and that they took longer to redissolve in big versus small tubes. It seems likely that the same will happen in xylem conduits.

Conclusions

This review discusses some of the selective constraints of xylem evolution. There are tradeoffs between the features that make xylem an efficient conductor of water and that make it vulnerable to dysfunction. In terms of the Hagen-Poiseuille law, large xylem conduits are more efficient for water conduction than small. The Hagen-Poiseuille law seems to put a constraint on the lower limit of conduit diameter of about 10 μm because if conduits were smaller, much more wood would have to be constructed and maintained for adequate hydraulic conductance than needed for mechanical support. There seems to be an upper limit of conduit diameter reached in several families of woody plants (D = 300 to 500 μm). The efficiency of water transport increases with the D^4. The smallest mean D in conifers is about 10 μm and the largest mean in vessels is about 100 μm in the small branches of trees. This means that some angiosperms have vessel lumina that are 10^4 times more conductive than tracheid lumina.

Some selective advantage can be gained for large conduits in that less carbon needs to be invested in wood to provide an equal amount of water. But wood also provides mechanical support so we presume that for trees not much can be gained in having vessel diameters > 100 μm. Given that a minimal amount of wood might be needed for support, then the tradeoff for larger more efficient vessels might be loss of pathway redundancy. With less pathway redundancy cavitation events have a larger negative impact on hydraulic conductance. Only when the constraint for mechanical support is reduced as for lianas, are mean vessel diameters > 100 μm common.

In the above discussion we have ignored the fact that vessels in tree trunks tend to be wider than in branches because most of the pressure drop in shoots of trees is confined to the smaller branches. Thus small branches have the most negative Ψ_{xp} values and are more likely to cavitate than vessels in the trunk. At this point we assume that evolu-

tionary selection is most likely to be strongest in small branches, but future studies of xylem dysfunction in root and trunk xylem are needed to test the assumption.

Another possible reason for an effective upper limit of 100 μm diameter might be explained in terms of vascular versus non-vascular resistances in large plants. There is a growing body of evidence that non-vascular resistances in leaves and in the absorbing zone of roots equals the vascular resistance in trees (Tyree & Yang 1994; Tyree et al. 1994). So a doubling of mean size of vessels from 100 to 200 μm in trees would decrease vascular resistance by 16-fold but would decrease whole-plant resistance by less than half because of the substantial non-vascular resistance. So loss of redundancy might be the main selective force against large conduits given that whole-plant resistances are not decreased much for vessels > 100 μm.

There is little evidence that the negative impact of large conduits is increased vulnerability to drought-induced cavitation. In a comparison of 60 taxa there is only a weak (though statistically significant) relationship between vulnerability to cavitation and conduit diameter.

In colder boreal and alpine environments there might be selection for small conduits over large conduits because small conduits are less likely to remain embolised after a freezing episode than large conduits. Furthermore, in cold environments, evaporative demand is likely to be less than in warm environments so selection for efficient rather than safe conduits is less likely to occur.

The time is ripe for a very productive reevaluation of the major Baileyan trends in the fossil records. This review provides some of the biophysical background needed for the reevaluation, but leaves many of the important questions unanswered. For example, the fossil record and extent of ecological patterns both show major trends in scalariform perforations between vessel elements (Wheeler & Baas 1992). Baileyan trends show an evolution from long vessel elements with scalariform perforations to shorter elements with simple perforations. Perforation plates will increase the resistance to water flow in vessels, so the trend towards simple plates can

be understood in terms of increased hydraulic efficiency. But why do perforation plates persist? Some fluid mechanical models of perforation plates with widely spaced bars suggest that they contribute a negligible amount to increased resistance. But really primitive perforation plates still exist with closely spaced bars, leaving hardly any space for water to pass. Future models of fluid mechanics may reveal the cost of such bars, but will not address the issue of their advantage(s). One possible advantage may be the role of perforation plates in limiting freezing-induced dysfunction. Narrow perforation plates may prevent the coalescence of embolisms when vessels thaw out, thus increasing the likelihood of the embolisms dissolving before tension resumes.

The fossil record also shows some interesting evolutionary experiments in primitive xylem structures in now largely extinct gymnospermous groups which had quite different vesselless wood with different types of pits than found in modern gymnosperms and, perhaps, with reduced redundancy. Experiments should be undertaken on existing taxa that retain primitive structures. Such experiments will increase our biophysical understanding of the constraints on evolution and may help us to interpret the fossil record better.

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