

Pit Membrane Degradation and Air-Embolism Formation in Ageing Xylem Vessels of *Populus tremuloides* Michx.

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ABSTRACT

Air-embolism formation in xylem vessels of *Populus tremuloides* Michx. was quantified by its reduction of hydraulic conductivity in branch segments. Embolism was induced by increasing xylem tension in drying stems, or by inserting one end of a hydrated stem in a pressure bomb and increasing air pressure in the bomb. Both treatments produced the same response suggesting that embolism by water stress was caused by air entering water-filled vessels, presumably through inter-vessel pits. In rapidly-growing *P. tremuloides* branches, the vessels of the outer growth ring were functional whereas vessels in older xylem were mostly embolized. This selective embolizing of older vessels was associated with a marked increase in permeability of their inter-vessel pits to air, relative to pits of younger vessels. Air-injection pressures less than 1.0 MPa completely embolized older vessels that had been re-filled in the laboratory, whereas pressures over 4.0 MPa were required to embolize young vessels. Greater permeability of old vessels was due to degradation of their pit membranes as seen in the scanning electron microscope; large openings were present that were not seen in pit membranes of young vessels. These holes would allow air to penetrate vessel ends at low pressure differences causing embolism. Degradation of pit membranes causing the selective dysfunction of older sapwood may be a general phenomenon initiating heartwood formation in many species.

Key words: Xylem embolism, hydraulic conductivity, heartwood formation, cavitation, *Populus tremuloides* Michx.

INTRODUCTION

Xylem vessels and tracheids cease to function in water transport when they become air-filled, or embolized (cavitated). Water stress has been found to cause embolism when xylem pressure drops low enough to pull air into water-filled xylem conduits from adjacent air-filled ones through inter-conduit pit membranes (Crombie, Hipkins, and Milburn, 1985; Sperry and Tyree, 1988, 1990). This mechanism becomes possible once even a few conduits become air-filled; for example, as a result of leaf abscission.

Embolism is thought of as a stress response, but in many species it must also occur in the course of normal xylem senescence as one of the first steps in heartwood formation. At the sapwood–heartwood transition in conifers, the number of aspirated inter-tracheid pits increases dramatically from sapwood to heartwood and there is a corresponding decrease in moisture content (Hillis, 1987; Coutts, 1977). This indicates massive embolism formation

over a narrow increment of xylem. In many angiosperm trees there is also a decrease in moisture content from sapwood to heartwood that may be accompanied by tylose formation in heartwood vessels (Hillis, 1987). Again, this suggests a developmentally-prescribed formation of embolism that de-activates the sapwood and initiates heartwood formation. This embolism necessarily occurs under conditions that do not embolize younger conduits.

Understanding how this ‘endogenous’ embolism arises is necessary for understanding how a tree regulates its sapwood area. Sapwood area, in turn, sets a limit to how much foliage can be supplied and in this way influences tree vigour. This regulatory role of the vascular system is perhaps not widely appreciated; yet it must play a part in how a tree responds to its environment.

In this paper, we explore the mechanism of embolism formation in vessels of *Populus tremuloides* Michx., and

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determine the basis for its selective occurrence at the sapwood–heartwood boundary. *P. tremuloides* does not form true heartwood, distinguishable by colour and lack of living cells; rather it forms ‘ripewood’ which is similar in colour to sapwood, but may have a different moisture content and retains living cells (Hillis, 1987). For our purposes, we define sapwood as the region of xylem functioning in water transport.

MATERIALS AND METHODS

Observations and experiments were made on *P. tremuloides* branches collected in the Wasatch Mountains of Utah, USA. Most were taken near Brighton at c. 2825 m; others were collected at c. 1370 m in Red Butte Canyon Research Area near the University of Utah in Salt Lake City. Branches were transported to the laboratory in plastic bags and used within 3 d of collection.

Midday and diurnal courses of xylem tension were measured in the field using a pressure bomb in order to determine the approximate range of xylem tensions.

Vessel lengths

Vessel length distributions were measured by a modification of the paint-infiltration method of Zimmermann and Jeje (1981). A 1% (w/w) suspension of pigment particles in water was filtered to 11 μm and forced into cut branches at c. 30 kPa for three or more days. Particles did not penetrate inter-vessel pit membranes and thus occluded and marked vessels continuous from the point of application. Following perfusion, branches were cut into 5.0 cm segments and the number of pigment-filled vessels counted at distances of 0, 5, 10, and 15 cm from the point of application. Using the geometric analysis of Zimmermann and Jeje (1981) these counts yielded the percentage of vessels at the application point belonging to specified length classes.

Pooled results for six branches with outside diameters (at application) between 0.50 and 1.57 cm were as follows: 95.5% of the vessels were shorter than 5.0 cm; 4% were between 5 and 10 cm long; the remainder were 10 to 15 cm long.

Measurement of embolism in native branches: ‘flushing’ method

In order to estimate embolism in branches in their natural (‘native’) condition, hydraulic conductivities were measured on excised branch segments before and after removal of air in embolized vessels by a series of high-pressure (c. 175 kPa) flushes of solution through the segments. The per cent the initial conductivity was below the final maximum value gave the percentage loss in hydraulic conductivity due to reversible embolism (for details, see Sperry, Donnelly, and Tyree, 1987).

Segments (7–10 cm in length) were cut from branches underwater to prevent additional embolism. Hydraulic conductivity (mass flow rate/pressure gradient; Kg m/MPa s) was measured on a tubing manifold allowing 15 stems to be mounted in parallel so that flow could be directed through any or all segments by stopcocks. Solution used was 10 mM oxalic acid; its low pH (1.8) prevented microbial growth that otherwise would clog the xylem. Solution was de-gassed by drawing it into an evacuated carboy through a tapered pipette. It was filtered to 0.22 μm immediately prior to use. During a measurement, solution was routed through segments one at a time under pressures induced by gravity (3–5 kPa). For each segment mass flow rate was measured by flow of solution into a reservoir on an electronic balance, and hydraulic conductivity computed.

During a flush, solution was routed through all stems at once from a solution-filled bladder enclosed in a pressurized air tank.

Measurement of embolism in water-stressed and air-injected branches: ‘adjacent segment’ method

In order to distinguish between embolism already present in a branch at collection and additional embolism induced in the laboratory by dehydration and air-injection, a modification of the hydraulic method was used that compared hydraulic conductivity on adjacent segments of the same branch. An ‘a’ and ‘b’ segment each 7.0 cm long and separated by 5.0 cm were marked off on a length of branch lacking side-branches. First, the ‘a’ segment was cut underwater and its hydraulic conductivity measured. Then, the remainder of the branch with the ‘b’ segment located 5.0 cm from the new cut end was either dehydrated, or air-injected (see below). Following this treatment, the ‘b’ segment was cut underwater and its hydraulic conductivity measured. The per cent the conductivity of the ‘b’ segment was below that of the ‘a’ segment gave the per cent reduction of conductivity due to the treatment. The location of the ‘b’ segment 5.0 cm from the cut end of the branch during the treatment insured that very little air entered the segment through vessels continuous with the cut end because 95% of the vessels were shorter than 5.0 cm (see ‘vessel lengths’ above).

For the water stress treatment, branches with the ‘b’ segment were dried in the laboratory with the distal foliage attached. After drying, branches were bagged in plastic for 2 h to promote equilibration of xylem tensions; these were measured with a pressure bomb.

Air-injected branches were cut to c. 20 cm lengths, the ‘b’ segment and additional 5.0 cm inserted inside a pressure bomb; the other end was outside the bomb. Bomb pressures were raised to the desired value and held for 20 min. After releasing the pressure, the branch was removed, the ‘b’ segment cut underwater and prepared for the hydraulic conductivity measurement. During the entire experiment, the stem was wrapped in wet towelling and plastic wrap to prevent water loss. In addition, water was continuously supplied to the stem outside the bomb via tubing.

In a control experiment, hydrated branches were entirely enclosed in the bomb under pressure and then measured for embolism. None was found, suggesting that embolism did not occur simply by oversaturation of water under pressure.

In one set of air-injection experiments, native branches were used. These measured embolism caused by air-injection over and above what had occurred under natural conditions. In a second set, branches were flushed for 2 h in order to re-fill embolized vessels before the air-injection. These results indicated the maximum embolism caused by air injection, because embolism was eliminated before the experiment.

Dye perfusions

Branch segments were perfused with 0.1% (w/v) safranin dye filtered to 0.22 μm to localize functional xylem. After segments were measured on the conductivity apparatus, they were fitted at one end to a water-filled tubing manifold. The other ends were immersed in dye, and dye was siphoned through the segments for 30 min under a 3.0 kPa pressure gradient induced by lowering the free end of the water-filled manifold below the dye surface. Permanent thin-sections were prepared for high-magnification detail, as well as thicker discs for observation under a stereomicroscope.

Thin sections were used to count the number of stained (functional) and non-stained (embolized) vessels. These numbers were expressed as the percentage of functional vessels versus

growth ring age for branch segments. Rather than counting the total number of vessels in each ring, a single wedge of xylem defined by rays continuous from pith to cambium was selected, and counts were made within this wedge for each growth ring.

Air permeability experiments

Conductivity of air at constant applied pressure was measured in xylem of different ages to assess directly the differences in permeability of vessel ends to air. Branches 25 cm in length (longer than the longest vessel) and between 5 and 8 years of age were selected. One end was inserted in a pressure bomb; the other end was trimmed in 'steps' so that a single length of tubing could be fitted over any of four progressively smaller portions of xylem. The first step included all the xylem, the second and third steps excluded progressively more of the younger xylem, the fourth step excluded all except the oldest year or two of growth.

Air pressure in the bomb was held at 0.5 MPa, and air flowing through the stem (through vessel ends) was routed via tubing to a burette having one end immersed in water and holding a hanging column of water. The rate at which air displaced the column was measured and this was divided by the air pressure gradient across the stem (0.5 MPa per stem length) to obtain the conductivity of air. Air penetrating the pith was vented by drilling a small hole into the pith from the outside near the middle of the stem. Problems due to compressibility of air were minimized by making all measurements at the same pressure gradient.

Conductivity of air through the stem at each of the four steps was measured sequentially starting at the first step, concluding at the fourth step, and then re-measuring the first step. This cycle was continued until repeated measurements at the first step were within 3% of each other; this was achieved within the first three cycles. The values at each step were related to the per cent of total xylem area removed at each step as measured from transverse sections. In addition, the step at which the youngest two growth rings were absent was noted.

Scanning electron microscopy (SEM)

In order to observe structural differences in pit membranes, specimens were prepared for SEM. A branch was flushed to maximum conductivity and air-injected at 0.5 MPa. Safranin dye was perfused through the segment, and longitudinal slivers of dye-stained (non-embolized; Plate 1A) and non-stained (embolized) xylem were made using a sliding microtome or a razor blade. These were dehydrated in ethanol (which removed dye), and critical-point dried before being coated with gold-palladium and observed in the SEM.

RESULTS

Figure 1A summarizes experiments using the adjacent-segment method on native branches. Embolism induced by water stress in drying branches (solid symbols) was compared with embolism caused by injection of air (open symbols). The responses were identical: air was forced into vessels of hydrated stems at the same pressure difference that vessels embolized in water-stressed stems. This suggested that embolism occurring during water stress was caused by air pulled into the vessel. The likely point of air entry was through pit membranes between air- and water-filled vessels where the vessel wall is thinnest and more porous.

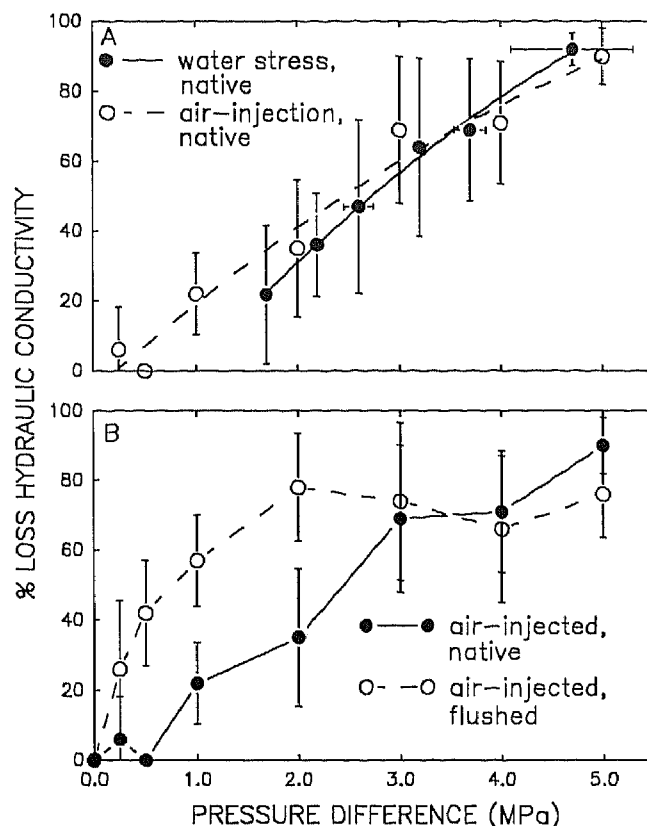


FIG. 1. Percentage loss of hydraulic conductivity versus pressure difference (MPa) in *P. tremuloides* branches. (A) Native branches that were water-stressed (solid symbols, solid line), and air-injected (open symbols, dashed line). Pressure difference refers to xylem tension for water-stressed branches, and to air-injection pressure for air-injected branches. Error bars are 95% confidence limits ($n \geq 9$). (B) Air-injected branches that were in native condition (solid symbols, solid line), and flushed to reverse previous embolism (open symbols, dashed lines). Pressure difference refers to air-injection pressure in both cases. 95% confidence limits shown ($n \geq 9$).

In Fig. 1A, there was significant embolism at pressures as low as 1.0 MPa despite previous exposure of these branches to field xylem tensions up to 1.8 MPa (data not shown). If embolism was a function of xylem tension alone, none should have been seen until field values were exceeded. The additional embolism may have been due to the use of cut branches in the experiments; this maximized exposure of vessel-ends to air and may have maximized the embolism response at a given xylem tension or air-injection pressure.

In Fig. 1B, the air-injection curve of native branches (solid symbols) shown in Fig. 1A is compared with the air-injection response of branches previously flushed to maximum conductivity (Fig. 1B, open circles). At pressures above 3.0 MPa the response was the same; at lower temperatures, however, the flushed stems showed significantly higher loss of hydraulic conductivity than the native ones. This means the flushing procedure re-filled a population of vessels that were highly permeable to air

and so re-embolized at low injection pressures. This population was not detected in native stems because they were already embolized.

Dye perfusions revealed that this population of permeable vessels was confined to older xylem. Branch segments flushed to maximum conductivity and perfused with dye were fully-stained in transverse section (Plate 1A, left). Adjacent segments that were flushed, air-injected at 0.5 MPa, and then perfused with dye were only completely stained in the youngest one or two annual rings; the older xylem was mostly non-stained, especially in the earlywood (Plate 1A, right). The same pattern was seen for native branches perfused without treatment (Fig. 4). Longitudinal sections showed an abrupt transition between water-filled vessels and fibres in the younger xylem, and air-filled vessels and fibres in the older tissue (Plate 1B).

Results of air-flow experiments, summarized in Fig. 2, provided additional evidence of greater air-permeability of vessel pits in older vessels relative to younger ones. In these experiments, air applied at low pressure (0.5 MPa)

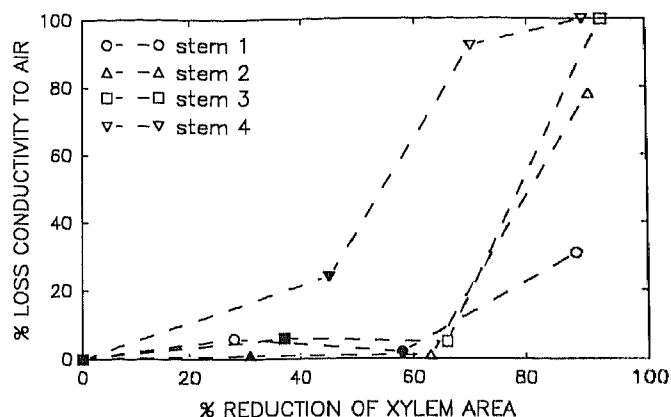


FIG. 2. Percentage loss in conductivity to air flow through xylem versus percentage reduction in transverse-sectional xylem area for *P. tremuloides* stems. Stems were 25 cm in length and longer than the longest vessel; air flowing through xylem had to pass vessel ends through inter-vessel pits. Air flow was measured from progressively smaller diameters of the xylem, in this way assessing the contributions of young versus old vessels to total air flow. Solid symbols represent the first measurement excluding the two youngest annual rings.

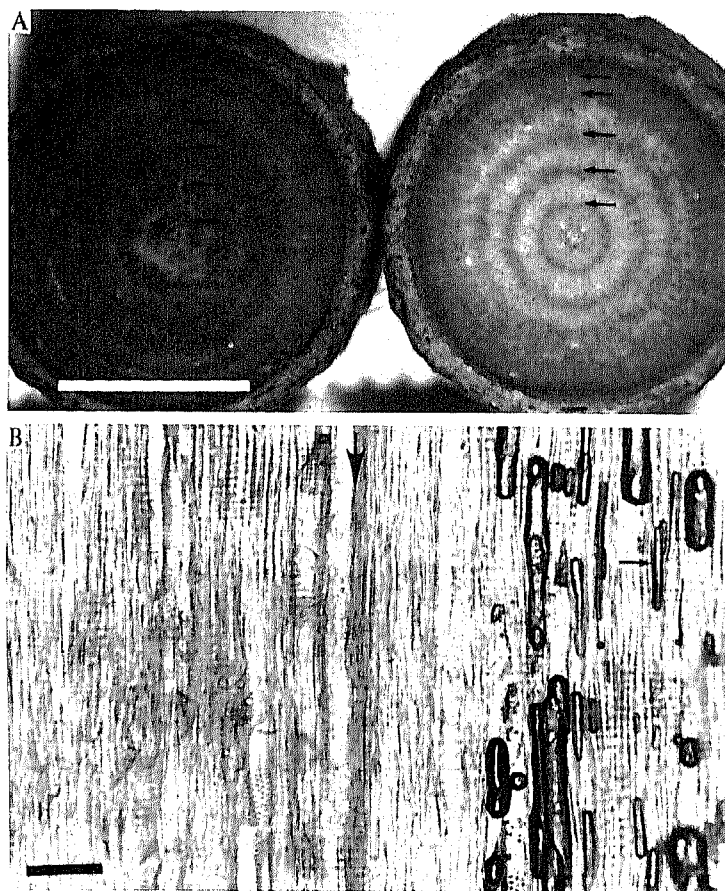


PLATE 1. (A) Transverse sections of adjacent segments from the same branch of *P. tremuloides* that had been flushed to re-fill embolized vessels. Arrows mark growth ring boundaries; left-hand segment was lower on the branch than the right-hand one and one year older. Scale bar is 0.5 cm. (Left) Segment stained with dye; all xylem is evenly stained and functional. (Right) Segment stained after injection with air at 0.5 MPa. Only the youngest (outer) two growth rings are evenly stained and functional; older rings contain non-stained and embolized regions, mostly in earlywood. (B) Fresh radial section of xylem from *P. tremuloides*. Large arrow marks growth-ring boundary between younger annual ring (left of arrow), and older ring (right of arrow). Scale bar approximately 100 μ m. No air bubbles are visible in younger ring; many are present in vessels of older ring; some fibres appear air-filled as well (arrow).

was observed to flow selectively through the vessel ends of older vessels. In stems 1, 2, and 3, less than 5% of the air flow occurred in the outer 60% (by area) of the xylem; this included the most recent two years of growth. In stem 4, the pattern was similar but not as dramatic: 25% of the conductivity was in the outer 55% of the stem; including the latest two years of growth.

The observation that older xylem is more vulnerable to embolism predicted progressively larger amounts of embolism in branch segments of increasing age. This was verified as shown in Fig. 3; in these measurements the amount of embolism in native stems was quantified using the flushing method. The current year's xylem averaged about 20% embolism compared to peak values near 60% in segments that were 3 or more years old. This pattern did not represent greater water-stress in older segments, but reflected the greater vulnerability of older xylem. The plateau seen for segments older than three years suggests there is some occlusion of embolized xylem, preventing complete re-filling despite the flushing procedure. This was also seen in some dye perfusions where the innermost xylem conducted no dye despite repeated flushing.

Scanning electron microscopy revealed degradation of inter-vessel pit membranes with age. Membranes between young vessels lacked conspicuous pores, and appeared uniform and smooth (Plate 2A, B). Plate 2C shows a membrane from a young vessel torn by sectioning; where the intact portion spans the pit aperture, pores approximately $0.08\ \mu\text{m}$ in diameter can be resolved (Plate 2C; arrow).

In older vessels the inter-vessel pit membranes were extremely fragile; it was hard to find ones not torn along their margins. It was difficult to know if this tearing was an artifact of preparation, or indicative of the native condition. However, in obviously non-torn areas of the membrane, pores up to $0.5\ \mu\text{m}$ in diameter were observed (Plate 2D, E, F; arrows). In addition, many older mem-

branes had a fibrillar texture as if matrix materials had been removed between the cellulose framework of the membrane (Plate 2F).

A seasonal survey of vessel function indicated that vessels produced in 1989 remained fully functional (i.e. conducted dye) until new xylem was produced in July 1990 (Fig. 4; 1990 xylem not shown). These branches were approximately 1.0 cm in diameter (including bark) and between 3- and 6-years-old; they were judged to be vigorously-growing branches based on their relatively wide growth rings (an example is shown in Plate 1A). Given that in November of 1989 most of the xylem from 1988 was embolized (Fig. 4A), we project that 1989 xylem would begin to embolize between July 1990 and early winter 1990.

A different pattern was seen in slow-growing branches. These were of similar diameters to the vigorous branches of Fig. 4, but between 11- and 16-years-old. These branches had extremely narrow growth rings (*c.* $150\ \mu\text{m}$) and showed more gradual development of embolism with age (Fig. 5). Xylem up to 6-years-old maintained on average 80% functional vessels. Older xylem showed a highly variable decrease in functional vessels down to a minimum of 9% after 12 years.

DISCUSSION

The results indicated for *Populus tremuloides* that xylem tensions in water-filled vessels were limited by air-entry through pits adjoining air-filled vessels (Fig. 1A). Embolism tension is a function of permeability of pit membranes to air-water interfaces. This is in agreement with previous results for both vessel-bearing angiosperms and tracheid-bearing gymnosperms (Sperry and Tyree, 1988, 1990). Vulnerability of xylem to cavitation by water stress is *not* due to conduit size, type (i.e. vessel or tracheid), or wall thickness, as has been suggested (Zimmermann and Brown, 1977).

The results also showed that embolism in *P. tremuloides* was selectively induced in ageing xylem by increases in the permeability of inter-vessel pit membranes to air-water interfaces (Figs 1–3). Progressive degradation of the membrane created pores at least $0.5\ \mu\text{m}$ in diameter (Plate 2) in older xylem. This means that embolism occurs not only in response to water stress, but also in response to developmentally-prescribed increases in pit membrane permeability. This is the mechanism by which *P. tremuloides* de-activates its sapwood.

The relationship between pore size in the membrane and permeability to air can be estimated by applying the capillary equation. Assuming a contact angle of 0° between xylem sap and pore wall, the pressure (P , in MPa) needed to displace an air-water interface from a circular pore of diameter D (μm) can be calculated (Sperry and Tyree, 1990):

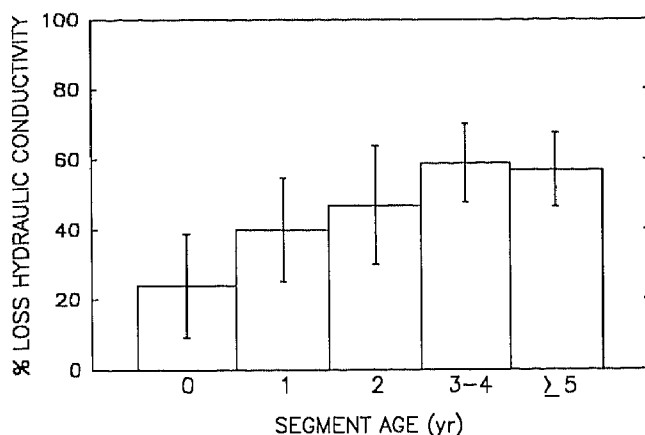


FIG. 3. Percentage loss of hydraulic conductivity versus branch segment age for *P. tremuloides*. Error bars are 95% confidence limits ($n \geq 12$).

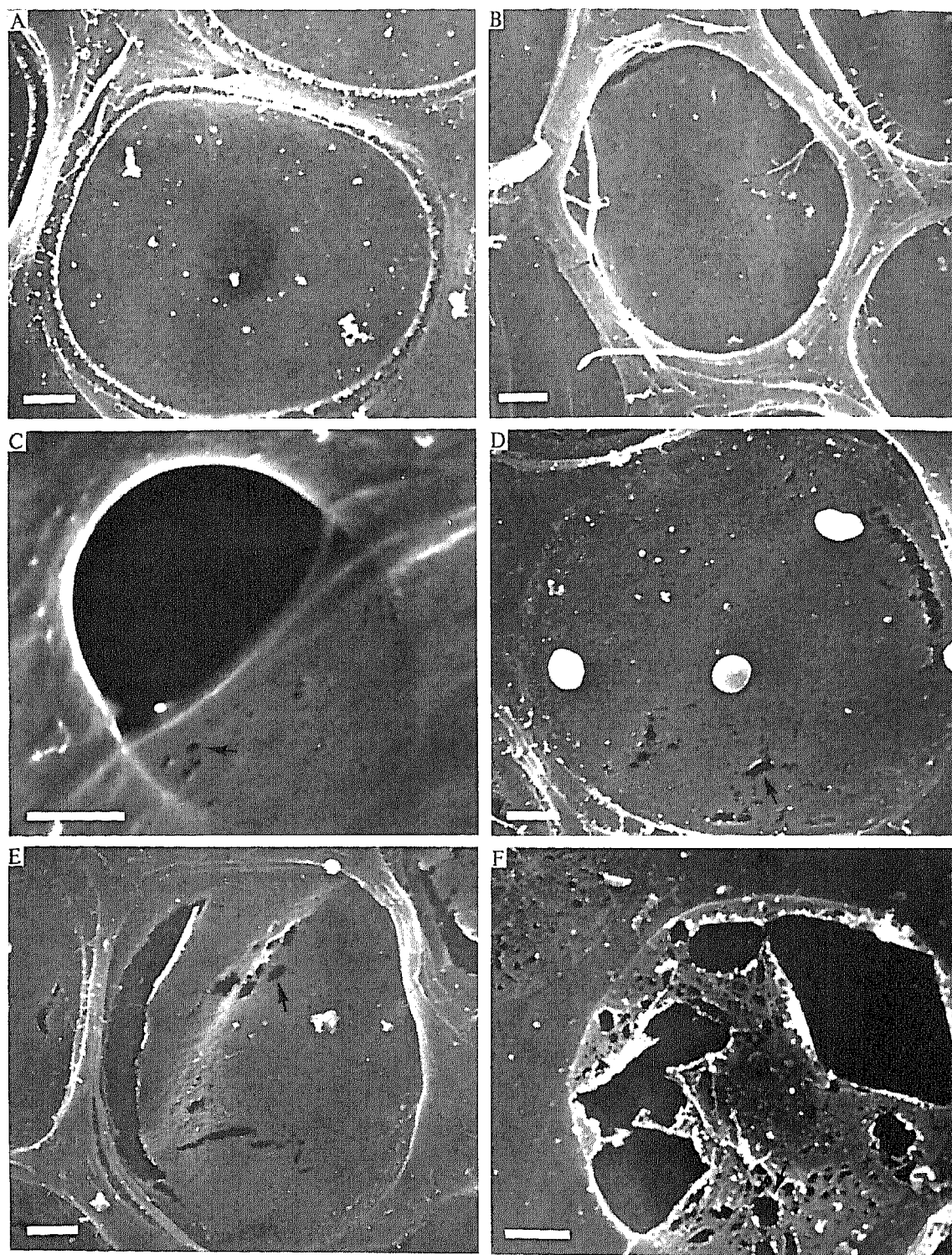


PLATE 2. SEM micrographs of inter-vessel pit membranes from *P. tremuloides*; secondary wall of one vessel has been removed revealing entire membrane. (A, B) Membranes in young, functional xylem vessels; scale bar 1.0 μm . (C) Torn membrane from young and functional vessel draped across pit aperture. Pores (arrow) visible where the membrane spans the aperture; scale bar 0.5 μm . (D-F) Membranes from old, non-functional vessels. Relatively large pores (arrows) visible even in non-torn parts of membranes. Fibrous structure of membrane revealed in (F). Scale bars 1.0 μm .

$$P = 4 T/D$$

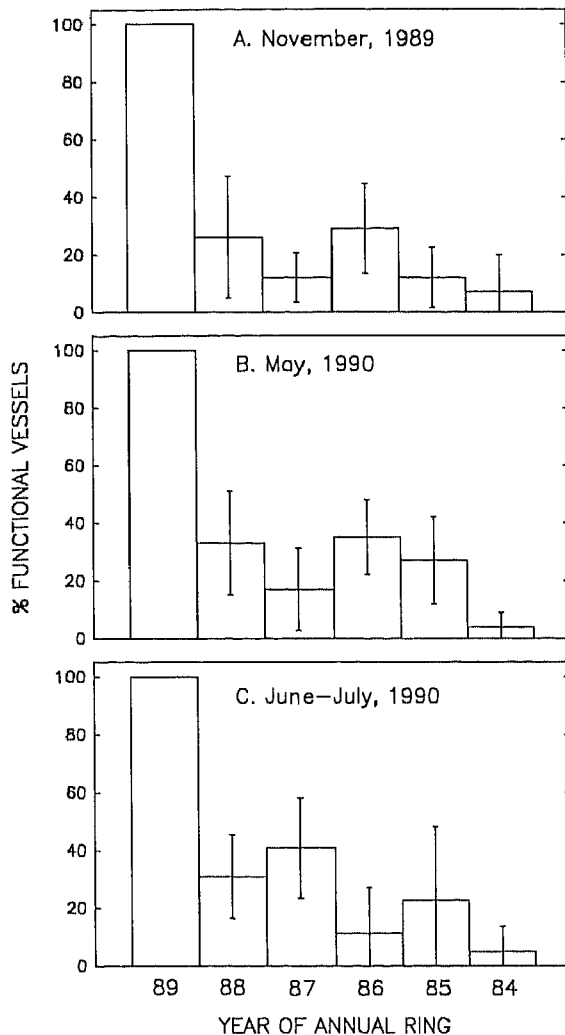


FIG. 4. Percentage of functional (dye-stained) vessels versus annual ring for *P. tremuloides* branches between 3- and 6-years-old and approximately 1.0 cm diameter. Error bars are standard deviations ($n \geq 5$).

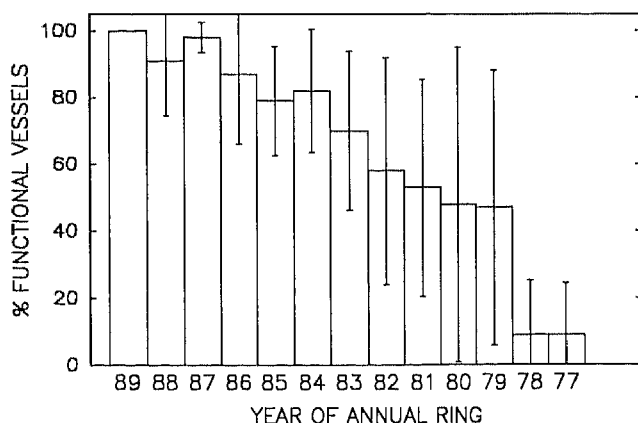


FIG. 5. Percentage of functional (dye-stained) vessels versus annual ring for *P. tremuloides* branches 11-16-years-old and approximately 1.0 cm diameter. Error bars are standard deviations ($n \geq 5$).

where T is the surface tension of water (0.072 N m^{-1} at 20°C). From this equation, the largest pores seen in the young pit membranes ($D = 0.08 \mu\text{m}$) would be permeable to air at pressures of 3.6 MPa or more. In other words, young vessels could sustain xylem tensions of at least 3.6 MPa before embolizing. For larger pores seen in the older xylem ($D = 0.5 \mu\text{m}$), embolism would begin at tensions as low as 0.6 MPa. This wide range of predicted embolism pressure corresponds with the gradual slope of the embolism response in Fig. 1A: embolism at low pressure occurring in older vessels, embolism at higher pressures occurring in younger vessels.

Increased permeability of pit membranes was not a simple function of pit membrane age because while it occurred after roughly one year in vigorously-growing branches, it was much delayed in slower-growing ones (compare Figs 4, 5). It may be related more to the immediate physiological environment of the xylem; perhaps a progressively lower oxygen level as the new xylem is added. The cause of the degradation is unknown, but structural observations suggest there was removal of material between cellulose fibrils (Plate 2F). One possible explanation is pectinases secreted by xylem parenchyma gradually break down middle lamella components in the pit membrane. Pectinases have been implicated in the increase of cell-wall porosity in soybean (Baron-Epel, Gharyal, and Schindler, 1988).

Increases in pit permeability with age may occur in other tree species as well, though not necessarily by obvious structural degradation. Earlier work on *A. saccharum*, *Abies balsamea*, *Picea rubens*, and other species, has shown that 5–20 mM oxalic acid together with added calcium increases membrane permeability without obvious structural changes; it acts by increasing pit membrane flexibility (Sperry and Tyree, 1988, 1990). Coutts (1977), also observed massive cavitation in response to oxalic acid injection in *Pinus nigra* var. *maritima*. The oxalic acid response, or something similar, may be involved in sapwood de-activation in some species. Preliminary work has indicated the oxalic effect is not pronounced in *Populus tremuloides*.

If xylem transport capacity is closely coupled with leaf physiology as modelling studies suggest (Tyree and Sperry, 1988), loss of sapwood function via developmentally-triggered embolism in *P. tremuloides* will be balanced with demand placed on the xylem by transpiration. In vigorously growing branches, there was only one annual ring of xylem available for conduction (Fig. 4), from late May 1990 when leaves flushed to early July when new xylem began to mature. This would seem to limit the foliage mass of *P. tremuloides* relative to other diffuse-porous species having several functional growth rings (e.g. *Acer saccharum*). This may be especially true given

the small diameter (40 μm) and short length (<5.0 cm) of the vessels in *P. tremuloides*. Ring-porous trees rely on a single functional ring, but compensate by having very large diameter (up to 400 μm) vessels that are also very long (several metres; Ellmore and Ewers, 1986).

In view of the disadvantage of limiting foliar area, advantages of the *P. tremuloides* pattern of xylem function are not obvious. Perhaps a large foliage mass would create too much mechanical stress on the trunk during the high winds common in its high-elevation habitat. The large apoplastic space created by the air-filled vessels and fibres no doubt increases water storage capacity. The gas volume of the trunk may also play a role in how the xylem survives the winter by accommodating expansion of water on freezing.

In conclusion, the pit membrane is clearly much more than a route for water flow between xylem conduits. It not only sets the cavitation threshold of the vascular system, but it is also the site where this threshold can be regulated. The key to what controls sapwood volume and heartwood formation in *P. tremuloides* lies in the signal that initiates pit membrane degradation. The same may be true for other tree species as well.

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LITERATURE CITED

- BARON-EPEL, O., GHARYAL, P. K., and SCHINDLER, M., 1988. Pectins as mediators of cell wall porosity in soybean cells. *Planta*, **175**, 389–95.
- COUTTS, M. P., 1977. The formation of dry zones in the sapwood of conifers. II. *European Journal of Forest Pathology*, **7**, 6–12.
- CROMBIE, D. S., HIPKINS, H. F., and MILBURN, J. A., 1985. Gas penetration of pit membranes in the xylem of *Rhododendron* as the cause of acoustically detectable sap cavitation. *Australian Journal of Plant Physiology*, **12**, 445–53.
- ELLMORE, G. S., and EWERS, F. W., 1986. Fluid flow in the outermost xylem increment of a ring-porous tree, *Ulmus americana*. *American Journal of Botany*, **73**, 1771–4.
- HILLIS, W. E., 1987. *Heartwood and tree exudates*. Springer, Berlin, 268 pages.
- SPERRY, J. S., DONNELLY, J. R., and TYREE, M. T., 1987. A method for measuring hydraulic conductivity and embolism in xylem. *Plant, Cell and Environment*, **11**, 35–40.
- and TYREE, M. T., 1988. Mechanism of water stress-induced xylem embolism. *Plant Physiology*, **88**, 581–7.
- — — 1990. Water-stress-induced xylem embolism in three species of conifers. *Plant, Cell and Environment*, **13**, 427–36.
- TYREE, M. T., and SPERRY, J. S., 1988. Do woody plants operate near the point of catastrophic xylem dysfunction caused by dynamic water stress? Answers from a model. *Plant Physiology*, **88**, 574–80.
- ZIMMERMANN, M. H., and BROWN, C. L., 1977. *Trees: structure and function*, Springer, Berlin.
- and JEJE, A. A., 1981. Vessel-length distribution in some American woody plants. *Canadian Journal of Botany*, **59**, 1882–92.