Water-stress-induced xylem embolism in three species of conifers

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Abstract. The mechanism of water-stress-induced xylem embolism was studied in three species of conifers: *Abies balsamea* (L.) Mill., *Picea rubens* Sarg. and *Juniperus virginiana* L. Each species showed a characteristic relationship between xylem tension and the loss of hydraulic conductivity by air embolism. *Abies balsamea* and *Picea rubens* began to embolize at tensions between 2 and 3 MPa and were completely non-conducting between 3 and 4 MPa. *Juniperus virginiana* was least vulnerable, beginning to embolize at 4 and still retaining approximately 10% conductivity at 10 MPa. As with a previous study of the vessel-bearing *Acer saccharum* Marsh., a brief perfusion of branch segments with an oxalic acid and calcium solution (10 and 0.1 mol m^{-3}, respectively) increased the vulnerability of the xylem to embolism; this was especially pronounced in *Abies balsamea*. In order to test whether embolism was caused by aspiration of air into functional tracheids from neighbouring embolized ones (the 'air-seeding' hypothesis), hydrated branch segments were injected with air at various pressures and measured for embolism. Results supported the air-seeding hypothesis because the relationship between injection pressure and embolism for both native and oxalic-calcium-treated segments was essentially the same as for embolism induced by xylem tension. Structural and experimental evidence suggested the air seeding occurred through inter-tracheid pit membranes when the thickened torus region of the membrane became displaced from its normal sealing position over the pit aperture. Thus, the embolism-inducing tension may be a function of pit membrane flexibility. This tension is of ecological significance because it reflects to some extent the range of xylem tensions to which a species is adapted.

Key-words: Abies balsamea (L.) Mill.; *Picea rubens* Sarg.; *Juniperus virginiana* L.; water stress; xylem embolism; hydraulic conductivity; conifers; wood anatomy.

Introduction

The ability of xylem conduits (vessels and tracheids) to maintain the xylem tensions required for water transport in plants is limited by their tendency to

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become gas-filled or 'embolized'. The relatively high tensions associated with water stress can lead to a large loss in hydraulic conductivity by embolism. Species vary considerably in their vulnerability to water-stress-induced embolism; not surprisingly, the more vulnerable a species the lower the xylem tensions it experiences under natural conditions (Sperry, Tyree & Donnelly, 1988b).

Although there are a number of possible explanations for the mechanism of water-stress-induced embolism (for a review, see Pickard, 1981), the air-seeding hypothesis most recently proposed by Zimmermann (1983) has received the most experimental support. According to this explanation, embolism is caused by air aspirating into functional conduits from neighbouring air spaces through pores in the conduit wall. Crombie *et al.* (1985) were the first to provide evidence that these adjacent air spaces could be previously embolized conduits and that seeding could occur through the pores in the inter-vessel pit membranes. They showed that the gas pressure required to penetrate inter-vessel pits in hydrated *Rhododendron* stems was equal to the xylem tension required to induce embolism during dehydration; embolism was detected acoustically by monitoring vibrations produced by the rapid pressure changes in an embolizing vessel. We have recently confirmed these results using sugar maple (*Acer saccharum*) and measuring embolism by how much it reduced hydraulic conductivity (Sperry & Tyree, 1988). Other studies have found support for the air-seeding hypothesis in plants as diverse as grapevines (*Vitis*; Sperry *et al.*, 1987b), mangroves (*Rhizophoraceae*; Sperry *et al.*, 1988b) and *Sphagnum* mosses (Lewis, 1988).

With the exception of the *Sphagnum* project which dealt with water storage cells, the studies cited above have all concerned air seeding in the vessel-type of xylem conduit that can measure up to several meters in length and has a relatively homogeneous pit membrane structure. In this paper, we consider water-stress-induced embolism in the tracheid-type of conduit that occurs in conifers. The typical conifer tracheid is about 3 mm long and has a pit membrane consisting of a porous margo surrounding a central, thickened torus (Figs 8–10). When a tracheid embolizes, capillary forces at the membrane deflect it against the pit chamber wall and the torus covers the

pit aperture, presumably minimizing air seeding (Dixon, 1914).

However, the sealing action of the torus has its limits because air has been shown to penetrate the inter-tracheid pit membranes of a variety of conifer species at pressures between 1 and 4.5 MPa (Stamm, 1964). Edwards & Jarvis (1982) estimated that applied air pressures of 1.5–3.0 MPa caused embolism by air seeding in *Pinus contorta* and *Picea sitchensis*, and suggested that embolism occurred at lower pressures by other mechanisms.

Our main objective was to determine the relationship between water stress and embolism for the three conifer species *Abies balsamea* (L.) Mill., *P. rubens* Sarg., and *Juniperus virginiana* L. We tested whether embolism was caused by air seeding at inter-tracheid pit membranes by comparing the air permeability of pits to the xylem tension required to induce embolism in tracheids. If experiments supported the air-seeding mechanism, we hoped to learn how the air penetrated the membrane given the barrier posed by the torus.

Materials and methods

Plant material

Branches from mature individuals of each species were collected from the same sites in northern Vermont, U.S.A., throughout the study. *Juniperus virginiana* was gathered from an abandoned pasture in South Burlington, *A. balsamea* from a state nursery in Essex junction, and *P. rubens* from a forest in Duxbury. Branches were cut near the bole and brought to the laboratory in plastic bags.

Induction of embolism

We tested the air-seeding mechanism by comparing embolism induced in dehydrated branches by water stress with that induced in hydrated branches by air injection. The hypothesis predicts the same pattern of embolism will result from both treatments. Water stress was induced by drying branches for various times. Dried branches were wrapped in plastic bags overnight to reduce evaporation and promote equilibration of xylem pressures. The next day, xylem pressure was measured on excised shoots with the pressure bomb, and embolism was measured in a 5 cm segment located 1 cm from the cut base of the branch (see below). This segment was cut from the branch underwater to prevent additional embolism; its location 1 cm from the original branch base ensured that it contained no tracheids embolized by the initial cut.

Branches for air injection were cut to a 30 cm length and inserted 8 cm into a pressure bomb. The 5 cm segment to be used in the embolism measurement was located 1 cm from the end of the branch in the bomb. The other end was fitted with water-filled tubing, and the whole branch wrapped in wet paper towelling and plastic wrap to minimize dehydration during air injection. Air pressure in the bomb was raised to a desired value (from 0.5 MPa to as high as 9 MPa in *J. virginiana*) and held for 20 min before release. The 5 cm segment was cut out underwater and left in water overnight to allow for equilibration of pressure in internal air-filled tracheids before being measured for embolism. Preliminary experiments indicated that even at the highest air pressures used, dehydration due to injection resulted in xylem tensions less than 0.35 MPa. Control segments enclosed entirely within the bomb and pressurized for the same time period and at the same pressures as injected segments showed no significant change in conductivity before and after pressurization.

In addition to inducing embolism in branches as they came from the field ('native' condition), we also used branches initially perfused with an oxalic acid and calcium (OAC) solution (10 and 0.1 mol m^{-3}, respectively; calcium added as $CaCl_2$). In previous work on sugar maple (*A. saccharum*), this treatment increased the permeability of inter-vessel pits to air, and as predicted by the air-seeding hypothesis, also increased the vulnerability of the xylem to embolism. Oxalic acid alone, which we use in a 10 mol m^{-3} solution to measure hydraulic conductivity (see below), has no effect except to reverse the action of oxalic with added calcium (Sperry & Tyree, 1988). The OAC response occurs regardless of the anion accompanying the calcium. Branches were perfused with OAC solution through tubing fixed to their base. The branch tips were cut off and solution forced through for 45 min at 175 kPa.

Measurement of embolism

Two methods were used to measure the embolism induced by water stress and air injection in branches. In the 'flushing' method, hydraulic conductivity of a segment was measured before and after a series of high-pressure (ca. 0.175 MPa) flushes of the measuring solution (10 mol m^{-3} oxalic acid in purified water); these flushes promoted the removal of air emboli in the tracheids. Hydraulic conductivity (kg m s^{-1} MPa^{-1}) was defined as the mass flow rate of solution through the stem (kg s^{-1}) divided by the pressure gradient (MPa m^{-1}). For most measurements, the solution was de-gassed prior to use by placing it under vacuum 2 dm^3 at a time and agitating it vigorously with a magnetic stirrer for 30–45 min. The flushing method has been used successfully for quantifying embolism in a variety of vessel-bearing species and has been described in detail elsewhere (Sperry, Donnelly & Tyree, 1987a).

The other 'adjacent-segment' method compared hydraulic conductivities of two 5-cm segments of the same branch separated by 1 cm. The first segment was measured before the water-stress or air-injection treatment, the second was measured after. The central 1 cm section was cut from the second segment

following the treatment in order to remove all tracheids exposed at the cut end during the treatment. Segments for conductivity measurements were always cut from the branch underwater to prevent additional embolism. The percentage by which the conductivity of the second segment was below the first gave the percentage loss in conductivity due to the treatment. Preliminary experiments evaluated the variation in conductivity between adjacent segments in the absence of an embolism treatment. For each species, the mean difference in conductivity between segment pairs was not significantly different from zero ($n = 10$ pairs, $P = 0.05$).

Identifying embolized tracheids and measurement of specific conductivity

Tracheids not functioning in water conduction were not stained when 0.05% safranan dye (filtered to $0.2 \, \mu m$) was perfused through the stem. Dye was pulled through stems with ca. 0.05 MPa vacuum; untreated and treated (e.g. water-stressed, or air-injected) stems were perfused simultaneously for equal time periods (10–20 min). Freshly cut longitudinal sections indicated whether non-stained tracheids were air-filled or otherwise occluded. Completely embolized segments conducted no dye indicating that re-filling did not occur during perfusion attempts.

Specific conductivity (kg s^{-1} MPa^{-1} m^{-1}) was defined as hydraulic conductivity per transverse-sectional area of functional xylem (m^2). Functional xylem area was determined from a transverse section through the middle of a dye-perfused branch segment. Area of stained xylem was measured using a bit pad (Ziess Zidas) and microscope with camera lucida. This method measured known areas with greater than 2% accuracy. Segments used were either untreated, or subject to various degrees of water stress or air injection.

Structural investigation of the pit membrane

In order to see how air-seeding might occur in inter-tracheid pits, wood of each species was studied in the scanning electron microscope (SEM). The following preparations were used: untreated wood, critical point-dried; untreated wood, air-dried; wood partially air-dried to embolism-inducing tension and then critical-point dried. These same treatments were repeated for samples previously perfused with OAC solution.

Measurement of the flexibility of inter-tracheid pit membranes

Structural investigations described in the 'Results' section suggested air seeding could be a function of membrane flexibility rather than membrane pore size. To study membrane flexibility, we compared the force required to seal the torus over the pit aperture for equal-length segments of each species. The torus was sealed by gradually increasing the hydraulic pressure gradient forcing solution through the stem. As the torus is progressively sealed, hydraulic conductivity should decline due to the increased resistance to water flow between tracheids. The relative pressures at which sealing occurred would be a function of the flexibility of the membrane. Experiments were also conducted on vessel-bearing species (*A. saccharum, Cassipourea elliptica* [Sw.] Poir.) to see if hydraulic conductivity was independent of applied pressure gradient when a torus was absent.

In practice, an 8-cm-long stem segment (freshly gathered) was inserted 3 cm into a pressure bomb and fixed at both ends to solution-filled tubing. Hydraulic conductivity was first measured at a relatively moderate pressure of approximately 0.008 MPa induced by gravity. Then the bomb was sealed and pressure raised to a series of pressures between 0.15 and 3.05 MPa and lowered back again. Each of these pressures was held for 5 min before a hydraulic conductivity measurement was made. The final measurement utilized the same gravity-induced pressure used initially.

Results

Embolism and the air-seeding hypothesis

The flushing method tended to under-estimate embolism in water-stressed conifer branches. An initial problem was a decline in conductivity following each flush. This was solved when the measuring solution was de-gassed prior to use. However, a second problem remained: it proved impossible to restore conductivity to expected pre-embolism values. In other words, there was some irreversible loss of conductivity caused by the dehydration. This is shown in Fig. 1 for *J. virginiana*. Dehydrated and flushed stems had conductivities below those expected judging from measurements of untreated stems of similar diameters. This was most noticeable when stems were dried to very high xylem tensions as were those in Fig. 1 (> 7 MPa). To the extent that a true maximum conductivity was not achieved by flushing, the percentage loss in conductivity due to embolism was under-estimated. We suspected the loss of conductivity was not simply due to persistent air bubbles, but to the sealing of the torus as embolism was induced during dehydration. If the torus remained sealed after rehydration, it could have resulted in lower conductivity. In view of the ambiguity of the flushing method, we used the adjacent-segment method exclusively.

Figures 2–4 show embolism curves for native (Figs 2a–4a), and OAC-perfused (Figs 2b–4b) branches of each species. Embolism was induced by water stress (solid lines, solid symbols) and by air injection (dashed lines, open symbols). In the native
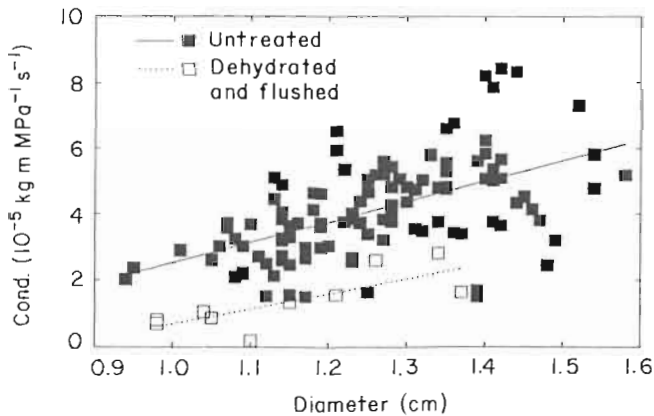
Figure 1. Hydraulic conductivity (COND.) vs diameter (without bark) for branch segments of *J. virginiana*. Untreated branches (solid squares) had higher conductivities than similar-sized branches that were dehydrated to xylem tensions greater than 7 MPa before being flushed to maximum conductivity (open squares). This suggests the flushing procedure was inadequate for reversing the effects of dehydration.

condition, *A. balsamea* was the most vulnerable of the three species to water stress, embolizing at xylem tensions between 2 and 3.5 MPa (Fig. 2a, solid line). *Juniperus virginiana* was by far the most resistant, embolizing between 4 and 10 MPa (Fig. 4a, solid line). For each species, air injection of hydrated stems produced almost the same embolism curve as did water stress (Figs 2a–4a, compare solid and dashed lines), as predicted by the air seeding hypothesis. The close correspondence of these two curves indicated that air seeding can explain all the embolism observed in these species; there was no evidence for additional mechanisms as suggested by Edwards & Jarvis (1982).

The air-seeding hypothesis was also supported by results from branches perfused with OAC solution (Figs 2b–4b). In each species, the solution increased vulnerability to embolism whether it was induced by water stress or air injection (compare Figs 2b–4b with 2a–4a). The results were most convincing in *A. balsamea* (Fig. 2b) where there was a large increase in vulnerability relative to native branches and the response was very similar for both water-stressed and air-injected twigs (solid and dashed lines, respectively). The solution had less of an effect on *J. virginiana*, with more embolism caused by air injection than by water stress, especially at low pressures (Fig. 4b). This same disparity was also seen in *P. rubens* where much more embolism was caused at low pressures by air injection than by water stress (Fig. 3b). This discrepancy may be due to differences in how rapidly the pressure difference was increased: over a span of a few minutes in air-injected branches as opposed to several days for water-stressed ones.

Dye perfusions indicated that early-wood tracheids were more vulnerable to embolism than late-wood ones whether embolism was induced by water stress

or air injection (Fig. 5). In each species, as a branch became progressively more embolized its specific conductivity decreased in direct proportion (Fig. 6). Thus, the late-wood tracheids that were most resistant to embolism were also least efficient in water conduction. This same relationship held between the three species: *J. virginiana* was most resistant to embolism (Fig. 4a) and it also had the lowest specific conductivity (Fig. 7).
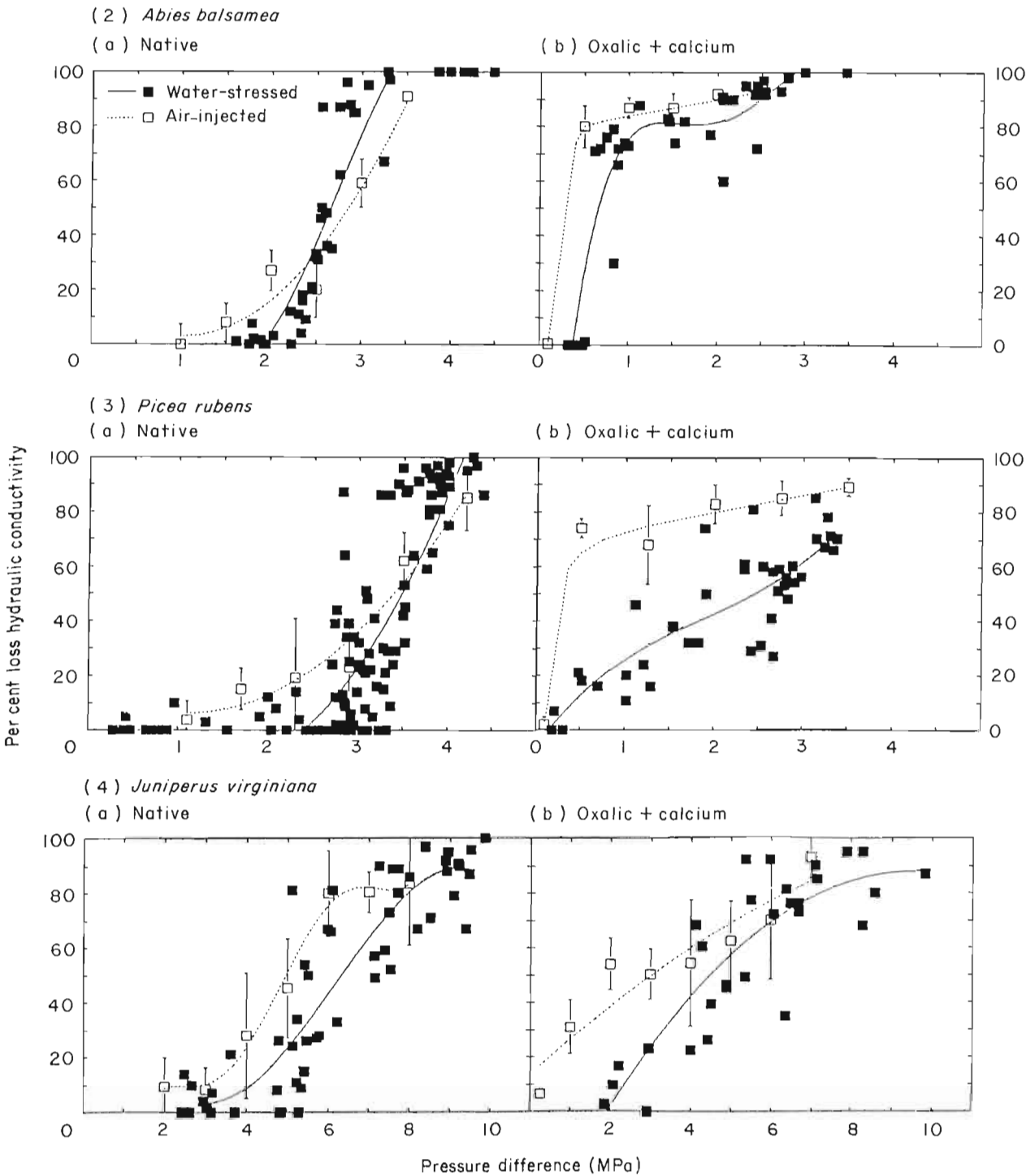
Possible mechanisms of air-seeding

Figures 8–10 show the structure of the inter-tracheid pit membranes in early-wood for the three species as viewed with the SEM. In each case, the membrane is pressed against the pit chamber wall and the torus is covering the pit aperture. In Figs 8 and 9, the impression of the underlying aperture is evident in the torus. If air seeding was occurring, it probably did not happen through the torus, which in each species looks to be without pores and forms a tight seal over the aperture. It is also doubtful that seeding occurred through pores in the margo prior to the sealing of the torus, because these pores appear too big to account for the observed embolism-inducing pressures. The air-seeding pressure (P, MPa) of a circular pore can be predicted from pore diameter (D, microns) using the following version of the capillary equation:

$$P = 4\,T/D$$

where T is the surface tension of the xylem sap (0.072 N m^{-1} for water at 20 °C). This equation assumes a 90° contact angle between the air-water meniscus and the pit membrane. For non-circular pores, Stamm (1966) has shown empirically that the equation is satisfied by using the equivalent circle diameter of the largest inscribed ellipse. For the larger margo pores in obviously undamaged areas of the pit membrane, calculated air-seeding pressures in *A. balsamea*, *P. rubens*, and *J. virginiana* were 0.29, 0.34 and 1.92 MPa, respectively. In each case, this is over 60% below the observed embolism-inducing pressure (Figs 2a, 3a & 4a). A discrepancy of this magnitude is probably not due to changes in pore diameters caused by the SEM preparation, but rather to air seeding occurring by some other mechanism.

When branches were dehydrated to embolism-inducing xylem tensions before being critical-point dried and viewed in the SEM, inter-tracheid pits were frequently seen where the torus was displaced from its sealing position. In some cases, the torus was still held by strands of the margo (Fig. 11); in other cases, the torus had broken free of the margo and the membrane looked as if it had ruptured. This suggested that air seeding occurred by the displacement of the torus by a critical pressure difference. It was unclear by observation whether this was due to rupture or stretching of the intact membrane, because the rupture observed could have been an artifact of specimen preparation.

(2) *Abies balsamea*

(a) Native

(b) Oxalic + calcium

(3) *Picea rubens*

(a) Native

(b) Oxalic + calcium

(4) *Juniperus virginiana*

(a) Native

(b) Oxalic + calcium

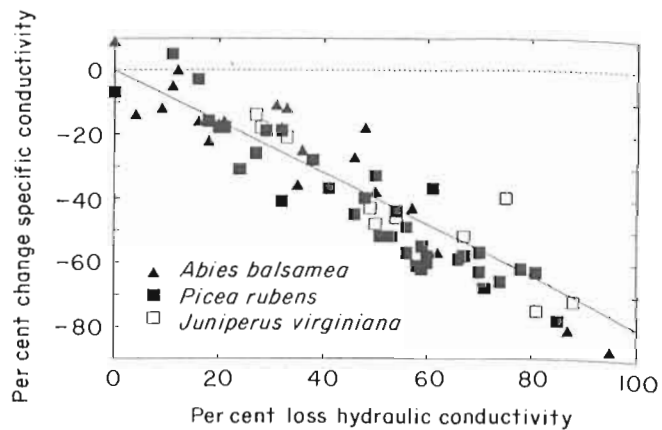Per cent loss hydraulic conductivity

Pressure difference (MPa)

Figures 2–4. Percentage loss in hydraulic conductivity due to water stress (solid squares, solid curves) and air injection (open squares, dashed curves) of branch segments from *A. balsamea* (2), *P. rubens* (3), and *J. virginiana* (4). The x-axis is the pressure difference between water in intact tracheids and the surrounding air (including air in severed tracheids at the cut end of the branch). In water-stressed branches, the pressure difference was increased by increasing xylem tension in intact tracheids; in air-injected branches pressure difference was increased by raising external air pressure while keeping xylem tension near zero. In native branches (2a, 3a & 4a), the good correspondence between the water-stress and air-injection curves suggests embolism occurred by air entry into tracheids. Branches perfused with oxalic and calcium solution (10 mol m⁻³ oxalic acid and 0.1 mol m⁻³ CaCl₂) showed an increase in vulnerability to embolism by both water stress and air injection (2b, 3b & 4b), especially in *A. balsamea*. This also supports the air-seeding hypothesis because the increase in the permeability of tracheids to air corresponded to an increase in vulnerability of branches to water-stress-induced embolism.
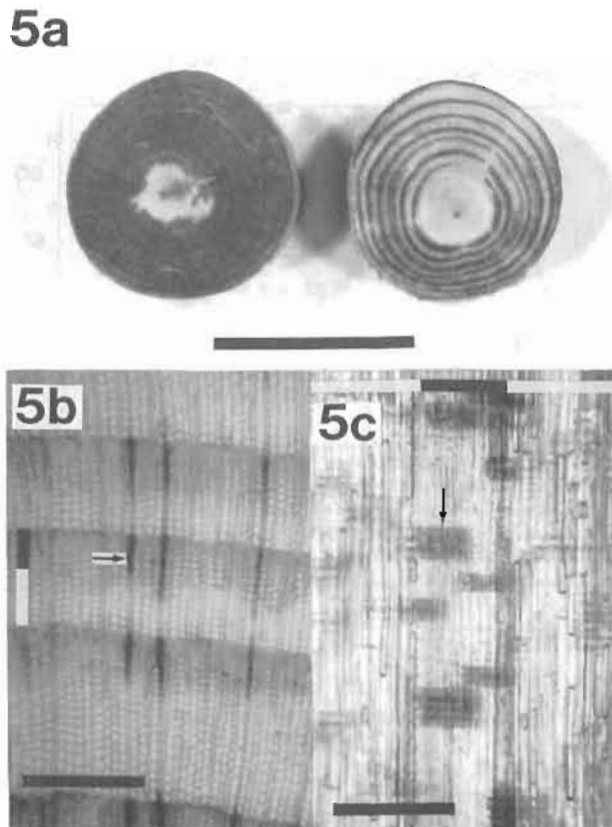
Figure 5. (a) Dye staining patterns of branches before (left) and after (right) dehydration. More vulnerable non-stained xylem is arranged in concentric bands. This same pattern occurred when branches were embolized by air injection. Scale bar is 1 cm. (b) Embolized bands correspond to larger diameter early-wood tracheids. White and black parts of vertical bar spanning the central growth ring correspond to non-stained earlywood and dye-stained late-wood, respectively. Dye staining is also evident by concentration of dye in ray parenchyma (arrow). Scale bar is 0.5 mm. (c) Longitudinal section of freshly-stained branches show non-stained, air-filled, early-wood tracheids (corresponding to white parts of bar across top of photograph) and dye-stained and water-filled late-wood tracheids (corresponding to black part of bar). Dye-stained region is also indicated by dark-staining ray cells (arrow). Embolized area has bubbles in tracheids. Scale bar is 0.25 mm.

Figure 6. Percentage change in specific conductivity (hydraulic conductivity per transverse sectional area) vs percentage loss of hydraulic conductivity for each species studied. Specific conductivity decreases as branch segments become increasingly embolized indicating that the tracheids most resistant to embolism (latewood tracheids, Fig. 5) were also least efficient in water conduction. Dashed line indicates result if specific conductivity were constant.

membrane. We tested membrane flexibility by measuring hydraulic conductivity as a function of hydraulic pressure gradient (Fig. 12a c). Results for the vessel-bearing *A. saccharum* showed that with the exception of a transient increase at moderate pressures, conductivity was constant over the range of applied pressure gradients (Fig. 12a, *A. saccharum*). The same response was seen in *C. elliptica*, a vessel-bearing tropical tree (data not shown). This is the expected result if there is no change in the geometry of the flow path. *Abies balsamea* and other conifers behaved very differently. As pressure was increased, conductivity gradually dropped off by more than 80% at the highest pressure used (2.75 MPa); as pressure was decreased, conductivity returned with some hysteresis to its initial value (Fig. 12a, *A. balsamea*). This response occurred whether or not the measuring solution was de-gassed so it was apparently not caused by bubbles coming out of
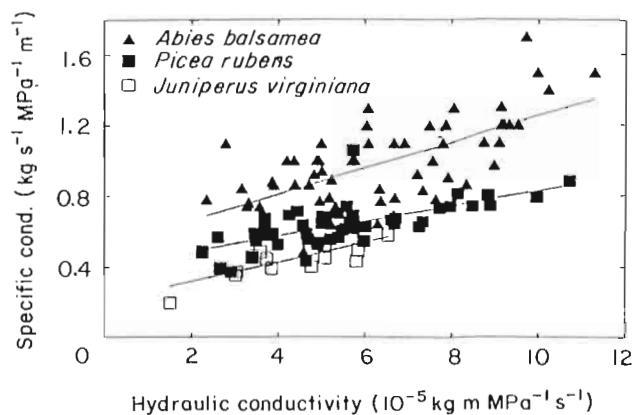
To test whether air seeding occurred by membrane rupture, the conductivity of air through branch segments of *A. balsamea* was measured at a pressure difference of 1 MPa before and after a 45-min injection of air at 3.5 MPa. This high-pressure injection was sufficient to completely embolize the branch segment (see Fig. 2a, air-injected curve). If membrane rupture caused embolism, then the conductivity of air at 1 MPa should have been greater following the injection than before because broken membranes would be more permeable to air. This did not happen, suggesting that membrane rupture did not account for air seeding.

If air seeding occurred by displacement of the torus without membrane rupture, then air seeding pressure should be a function of the flexibility of the
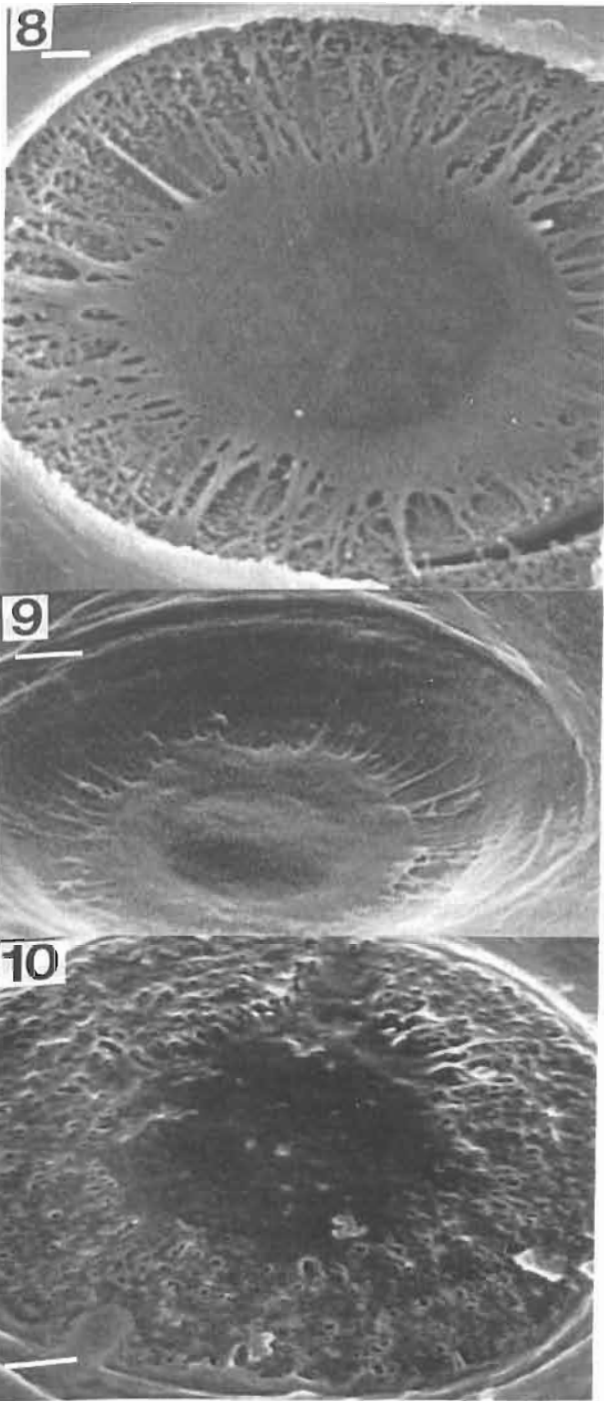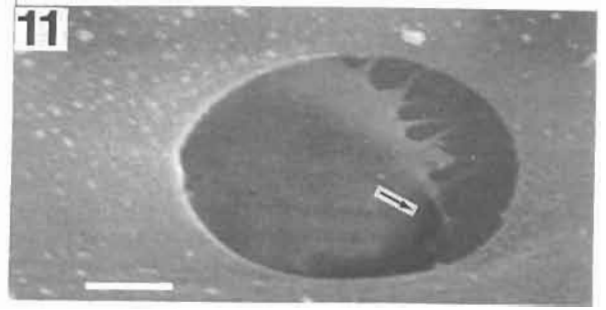

Figure 7. Specific conductivity (hydraulic conductivity per transverse sectional area) vs hydraulic conductivity for each species.

Figures 8–10. Scanning electron micrographs of early-wood inter-tracheid pit membranes in *A. balsamea* (8), *P. rubens* (9), and *J. virginiana* (10). Scale bar is 1 μm. One side of the pit has been cut away exposing the membrane to view. In each case, the membrane has been pressed against the wall of the pit chamber and the torus has covered the pit aperture. Sections were either air-dried (9 & 10), or partially air-dried before being critical-point dried (8).

solution. The response is probably not due to transition from laminar to turbulent flow because the flow rate at the higher pressures became independent of the pressure gradient (data not shown) rather than

Figure 11. Intact inter-tracheid pit in *A. balsamea;* scale bar is 1 μm. The section was from a branch dehydrated to embolism-inducing xylem tensions before being critical-point dried. Through the facing aperture of the pit, the torus can be seen in sealing position over the opposite aperture. At one point (arrow), the torus has slipped through this aperture though still held by strands of the margo. This was seen frequently when xylem was embolized prior to sectioning and viewing.

proportional to the pressure raised to the 0.57 power (Siau, 1984). We interpret the drop in conductivity in conifers to be due to the gradual sealing of the torus against the pit aperture as the pressure gradient was increased. When the pressure was decreased, the torus eventually returned to its central position and conductivity was restored.

Assuming this is the correct interpretation, this response can be used to evaluate the flexibility of the inter-tracheid pit membrane. A more flexible membrane would show a drop in conductivity at lower pressures than a more rigid one because the torus would seal more readily. The response for *Picea rubens* was essentially identical to *Abies balsamea* (data not shown), suggesting these two species are almost identical in the flexibility of their pit membranes. However, *Juniperus virginiana* apparently has considerably less flexible membranes than either *A. balsamea* or *P. rubens* because a higher pressure gradient was required to lower its hydraulic conductivity (Fig. 12b). In addition, when the pressure was lowered, the conductivity in *J. virginiana* returned to its initial value more readily than did the other species. The rigidity of the *Juniperus* membrane may confer high resistance to air seeding by holding the torus in sealing position against large pressure differences.

More evidence for the causal relationship between membrane flexibility and vulnerability to air seeding is shown in Fig. 12c. In addition to causing a large increase in the vulnerability of *A. balsamea* xylem to embolism (Fig. 2), OAC solution also appeared to increase the flexibility of the inter-tracheid pit membrane. This solution induced a premature drop in hydraulic conductivity with increasing pressure relative to controls treated with oxalic acid alone (Fig. 12c). In addition, initial conductivity in OAC-treated branches was not restored as it was for controls when pressure was decreased. This indicates that the torus did not return to its normal position but remained sealed against the aperture. The solu-
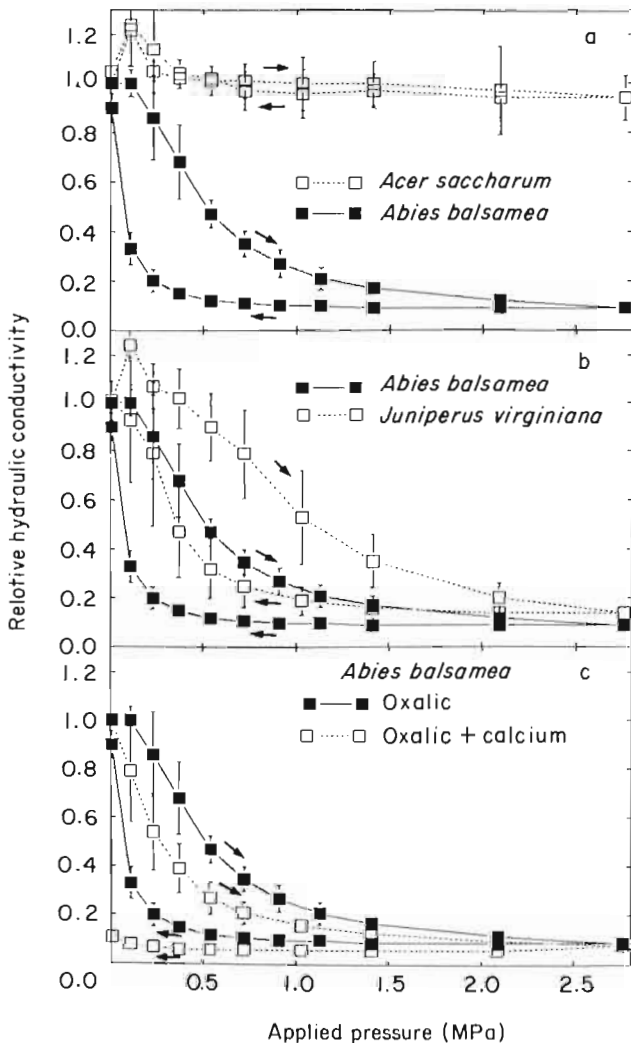
Figure 12. Relative hydraulic conductivity vs applied hydraulic pressure gradient. Conductivity is expressed relative to its initial value at minimum applied pressure. Arrows along curves indicate increasing pressure and decreasing pressure. (a) For *A. saccharum*, conductivity was essentially independent of applied pressure indicating a fixed flow path throughout the experiment. In *A. balsamea* and other conifers, conductivity decreased with increased pressure (arrows pointing right), and returned to its initial value (with some hysteresis) as pressures were decreased (arrows pointing left). This was probably due to sealing of the torus against the pit aperture as applied pressure was increased, and unsealing as pressures were decreased. (b) Decrease in conductivity with increasing pressure occurred at higher applied pressures for *J. virginiana* than for *A. balsamea* (arrows right); when pressure was decreased, *J. virginiana* returned to its initial conductivity at higher pressures than *A. balsamea* (arrows left). This implies that the pit membrane was less flexible in *J. virginiana* than in *A. balsamea*. (c) In *A. balsamea*, treatment with oxalic and calcium solution (10 mol m⁻³ oxalic acid and 0.1 mol m⁻³ CaCl₂) caused conductivity to drop at lower pressures than controls treated with oxalic alone (10 mol m⁻³) as pressure was increased (arrows right). As pressure was decreased (arrows left), OAC treated stems did not return to initial conductivity. This suggests the solution increased membrane flexibility and reduced membrane elasticity. This may explain why the solution also caused dramatic increase in embolism vulnerability in this species (see Fig. 2).

tion, in addition to increasing flexibility, may have also eliminated the membrane's normal tendency to return to its unsealed position. We hypothesize that the increase in flexibility caused by the OAC solution led to increased vulnerability to air seeding (Fig. 2) because it allowed the torus to be more easily displaced from its sealing position.

Discussion

The results indicate that water-stress-induced embolism in tracheid-bearing plants occurs by air seeding at inter-tracheid pit membranes. This is the same conclusion reached by other studies on a variety of vessel-bearing species (Crombie *et al.*, 1985; Sperry & Tyree, 1988; Sperry *et al.*, 1988b). Our results are less conclusive on how the air gets through the membrane. They suggest that air-seeding pressure is not directly a function of pore size but of membrane flexibility, because the seeding may occur when the torus is displaced from its normal sealing position over the pit aperture. Perhaps the best evidence for this is that the OAC solution increased both membrane flexibility (Fig. 12c) and vulnerability to embolism (Figs 2a,b). In vessels, evidence indicates that the air-seeding pressure is a function of pore size in the pit membrane (Sperry & Tyree, 1988); however, flexibility and pore size are probably related given that pore dimensions may change (and new pores arise) as a membrane is stretched.

The way in which OAC solution appeared to increase flexibility in *A. balsamea* pit membranes (Fig. 12c) is unknown. The action of OAC is not a function of pH or surface tension of the bulk solution (Sperry & Tyree, 1988). Unpublished results indicate that the effect is not duplicated by treatment of stems with the chelator EDTA, or with malic acid and calcium. The increased flexibility may be caused by calcium oxalate complexes formed *in situ* at the cellulose microfibrils composing the membrane; this would disrupt ionic interactions between individual fibrils possibly allowing them to 'creep' as a pressure difference is applied across the membrane. This creeping, or non-reversible stretching, of the membrane would explain why it apparently remained in a sealed position even after the pressure was relieved in the experiment shown in Fig. 12c.

If the pores in the margo are too large to account for the observed air seeding as our results suggest, then it necessarily follows they are small enough to hold an air-water meniscus against pressures sufficient to deflect the membrane and seal the torus. This is one functional explanation for the observation that the more rigid the membrane the smaller the margo pores (compare *A. balsamea* to *J. virginiana*, Figs 8 & 10), because the smaller pores can withstand a higher pressure without seeding. Of course, this correlation makes sense from a purely structural standpoint as well. We can arrive at an upper estimate for the pressures required to initiate and complete membrane

deflection from the experiments summarized in Fig. 12. The experiments were performed on 8-cm segments. Taking an estimate for tracheid length of 3 mm, the pressure drop across each tracheid would be about 3/80 the applied pressure in Fig. 12. The pressure drop across each pit membrane will be no more than the pressure drop per tracheid. Dividing the pressures at the beginning and end of the drop in hydraulic conductivity for each species in Fig. 12 by 80 then gives a maximum estimate of the pressure needed to initiate, and complete membrane deflection, respectively. This works out to 0.0075 and 0.0564 MPa, for *A. balsamea* and *P. rubens*; and 0.015 and 0.075 MPa for *J. virginiana*. These pressures are well below the air-seeding estimates for margo pores of 0.29, 0.34 and 1.92 MPa for *A. balsamea*, *P. rubens*, and *J. virginiana*, respectively. Based on these estimates, the torus will seal off the pit before air would be drawn through the margo.

The same conclusion has been reached by wood technologists who are concerned with the permeability of wood to preservatives. For example, Gregory & Petty (1973) concluded from models based on anatomical measurements that, for early-wood pits of *P. sitchensis*, the margo pores can retain an air-water meniscus at pressures up to 0.1 MPa, whereas the membrane will completely deflect at a pressure of 0.033 MPa. Interestingly, this is not predicted to happen in late-wood pits (Gregory & Petty, 1973; Petty & Puritch, 1970). They are apparently more rigid than early-wood ones and essentially never seal over. This explains why preservatives forced through kiln dry wood penetrate the late-wood more readily than the early-wood (Petty & Puritch, 1970). If this is true, then the air seeding in these late-wood pits must occur through pores in the membrane in the same way as for inter-vessel pits. Although we did not make a systematic comparison between early- and late-wood pit membranes, other studies have found that the membrane pores in late-wood are much smaller than in early-wood (Petty & Puritch, 1970). Non-sealing of late-wood pits may account for the residual conductivity observed (about 20% of initial) after early-wood pits had presumably sealed over at high pressure gradients in Fig. 12.

The xylem tensions required to cause embolism in the three species we studied (Figs 2-4) indicate that conifers as a group are no more or less vulnerable to water-stress-induced embolism than vessel-bearing angiosperms. *Juniperus virginiana* is as resistant to embolism as any vessel-bearing species we have studied. However, unlike many vessel-bearing species, conifers may lack the ability to refill their conduits once they have been embolized. Even if conifers could generate the positive xylem pressures associated with embolism reversal in vessel-bearing species (Sperry, Donnelly & Tyree, 1988a; Sperry *et al.*, 1987), hydraulic conductivity would not return to normal if the torus remained sealed (Fig. 1).

Although the pits are permanently sealed in the heartwood of conifers (Siau, 1984), it is not clear whether sealing is irreversible in the sapwood in nature. There is some evidence that the longer the membrane is sealed, the harder it is to unseal (Siau, 1984, p. 116). Apparently, they unsealed in the experiments summarized in Fig. 12 (except for stems treated with OAC, Fig. 12c), although they were only in the sealed state for a matter of minutes. If embolism is irreversible in conifers, the sheer number of tracheids available for water conduction may confer enough redundancy to tolerate partial embolism.

The correlation we observed between high resistance to embolism and low specific conductivity (Figs 6 & 7) may have a causal explanation if pit membranes that are resistant to air seeding also have a high hydraulic resistance. This seems reasonable in that a denser and less porous margo would be more effective in holding the torus in sealing position against air seeding but would also afford more resistance to water flow than a more open margo. According to Calkin and co-workers (Calkin, Gibson & Nobel, 1986), resistance at the pit membrane is the most important determinant of the overall hydraulic conductivity of tracheid-bearing plants, outweighing other factors of tracheid length and diameter.

These considerations provide a tentative answer to the adaptive significance of the torus and margo structure of the tracheid pit. The central, thickened torus fulfills the requirement for safety from air seeding (up to a point), and the porous margo minimizes hydraulic resistance. Reducing this resistance is especially important for conifers because tracheids are only a few millimeters long and water must continually cross from one to the next. The trade-off between safety from embolism and efficiency of water conduction may also explain why the vulnerability of a species tends to correlate with the xylem tensions it experiences in nature. Xylem that is safer than necessary from embolism would place a species at a competitive disadvantage because it would cause an unnecessary reduction in water conducting efficiency.

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