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Mechanism of Water Stress-Induced Xylem Embolism[1]

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ABSTRACT

We investigated the hypothesis that water stress-induced xylem embolism is caused by air aspirated into functional vessels from neighboring embolized ones (*e.g.* embolized by physical damage) via pores in intervessel pit membranes. The following experiments with sugar maple (*Acer saccharum* Marsh.) support the hypothesis. (a) Most vessels in dehydrating stem segments embolized at xylem pressures < −3 megapascals; at this point the pressure difference across intervessel pits between air-filled vessels at the segment's ends and internal water-filled vessels was >3 megapascals. This same pressure difference was found to be sufficient to force air across intervessel pits from air injection experiments of hydrated stem segments. This suggests air entry at pits is causing embolism in dehydrating stems. (b) Treatments that increased the permeability of intervessel pits to air injection also caused xylem to embolize at less negative xylem pressures. Permeability was increased either by perfusing stems with solutions of surface tension below that of water or by perfusion with a solution of oxalic acid and calcium. The mechanism of oxalic-calcium action on permeability is unknown, but may relate to the ability of oxalate to chelate calcium from the pectate fraction of the pit membrane. (c) Diameter of pores in pit membranes measured with the scanning electron microscope were within the range predicted by hypothesis (≤0.4 micrometer).

Xylem embolism is the presence of air-filled tracheids and/or vessels, and it can result in a substantial impairment of xylem transport. Environmental causes of embolism include water stress and winter freezing; potential consequences include reduction of growth and dieback. In a seasonal study of sugar maple (*Acer saccharum*) in northern Vermont, we learned that even during a wet growing season trunk xylem became 30% embolized, and that by winter's end hydraulic conductivity of xylem was reduced by an average of 80% with many twigs 100% embolized (17).

Numerous mechanisms have been proposed for how water stress causes embolism. The simplest one is that as xylem pressure becomes increasingly negative it overcomes the cohesion between water molecules and vaporization occurs. However, theory predicts this to happen at pressures much more negative than those observed to cause embolism (13). Other mechanisms that could nucleate vaporization include mechanical shock and ionizing radiation, although Milburn (10) has shown radiation to be ineffective. In addition, embolism could be caused by air coming out of solution due to rapid pressure changes (13).

The explanation with the most experimental support is the air-seeding hypothesis (12, 20). According to this mechanism, embolism is triggered by air aspirated into the vessel via pores in the wall where it adjoins an air space. Once inside the vessel, the air disrupts the cohesion of the water column which retracts, leaving behind a vessel filled with water vapor and air. Eventually the vessel becomes completely air-filled as air comes out of solution from surrounding water. Embolism of adjoining vessels is prevented as long as pressure difference across intervessel walls does not exceed the surface tension of the air-water interface at pores in this wall. The largest pores are the most vulnerable to the penetration of air; these are in the pit membranes which under normal circumstances facilitate water flow between vessels (Fig. 1). The pit membrane consists of two primary cell walls and middle lamella, and is a membrane only in the general sense (*i.e.* it is not a lipid bilayer). If the air-seeding hypothesis is correct, the dimensions of pit membrane pores dictate the minimum xylem pressure that can be sustained by a plant without embolism spreading throughout the vascular system. A plant would be vulnerable to this mode of embolism any time even one of its xylem conduits became air-filled by physical damage (*e.g.* by an insect bite or broken branch).

The maximum pressure difference (ΔP, in MPa) withstood by a meniscus at an intervessel pore can be calculated from the pore diameter (D, in μm) and xylem sap surface tension (T, in N^2m^{-1}) using the capillary equation:

$$\Delta P = 4\ (T/D) \qquad (1)$$

We refer to the ΔP of a pit membrane pore as its bubble pressure. The bubble pressure can be reached by lowering the xylem pressure in the water-filled conduit (Fig. 1, functional) while keeping the air-filled one (Fig. 1, embolized) near atmospheric as would be the case in a transpiring plant, or conversely it can be induced experimentally by raising the air pressure while keeping xylem pressure near atmospheric.

Evidence for the air seeding hypothesis comes from a variety of sources and organisms. Lewis (9) has verified that Eq. 1 predicts the bubble pressure for pores in the water storage cells of *Sphagnum*. Sperry *et al.* (15) demonstrated that the bubble pressure of intervessel pit membrane pores in grapevine was reached when xylem pressure dropped to −2 MPa or less. This is the same pressure range known to cause embolism in grapes from independent work (S Salleo, MA LoGullo, personal communication). Crombie *et al.* (4) have shown in *Rhododendron* that the negative xylem pressure required to induce embolism as detected acoustically corresponded with independent measures of the bubble pressure for pit membranes. Furthermore, by decreasing the surface tension of the xylem sap and thus decreasing the bubble pressure, they also increased embolism.

In this paper, we report experiments with sugar maple that were designed to further test the air seeding hypothesis. In one type of experiment we lowered the bubble pressure of intervessel pits in order to see if corresponding decreases occurred in the negative xylem pressure (Ψ_{px}) required to induce embolism. We decreased bubble pressure by perfusing the stem segments either with a solution of low surface tension (T in Eq. 1), or with a

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[2] Abbreviations: N, newton; SEM, scanning electron microscope.

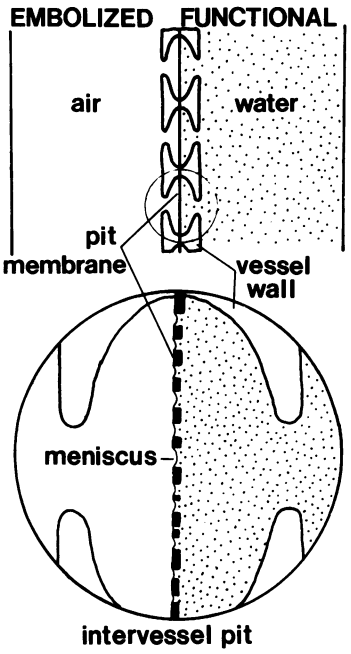

FIG. 1. Diagram of wall structure between adjacent xylem vessels showing intervessel pit structure. The porous pit membrane develops from primary cell walls of the two vessels and middle lamella; it is overarched by thick secondary walls to form a pit chamber that opens to the vessel lumen via a pit aperture (see Fig. 6). When a vessel is embolized, air is prevented from spreading to adjacent functional vessels by the capillary force of the air-water meniscus spanning pit membrane pores. If the pressure difference across this meniscus exceeds the force holding it there (which is dependent on pore diameter and sap surface tension), air is aspirated into the functional vessel and it becomes embolized. This is the air seeding mechanism of water stress-induced embolism.

solution of oxalic acid and calcium. The mechanism by which this latter solution decreases the bubble pressure is unknown, but it may be related to the interaction between oxalic acid and the calcium pectate of the pit membrane (see "Discussion"). We quantified embolism by measuring how xylem hydraulic conductivity was lost as branches were dehydrated to increasingly more negative Ψ_{px}. In other experiments we compared the bubble pressure to the negative Ψ_{px} required to induce embolism; bubble pressure was measured by raising air pressure relative to atmospheric xylem pressure. Finally, we measured pore diameters in intervessel pit membranes to see if they agreed with the range predicted by hypothesis.

MATERIALS AND METHODS

Plant Material. All experiments were done on 5- to 8-year-old sugar maple (*Acer saccharum* Marsh.) saplings growing in a nursery bed in Essex Junction, Vermont. Stem segments were cut from the main axis and were between 0.5 and 1.5 cm diameter (excluding bark).

Air Permeability Experiments: Measurement of Bubble Pressure. We measured the bubble pressure of intervessel pit membranes by measuring the pressure required to force air through hydrated maple stems longer than the longest vessel. In this way we were raising the air pressure in the open vessels at one end of the stem and keeping the water in the remaining vessels near atmospheric pressure. When the bubble pressure was reached, air penetrated intervessel pit membranes and passed through the stem. Air was not seen passing through the pith or bark. By measuring the conductivity of air as pressure was increased we could estimate the relative abundance of vessels whose bubble pressures had been reached. We plotted the relationship between applied air pressure and air conductivity in permeability curves.

In practice, a hydrated stem segment 40 cm long was inserted a few centimeters into a pressure bomb and both ends shaved with a sharp razor blade. The other end was fitted to an inverted side-arm flask equipped with a vent so it could be filled with water from a supply reservoir. The side arm connected with a small drain reservoir on an electronic balance via water-filled tubing. The bomb pressure was increased at 0.34 MPa intervals and held at each level for 5 min until the first streams of air bubbles were seen entering the flask from vessels. This signified the minimum bubble pressure for the stem. Beginning at this pressure and continuing at each increment up to 4.5 MPa, the conductivity of air (L_a) was measured at each level according to the equation (14):

$$L_a = Q_a\,[P\,l/(\Delta P\,\bar{P})]^{0.791} \quad (2)$$

where Q_a is the mass flow rate of water (in kg s^{-1}) displaced by air entering the side-arm flask from the stem, l is the stem length (m), P is the pressure at which Q_a was measured, ΔP is the applied air pressure (bomb pressure), and $\bar{P}$ is the average air pressure in the stem (= sum of absolute pressures at both ends of the stem divided by two; all pressures in MPa). Air permeability curves were plotted as relative conductivity of air (L_a relative to maximum for the stem) *versus* applied air pressure (ΔP).

The value of the exponent in Eq. 2 depends on whether air flow through the stem is laminar, nonlinear, or turbulent (exponent = 1, 0.5, or 0.57, respectively; 14); this in turn depends on the vessel geometry and flow rate. The correct exponent results in L_a being constant for any applied pressure (ΔP) for a flow path of fixed dimensions. We determined the exponent experimentally by measuring flow rate *versus* applied pressure in air-dried maple stems of the same dimensions used in the above experiments and calculating the exponent that gave us a constant value for L_a. Dry stems represented a flow path of fixed dimensions regardless of applied pressure because all intervessel pits lacked an air-water interface and all vessels could conduct air from the lowest pressure. Thus, the relationship between flow rate and applied pressure was solely dependent on the nature of air flow through the vessels (*i.e.* laminar *versus* turbulent) rather than the number of vessels conducting air. This approach necessarily assumes that the type of air flow through segments is dependent on the geometry of individual vessels rather than how many are conducting air.

For each of six dry stems we measured Q_a at each pressure from 0.34 to 4.50 MPa as previously described for hydrated stems. In order to keep the stems dry, we measured Q_a by diverting the air exiting the stem through a plastic tube to an inverted graduated cylinder filled with water and timed its accumulation. Potential flow of air through the dry pith was diverted by notching the stem into the pith a few centimeters below the end of the stem outside the bomb. The upper side of the pith and notch (the side away from the bomb) was sealed with epoxy to prevent back-flow of air from the stem end. The exponent was determined as the slope of a log-log plot of Q_a and the inverse of the pressure term in brackets in Eq. 2; its value of 0.791 is the average of 6 experiments (SD = 0.0167). This indicates air flow in our material was transitional between laminar and turbulent.

Effect of Lowered Surface Tension. The surface tension (T) of the xylem sap is probably near that of water (T = 0.072 N m^{-1} at 25°C). We determined the effect of lowering it on the air permeability curve by perfusing stems with 9.5% (w/w) *n*-butanol (T = 0.027 N m^{-1}), or 2% (v/v) Tween 80 (T = 0.045 N m^{-1}) at about 0.175 MPa for 15 min immediately prior to generating the curve as described above. In order to determine if the

solutions caused permanent damage to the pit membrane, after perfusing some stems with Tween or butanol, we rinsed them with a 3.5 h perfusion of water and determined whether their permeability curves were different from nontreated controls. Surface tension of these solutions, and the oxalic-calcium solutions referred to below, were measured relative to pure water (tap water passed through 2 deionizing and organic removal cartridges each) by comparing bubble pressures of 0.22 μm membrane filters soaked either in water or in the test solution.

A field experiment was designed to evaluate if injecting trees with 9.5% *n*-butanol or 2% Tween 80 was associated with subsequent development of embolism. On a sunny afternoon in late August, watertight collars were fitted around the trunks of 4 small saplings (dbh = about 2 cm). Two of these collars were filled with pure water, one with 9.5% butanol, and one with 2% Tween 80. A notch about 0.5 cm deep was cut into one side of each stem with a razor blade taking care the cuts were made under the surface of the solution in the collar. The trees were allowed to take up solution for 20 min before the solution was removed and the notch exposed to air for 1 h. The trees were then defoliated, cut at the base and brought to the laboratory with the cut ends in water. The 40 cm main axis segment immediately above the wound was cut from each tree taking care all cuts were made underwater to prevent additional embolism. Safranan dye (0.1% w/v) was perfused through each segment by dipping one end in dye and applying a suction force of about 15 kPa at the other end. Embolism was identified by nonstaining sapwood. Embolism was confirmed by making longitudinal sections of the nonstaining wood and checking for air-filled vessels.

Effect of Oxalic Acid and Calcium. We serendipitously discovered that a combination of oxalic acid (2–20 mM) and calcium (0.1–1.0 mM; from either calcium chloride or calcium carbonate) in pure water perfused through stems caused a significant change in the air permeability curve toward higher conductivity of air at lower applied pressure relative to controls (Fig. 4). The steps leading to this discovery are outlined in the "Results" section; the experiments were done by perfusing stems with a variety of solutions (Table I) at 175 kPa for 15 min prior to the generation of a permeability curve. The effect of oxalic and calcium perfusion on embolism was determined from embolism vulnerability experiments described below.

Vulnerability to Embolism. Vulnerability curves described the relationship between percent loss in hydraulic conductivity of the xylem and Ψ_{px}. Prior to determining the vulnerability curve, stems were perfused for 30 to 45 min at 175 kPa with either oxalic acid (20 mM) and calcium chloride (0.1 mM) in pure water, or pure water alone. The objective was to see whether the increased permeability to air caused by the oxalic-calcium solution corresponded with increased vulnerability to embolism.

Trees in batches of 10 were cut at the base, set in water, and brought to the laboratory. Sections of main axes 60 cm long were cut from trees leaving all lateral branches in place; all cuts were made underwater to prevent additional embolism. Stems were perfused and then placed on the laboratory bench to dry out. After dehydration, Ψ_{px} was allowed to equilibrate overnight by wrapping stems in a plastic bag, a damp cotton bag, and another plastic bag in that order. The next day, Ψ_{px} for each stem was measured in lateral branches with the pressure bomb. The stem, minus branches clipped for the pressure measurement, was placed in a tub of water, and two central and contiguous 15 cm segments cut out, taking care cuts were made underwater. The central portion was used in order to avoid air-filled vessels at the cut ends of the original 60 cm branch.

One of the two central segments per stem was perfused with 0.1% (w/v) safranan dye so we could locate the nonstaining, embolized xylem (16, 17). The other segment was attached to a tubing apparatus designed to measure its hydraulic conductivity before and after the removal of any air emboli in the xylem. This methodology has been described in detail elsewhere (16, 17). The percent by which the initial conductivity of a segment was below its maximum measured after embolism reversal gave us the percent loss in conductivity for the segment. We generated embolism vulnerability curves by plotting this value *versus* Ψ_{px} for each stem.

Measurement of Pit Membrane Pore Diameter. Diameters were measured from SEM photographs. Longitudinal sections (about 150 μm thick) of maple stems were soaked in pure water, or 20 mM oxalic acid and 0.1 mM calcium chloride in pure water for 1 h and then rinsed in pure water. Sections were air- or critical-point dried and coated with carbon followed by gold-palladium. Intervessel pit membranes were photographed with the SEM and pores in the membranes were traced on a bit pad (Zeiss Zidas) programmed to calculate the equivalent circle diameter.

RESULTS

Figure 2 shows representative air permeability curves for stems perfused with pure water, 9.5% (w/w) *n*-butanol, and 2% (v/v) Tween 80. The pure water, or control, curve shows that the bubble pressure for most intervessel pit membranes is above 3 MPa (see also Fig. 4). In contrast, both butanol and Tween curves show greater permeability, *i.e.* higher conductivity of air at lower applied pressures. This is most extreme in the butanol curve where maximum air conductivity is reached at about 1.5 MPa indicating the bubble pressure for all intervessel membranes had been reached. In the case of the Tween solution, increased permeability was strictly due to surface tension rather than a change in pit membrane structure because a water rinse restored normal permeability. For the butanol perfusion, however, a rinse only partially reversed the permeability increase. Thus, unless the rinsing was insufficient, there may have been some permanent physical alteration of the pit membrane.

Figure 3 shows the results of injecting 9.5% *n*-butanol, 2% Tween 80, and pure water into saplings. Whereas the two water-injected controls show no embolism associated with the notch (Fig. 3, arrows), both butanol- and Tween-injected trees show extensive embolism above the notch. This is much more extensive than could be caused by the cutting of the notch itself; 90% of the vessels in stems this size are shorter than 15 cm, and

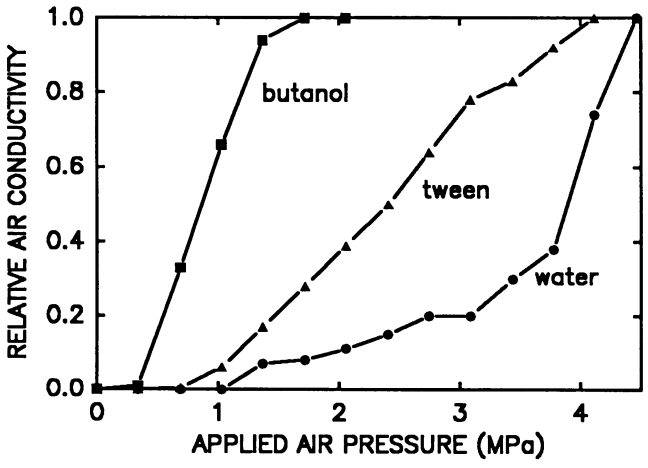

FIG. 2. Air permeability curves (see text) for a sugar maple stem perfused with: (a) pure water (surface tension, $T = 0.072$ N m^{-1}, 25°C), (b) 2% (v/v) Tween 80 ($T = 0.045$ N m^{-1}), and (c) 9.5% (w/w) *n*-butanol ($T = 0.027$ N m^{-1}). Decreasing the surface tension causes air permeability of the intervessel pit membranes to increase.

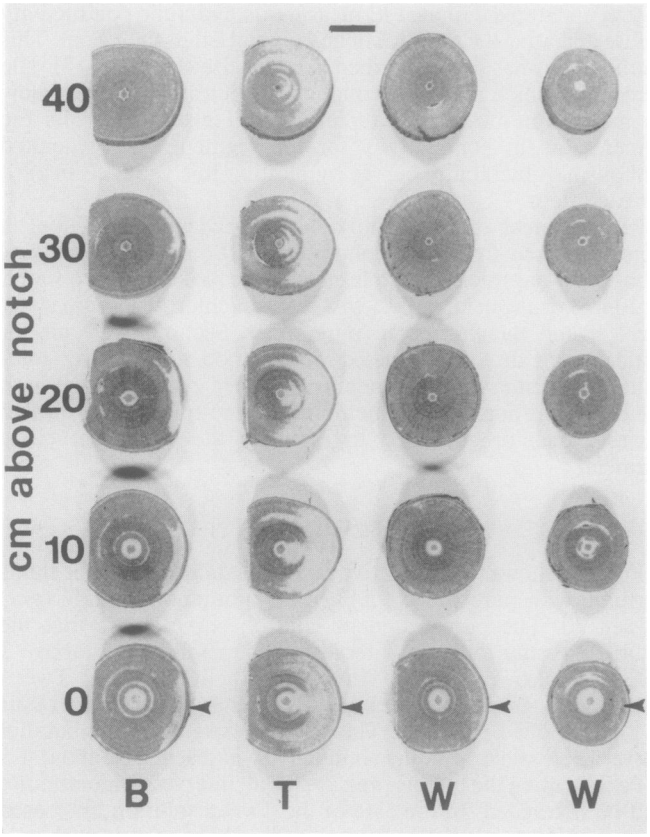

FIG. 3. Results of injecting pure water (*W*), 2% (v/v) Tween 80 (*T*), and 9.5% (w/w) *n*-butanol (*B*) into sugar maple saplings. Injection was made via a notch (at arrows). Subsequent dye perfusion showed the distribution of embolized (nonstained) xylem associated with the notch. Embolism above the notch occurred only in the Tween- and butanol-injected trees presumably because the low surface tension of these solutions caused air-seeding at intervessel pits. Scale bar is 1 cm.

nonstained xylem occurred well beyond 15 cm of the notch. Air-filled vessels observed in longitudinal sections of nonstained xylem made immediately after the dye perfusion confirmed that embolism was responsible for the lack of staining. The greater permeability of the butanol-perfused stem in Figure 2 suggested that we might see more embolism in butanol- than Tween-injected trees. Figure 3, however, indicates the contrary. Apparently the butanol did not penetrate as extensively into the xylem; possible explanations include a lower transpiration rate in this tree, and/or incompatibility between the butanol and the xylem sap.

Figure 4 shows the increased permeability caused by perfusion of a solution of oxalic acid (20 mM) and calcium chloride (0.1 mM) in pure water. We accidentally discovered this when we measured permeabilities of stems previously attached to our apparatus for measuring hydraulic conductivity. To inhibit microbial growth in the apparatus and stems we use a solution of 10 or 20 mM oxalic acid in tap water because of its low pH (<2; 16). To our surprise, this solution significantly increased air permeability of stems relative to controls not perfused at all, or perfused with either pure water, tap water, or 2 to 20 mM oxalic acid in pure water (Fig. 4; Table I). Thus, the increased permeability was due to interaction of oxalic acid with some chemical in tap water. Despite its effect on air permeability, the oxalic acid-tap water combination did not alter the hydraulic conductivity of stems previously measured using oxalic acid in pure water (data not shown).

Table I summarizes the results of the experiments from which

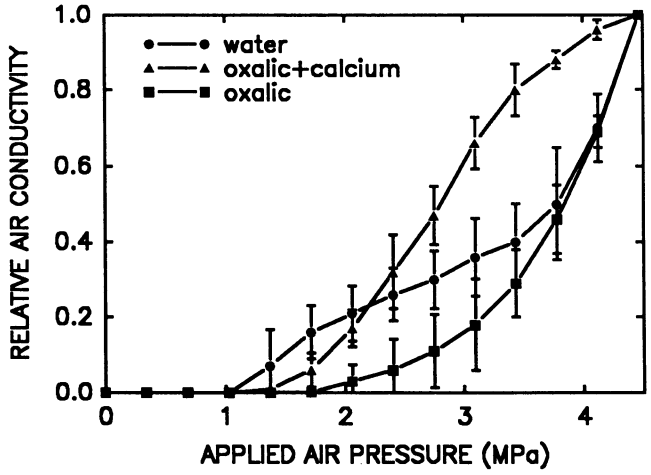

FIG. 4. Air permeability curves for sugar maple stems perfused with: (a) pure water, (b) 20 mM oxalic acid and 0.1 mM calcium chloride in pure water, (c) 20 mM oxalic acid in pure water. Curves are means for five replicates with 95% confidence intervals. Stems not perfused with any solution had the same curve as those perfused with pure water. Permeability is increased by the oxalic-calcium solution, and decreased by the oxalic acid solution relative to the water perfused stems.

Table I. *Solutions Tested for Causing Increased Air Permeability (+) in Sugar Maple Stems*

Stems were perfused with solution for 15 min at 175 kPa immediately (except for experiment 14) prior to making a permeability curve.

Experiment	Solution	Permeability
1	10 or 20 mM oxalic acid in tap water, pH 1.8–2.0	+
2	Tap water	−
Solutions in pure water:		
3	10 or 20 mM oxalic acid, pH 1.8–2.0	−
4	20 mM oxalic acid, 1 mM $CaCO_3$, pH 1.8–2.0	+
5	20 mM oxalic acid, 1 or 0.1 mM $CaCl_2$, pH 1.8–2.0	+
6	2 or 5 mM oxalic acid, 0.1 mM $CaCl_2$	+
7	0.1 mM oxalic acid, 0.1 mM $CaCl_2$	−
8	20 mM oxalic acid, 0.1 mM $CaCl_2$, pH 5[a]	+
9	1 mM $CaCO_3$	−
10	1 mM $CaCl_2$	−
11	1 mM $CaCl_2$, pH 1.88[a]	−
12	20 mM oxalic acid, 1 mM $CaCl_2$ followed by 1 mM $CaCl_2$	+
13	20 mM oxalic acid, 1 mM $CaCl_2$ followed by 20 mM oxalic	−
14	20 mM oxalic acid followed by 24 h wait	+
15	1 mM calcium oxalate, pH 1.91[a]	−
16	20 mM oxalic acid + 20 mM NaCl	−

[a] The pH of these solutions was adjusted with HCl and/or NaOH.

we conclude the following about the observed increased permeability: (a) it is caused by the combination of oxalic acid and calcium, nothing else we tried was successful (experiments 3–5, 9, 10, 15, and 16); (b) it requires a minimum oxalic concentration of somewhere between 0.1 and 2 mM for a calcium concentration of 0.1 mM (experiments 6 and 7); (c) it is not a function of pH (experiments 8 and 11); (d) it is reversible by subsequent perfusion of oxalic acid (20 mM) and pure water, but not by subsequent perfusion of calcium (1 mM) in pure water (experiments 12 and 13). Stems perfused with oxalic acid (20 mM) and pure water

that sat overnight also showed increased permeability (experiment 14). We conclude from this that endogenous calcium levels are sufficient to interact with the acid and create higher permeability.

There is some evidence that the oxalic-calcium effect may occur in nature. As noted above, oxalic acid in pure water reversed the increased permeability caused by the additional presence of calcium (Table I, experiment 13). Stems perfused with oxalic acid alone (Fig. 4, oxalic) were less permeable at many pressures than stems perfused with either pure water (Fig. 4, water), or not perfused at all (data not shown, but not significantly different from Fig. 4, water). This suggests that the oxalic acid reversed some naturally occurring enhancement of permeability.

Figure 5 shows that stems perfused with oxalic acid (20 mM) and calcium chloride (0.1 mM) were dramatically more vulnerable to embolism than those perfused with pure water. Oxalic-calcium stems were 95% embolized at Ψ_{px} of −2.0 MPa, pure water stems did not become this embolized until Ψ_{px} of −4.0 MPa. Dye perfusions showed that in both cases, the last vessels to embolize were in the latewood, or wood that was structurally similar but did not occupy the outer position in an annual ring.

For pure-water perfused stems there is good correspondence between the vulnerability curve and the permeability curve; the sharp increase in embolism at Ψ_{px} below −3 MPa (Fig. 5, water) reflects a sharp increase in air conductivity at applied pressures above 3 MPa (Figs. 2 and 4, water). This is the expected result if the bubble pressure of intervessel pits determines the Ψ_{px} at embolism as predicted by the air-seeding hypothesis. The correspondence is not as good for oxalic-calcium perfused stems. Although their greater permeability to air (Fig. 4, oxalic + calcium) corresponds to their greater vulnerability to embolism (Fig. 5, oxalic − calcium), they were much more vulnerable than predicted from the permeability curve. Whereas embolism increased to 100% over a Ψ_{px} range from −0.5 to −2.6 MPa, conductivity to air did not begin to increase until air pressures were greater than 1.7 MPa.

It was difficult to measure pit membrane pore sizes with the SEM because it was hard to find them exposed and yet undamaged by the sectioning knife. The best views were obtained when the knife sliced away one side of the vessel wall leaving the entire membrane exposed. In critical-point dried material, the membrane had a flat and featureless appearance, and pores were generally very difficult to resolve. Only when the membrane had been torn was its fibrillar nature obvious by its unraveling appearance (Fig. 6a). In air-dried sections the membrane was tight against the inner wall of the pit (aspirated) presumably because of surface tensions developed during dehydration. Pores could be easily seen and measured where the membrane spanned the pit opening (Fig. 6b). Pores are probably increased in size as the membrane stretches to become aspirated; this in itself may not represent an artifact, however, because it probably occurs when an embolized vessel is adjacent to a functional one in a transpiring tree. A more serious artifact may lie in the shrinkage of the individual cellulose fibrils by dehydration.

We saw no difference in the appearance or pore diameter of pit membranes between sections soaked in pure water or oxalic-calcium solution (all measurements were made on air-dried material). In both cases, most pores were much smaller than 0.05 μm diameter (*e.g.* Fig. 6b); this was true regardless of soaking solution. For the purposes of evaluating the air seeding hypothesis, we were most interested in the larger pores because they would be the first to seed air into functional vessels. Mean pore diameter for pores over 0.06 μm diameter was 0.13 ± 0.053 μm (4 membranes, n = 32 pores) for sections soaked in pure water, and 0.12 ± 0.065 μm (6 membranes, n = 58 pores) for sections soaked in oxalic-calcium solution; in both cases, the largest individual pore diameters were about 0.4 μm. Using the capillary

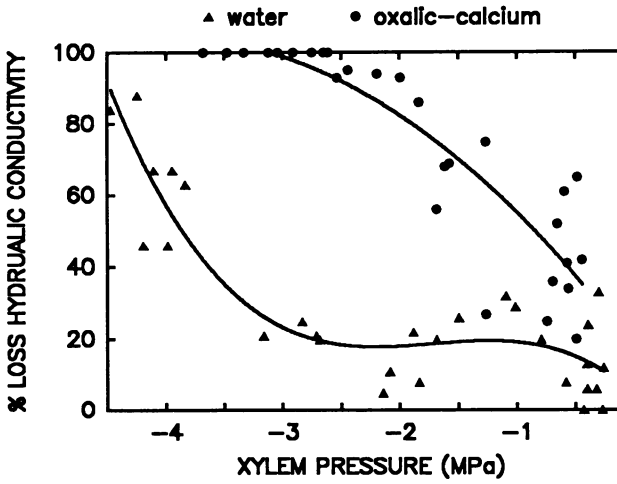

FIG. 5. Embolism vulnerability curves (see text) for sugar maple stems perfused with pure water, or 20 mM oxalic acid and 0.1 mM calcium chloride in pure water. The oxalic-calcium solution causes a dramatic increase in embolism vulnerability relative to the water-perfused controls. The fact that this solution also increased the air permeability of intervessel pits (Fig. 4) suggests that air seeding at these pits is causing water stress induced embolism.

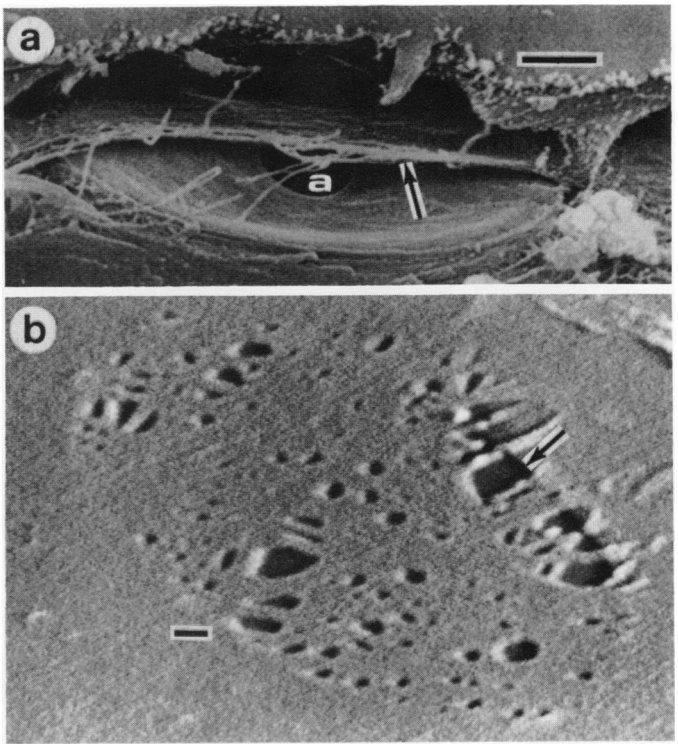

FIG. 6. a, Oblique cutaway view of an intervessel pit of sugar maple from a critical point-dried sample. The pit membrane (arrow) has been torn revealing its structure of cellulose microfibrils. The lower pit aperture (a) in the pit chamber wall opens to the lumen of the lower vessel; the upper pit aperture has been mostly cut away. Scale bar is 1 μm. b, Closeup view of pit membrane from an air-dried sample. The membrane is appressed against the pit chamber wall, and pores are evident where it spans the pit aperture; the aperture border (arrow) is evident behind what appears to be a damaged area of the membrane. Pores in the intact area are approximately 0.1 μm in diameter or smaller (scale bar is 0.1 μm). This is within the diameter range predicted by the air-seeding hypothesis.

equation (Eq. 1), these diameters correspond to a minimum bubble pressure of 1.2 MPa (largest individual pores), and an average minimum of about 2.2 MPa (mean diameter of pores > 0.6 μm) for both treatments. These estimates are consistent with the bubble pressures measured in permeability experiments for both treatments (Figs. 2 and 4, water, oxalic + calcium). Minimum bubble pressures were between 1 and 1.4 MPa, and the calculated average minimum bubble pressure (2.2 MPa) corresponds to an air conductivity roughly 21% of maximum for both treatments (Fig. 4).

DISCUSSION

The mechanism by which oxalic acid and calcium solution induced greater air permeability (Fig. 4) and greater embolism vulnerability (Fig. 5) in sugar maple xylem is unknown. The solution did not cause a permanent change in pit membrane structure because the use of oxalic acid alone reversed the effect (Table 1, experiment 13). The surface tension of the solution was found to be no different from water alone, which suggests that it may have increased pore diameters in the pit membrane. However, SEM studies gave no evidence of this; membranes looked the same regardless of whether they had been soaked in pure water or oxalic-calcium solution. This is consistent with our observation that the solution did not alter hydraulic conductivity which is to some degree a function of pit membrane structure. The oxalic-calcium effect seems to be a general phenomenon, because preliminary experiments indicate it occurs in balsam fir (*Abies balsamea*) as well as sugar maple.

The mechanism probably involves the disruption of calcium-mediated cross-linkage in the pectins of the pit membrane's middle lamella region. This could make the membrane more flexible and elastic (5), and allow for transient pore widening as a pressure difference is applied across an air-water meniscus at the membrane. In the absence of any stress to the membrane, its structure would essentially be unchanged. Oxalate could disrupt calcium cross-linkage by calcium oxalate formation *in situ*, or in solution as calcium is pulled away from the membrane. The fact that a small amount of calcium is needed in combination with oxalic acid suggests that exchange of calcium between solution and membrane pectates is required for oxalate to react.

Results (see discussion of Fig. 4) imply that the oxalic-calcium effect may occur to some extent in nature. The tree must prevent oxalic acid concentrations from becoming too high (*i.e.* $\geq$2 mM), or else the increase in embolism vulnerability could be lethal. Sugar maples typically have field Ψ_{px}'s down to −2 MPa, and if a maple tree had a vulnerability equal to that shown for oxalic-calcium perfused xylem in Figure 5, it would have difficulty surviving. A study of organic acid concentrations in sugar maple during the early spring reports oxalic acid concentrations of 9 μM or less (11).

A vascular pathogen, however, could exploit the oxalic-calcium effect. Oxalic acid secreted by a pathogen in combination with endogenous calcium could cause extensive embolism and dieback that would facilitate further invasion. In fact, oxalic acid in millimolar concentrations is produced by vascular pathogens (*e.g. Fusarium* sp.; 7, 8) and one early study showed that injections of oxalic acid into noninfected controls duplicated many of the symptoms of infected plants (8). Vascular, or wilt, pathogens are known to substantially decrease the hydraulic conductivity of host xylem (6, 18). Although it has always been assumed that this is due to occlusion of vessels by the fungus or host, our experiments suggest the initial blockage is due to induction of embolism.

The following results support the conclusion that one mechanism of water stress-induced embolism is air-seeding at intervessel pit membranes. (a) Increasing the air permeability of intervessel pit membranes by either lowering surface tension of the sap (Fig. 2) or perfusing the oxalic acid-calcium solution (Fig. 4) increased the vulnerability of the xylem to embolism (Figs. 3 and 5). (b) There is close correspondence for water-perfused stems between permeability and vulnerability curves (Figs. 4 and 5, water) indicating that embolism occurs when the pressure difference across intervessel pits is equal to their bubble pressure. (c) Pit membrane pore diameters were within the range predicted to cause embolism via air seeding (Fig. 6b).

The discrepancy between permeability and vulnerability curves for oxalic-calcium perfused stems (Figs. 4 and 5, oxalic-calcium) may lie in the different timing of the two experiments. The permeability curves were made by holding the stems at each pressure for a maximum of 5 min; the dehydration of the stems used in the vulnerability experiments necessarily proceeded much more slowly (3–5 d for minimum Ψ_{px} to be obtained). If there is a substantial hysteresis in the displacement of the air-water meniscus at the pit membrane, the longer time frame of the dehydration experiments could result in more complete meniscus-displacement for a given pressure difference. In addition, the effect of the oxalic-calcium solution could increase with time.

The possibility of air-seeding at intervessel pit membranes has long been recognized (3), but only recently has there been any significant experimental results demonstrating its occurrence. With this evidence we have the first clear knowledge of a structural feature of the xylem that is causally related to drought-induced embolism. It has been widely reported that larger diameter vessels and tracheids tend to be most vulnerable to embolism (1, 2, 19–21). Although we have no quantitative data, this conclusion is supported by our results which show the generally smaller-diameter latewood vessels (or vessels in structurally similar zones of xylem) were the most resistant to embolism. If this is due to smaller pit membrane pores as our results suggest, there must be a developmental link between the diameter of the vessel and its pit membrane pores. Preliminary evidence for this is that when the minimum bubble pressure was reached for a stem, the bubbling vessels were arranged in arcs that subsequent dye perfusion showed to correspond to the earlywood vessels; thus, these generally larger diameter vessels also must have larger pit membrane pores.

The air-seeding hypothesis is further supported by recent results from a concurrent study showing that within the family Rhizophoraceae, the greater vulnerability of the xylem to embolism in a rainforest species as compared to a mangrove species can be explained by differences in the permeability of the intervessel pit membranes to air (JS Sperry, MT Tyree, unpublished observations). Thus, the pore size of the pit membrane seems to be an adaptive feature that reflects the physiological demands of a species' habitat. Representing as it does the Achille's heel of the xylem, the functional implications of pit membrane chemistry, structure, and development deserve more study.

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