Collapse of Water-Stress Emboli in the Tracheids of *Thuja occidentalis* L.[1]

Ann M. Lewis*, Virginia D. Harnden, and Melvin T. Tyree

Department of Forestry and Wildlife Management, University of Massachusetts at Amherst, Amherst, Massachusetts 01003–4210 (A.M.L., V.D.H.); and Aiken Forestry Science Laboratory, United States Department of Agriculture-Forest Service, 705 Spear Street, P.O. Box 968, Burlington, Vermont 05402 (M.T.T.)

We report the kinetics of embolus formation and collapse in the tracheids of *Thuja occidentalis* L. stem segments. Radial wood sections were trimmed to 4 mm long paralleling the tracheids by 1 mm wide and 0.1 mm thick. They were observed under a dissecting microscope at 128× while sections were dehydrated and rehydrated. During dehydration, cavitations resulted in the formation of emboli in tracheids, but we concluded that the cavitated tracheids did not immediately fill with air at atmospheric pressure. This conclusion was based on the time required for the emboli to collapse after the rewetting of the dehydrated segment. By hypothesis, the time for the emboli to collapse should be proportional to the amount of air in the emboli. The time for all the emboli to collapse was a linear function of the dehydration time for times up to 15 min. For dehydrations greater than 80 min, the time for collapse after rewetting was constant, and we concluded that the tracheids have saturated with air by 80 min of dehydration. The kinetics of embolus formation is discussed in terms of the air-seeding hypothesis for cavitation, and collapse is discussed in terms of the physics of gas dissolution and diffusion. Embolus formation and dissolution in intact herbaceous and woody plants should follow the same physical laws.

Water stress can induce xylem dysfunction through cavitation (Tyree and Sperry, 1989; Tyree and Ewers, 1991). The dysfunction caused by cavitation is a loss of hydraulic conductance of xylem when fluid in xylem conduits (vessels or tracheids) is replaced by air bubbles (emboli). According to the air-seeding hypothesis (Zimmermann, 1983), a cavitation (the rapid replacement of water by gases in a xylem conduit) is seeded when an air bubble is sucked into a xylem conduit via a hole or pore from an adjacent air-filled region. The hypothesis assumes that the holes are usually the pores in pit membranes, and the adjacent air-filled region connected by the pore to the conduit lumen is an adjacent embolized conduit.

Lewis (1988) confirmed that air seeding can happen in plant material by direct microscopic observation of cavitation events in hyalocysts of *Sphagnum* spp. Other experiments, the results of which support the air-seeding hypothesis, involved blowing compressed air down the axes of excised stem segments and showing that the pressure required to make air pass through stem segments is of the same magnitude as the Ψ_x required to cause embolism or the loss of hydraulic conductance by cavitation (Crombie et al., 1985; Lewis, 1987; Sperry and Tyree, 1988, 1990). Cochard et al. (1992) forced air radially into stem segments while simultaneously passing water through the vessels at positive pressure and showed that this method produced the same reduced conductivity as tension-induced embolism. These results were consistent with the air-seeding hypothesis, which predicts the possibility of embolism induction without tension provided a pressure difference across the air-water interface is sufficient to push air into vessels. These results were inconsistent with other mechanisms (e.g. seeding at hydrophobic cracks and de novo cavitation) because they predict cavitation only under tension.

Among the questions that remain unanswered is, How much air is sucked into a conduit when a cavitation seeds? This is important because the amount and type of gas that is in the lumen should determine the time required for collapse of the embolus. If the embolus is filled only with water vapor, it should collapse rapidly once the water pressure rises to more than the partial pressure of water vapor in the embolus (about 2.3 kPa at 20°C). But an embolism filled initially with water vapor would eventually fill with air because Henry's Law would predict eventual equilibrium of gases in the embolus with air in solution in surrounding tissue, which ought to be near saturation. Once air has entered an embolus, the rate at which it can be dissolved will depend on the pressure of the fluid around the embolus, the rate of diffusion of air away from the embolus, and the amount of air to be dissolved. In many cases the rate of air dissolution will be limited by the rate of diffusion of air away from the embolus (see below).

Understanding the processes of air entry and exit from emboli is essential to a better understanding of the impact of drought-induced xylem dysfunction in the ecophysiology of

Abbreviations: D, diameter of tracheid lumen or of embolus (m); D_a and D_w, diffusion coefficient of air in pure water and wood, respectively ($m^2 s^{-1}$); D_s, diameter of a stem (m); P_a, absolute partial pressure of air in an embolus (Pa, 0 = perfect vacuum); P_x, absolute pressure of water in xylem (Pa); Ψ_x, xylem pressure potential (Pa, 0 = atmospheric pressure); τ, surface tension of water in air (Pa m^{-1}).

plants. Loss of hydraulic conductance in plants can decrease water supply to leaves, increase water stress in shoots, and reduce turgor pressure in growing tissue, which can reduce net carbon assimilation and growth (Hsiao et al., 1976; Dixon et al., 1988)

We hypothesized that the amount of air sucked in initially could be estimated by how long it takes emboli to collapse after rewetting a dehydrated sample of wood. This follows because the rate at which emboli collapse should be limited by the rate of diffusion of dissolved air away from the emboli. So the time required for emboli to collapse should depend on the amount of air (i.e. the partial pressure of air) in the emboli.

Emboli in water are inherently unstable because under most conditions the emboli will dissolve. This happens because surface tension places the air in an embolus at higher-than-atmospheric pressure (Epstein and Plesset, 1950). If P_a is the absolute partial pressure of air in the embolus and P_x is the absolute pressure of the fluid outside the embolus, then the capillary equation predicts that

$$P_a = 4\tau/D + P_x \tag{1}$$

where D is the diameter of the embolus and τ is the surface tension of water (= 0.072 Pa m^{-1} at 25°C). In the experiments in this paper, P_x is assumed to be atmospheric pressure (101.3 kPa) during embolus-collapse experiments, and the embolus is confined to a tracheid of $D = 15$ μm. Equation 1 predicts that $P_a \cong 121$ kPa. The embolus is unstable because Henry's Law predicts that the equilibrium concentration of air in water is proportional to the partial pressure of air next to the air-water interface. In a dynamic situation where the embolus is dissolving, Henry's Law applies to equilibration only at the air-water interface. Since the embolus air partial pressure is more than the atmospheric air partial pressure at the atmospheric interface ($P_a > P_x$), there will be a corresponding difference in the concentration of air (mainly O_2 and N_2) between the two interfaces. Thus, according to Fick's Law, there will be a continuous diffusion of air from the embolus through the surrounding liquid to the atmosphere causing the gradual collapse of the embolus.

The physics of embolus collapse has been fully described by Epstein and Plesset (1950) for physical systems, by Pickard (1989) for an isolated embolus in a plant, and by others for a large number of emboli in a woody stem (Tyree and Yang, 1992; Yang and Tyree, 1992). Pickard's (1989) analysis predicted that an isolated embolus will collapse in about 10^3 s or 17 min ($D = 15$ μm, $P_x = 101.3$ kPa, at 25°C), but no direct experimental observations of emboli collapsing were made. Yang and Tyree (1992) used iterative computer models to predict how long it would take all emboli in a fully embolized maple stem to collapse. Tyree and Yang (1992) also performed experiments to test the model, but embolus collapse was never monitored directly; embolus collapse was deduced indirectly from changes in hydraulic conductance and water content of stem segments. Lewis (1987) and Sperry (1986) directly observed embolus formation and collapse in monocots and dicots, and Lewis (1988) documented the development and collapse of emboli in the hyalocysts of the bryophyte Sphagnum spp. None of these studies rigorously linked direct observation with theory. In this study we offer more evidence in support of the air-seeding hypothesis and confirm through direct, microscopic observations of the collapse of emboli that emboli do collapse as described by Henry's and Fick's Laws.

MATERIALS AND METHODS

Dehydration-rehydration experiments were performed in the laboratory on thin sections of wood. The formation and collapse of emboli were observed in the thin sections using a microscope.

Stem segments (5–15 mm diameter) were cut from 4- to 6-year-old apical shoots of Thuja occidentalis L. saplings grown in a greenhouse. T. occidentalis was selected because of its relatively simple anatomy. The stems had few parenchyma, were free of resin ducts, and had short, fairly straight tracheids of the following dimensions: length = 1100 μm, diameter = 20 μm, lumen diameter = 15 ± 0.2 μm, and lumen area as fraction of total cross-section = 0.56 ± 0.04. After cutting segments, foliage was removed and the segments were submerged in water and hydrated overnight. After hydration, the segments were trimmed to a length of 1.5 to 2.5 cm. They were sectioned with a microtome into radial sections 106 ± 8 μm thick. After sectioning, the samples were trimmed with a razor blade to 1 mm wide and 4 mm long. Care was taken to keep the tissue flooded during processing to ensure full hydration, and the samples were sectioned parallel to the tracheids to ensure the maximum number of complete, uncut tracheids.

Each of the 79 observations was conducted on a separate tissue section as follows. A fully hydrated section was placed on a microscope slide. The drying process was initiated by removing excess water with a laboratory wipe. The section was covered with a weighted 1-mm mesh screen to reduce tissue movement during dehydration. The dehydration-rehydration process was viewed under a light microscope at 128X. A clear viewing area was selected between rays in the middle of the sample. The onset of dehydration was recognized by the abrupt change from a translucent to an opaque appearance of individual tracheids within the sample as emboli developed (Fig. 1). Time zero for the dehydration measurement was taken as the time at which the first embolus was observed in the field of view (typically 1 min after water was removed). Once embolism began, all tracheids developed emboli (cavitated) within 5 min. Dehydration time ended at rewetting.

At an arbitrarily predetermined dehydration time, ranging from 0.5 to 120 min after the first tracheids cavitated within the field of view, the tissue was flooded with water. Ideally, the shortest dehydration time should be 0 min, but the soonest that rewetting could be done reliably was about 0.5 min after the first embolus developed. Water was added as needed to replace water lost by evaporation. The rehydration time of each sample was defined as the time between rewetting and the collapse of the last embolus observable in the field of view (Fig. 2). Room temperature ranged from 25 to 30°C, and ambient RH ranged from 21 to 65%. We did not measure sample temperature, but it was presumed to be a little cooler than air temperature due to evaporative cooling
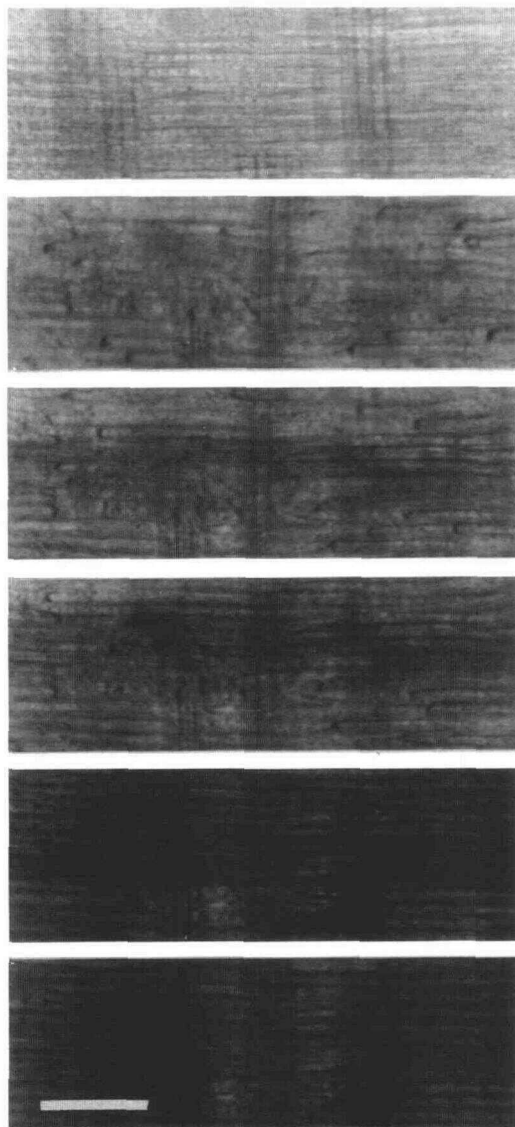
Figure 1. Development of emboli. This series shows a tissue sample as it progresses from full hydration, through the development of emboli, to complete dehydration. The tissue becomes more opaque as the water content decreases because water-filled tracheids are translucent but become opaque when air seeded. As the walls lose water, they also become more opaque. This sample was photographed through the microscope with its upper surface exposed to the atmosphere, in the same manner as the experimental samples. Individual tracheids are approximately twice as long as the frame is wide. Dehydration from the first to the last frame required about 5 min. Scale = 0.2 mm.

from the water surface. To reduce tissue warming, the microscope light was on only briefly during observations.

THE MODEL

The rate of embolus collapse is determined by the rate of diffusion of air in a given geometry and by the concentration of air in water at the boundary of the embolus, which is determined by the capillary equation and Henry's Law. We

calculated the time for collapse by iterative calculations using a grid size of 20 μm centered along the axis of the tracheid lumens. The method of computation and the defining equations for Fick's Law have been presented before (e.g. Tyree and Yianoulis, 1980; Yang and Tyree, 1992), so the derivation will not be given here. The program used to compute collapse times will be provided to anyone wishing to write to M.T. Tyree.

The diffusion problem for embolus collapse is more complicated than for simple diffusion in that there is a moving boundary, i.e. the air-water interfaces of the emboli move as the emboli contract. This moving-boundary problem has been solved for cylindrical geometry by Yang and Tyree (1992); the solutions in this paper differ only in that (a) we calculate diffusion for rectangular geometry and (b) we calculate diffusion only in one direction from the rectangle through an overlying layer of water.

Figure 3 illustrates the geometry and conditions used in many of the solutions. Figure 3A shows the edge-view of a five-tracheid-thick section of wood that is 100 μm thick. Note that the x and y axes are not drawn to the same scale. In many solutions, the embolized tracheids are assumed to be filled initially with air at 101 kPa partial pressure. Figure 3B illustrates what happens to the emboli immediately after flooding with a layer of water. The bubbles immediately collapse under the forces of surface tension to emboli at 121 kPa; according to the Ideal Gas Law, the emboli must contract to about 83% of their initial volume. We measured the thickness of the water layer held in place by the overlying screen to be 280 ± 43 μm (SE, $n = 5$). Figure 3C illustrates the state of the bubbles about halfway through the collapse (dissolution). The overlying layers of emboli prevent dissolution and diffusion of air from emboli directly underneath them, so the upper layer contracts fastest. The lowest layer is the last to disappear.

We used a geometry with a high degree of symmetry to reduce the complexity of the equations and the amount of computer memory required for the iterative solution. Tracheids are taken as perfectly aligned, as shown in Figure 3, and the emboli are assumed to be fixed at their centers and are assumed to contract equally from both sides as air dissolves. In reality, tracheids are staggered, and bubbles may contract unequally from the sides, making a more random distribution of emboli. When bubbles are spread out randomly, they might dissolve faster depending on the geometry. Air will dissolve fastest in plane geometry when they are spread out evenly over a plane perpendicular to the direction of diffusion. Solutions (not shown) for diffusion from a uniform plane predicted dissolution times about 10% shorter than in the symmetrical case shown in Figure 3A.

We started some computations with an initial partial pressure of air <101 kPa, which represented tracheids not fully embolized in Figure 3A. This affected the initial state immediately after adding water (Fig. 3B). If the initial partial pressure were 50.5 kPa (50% fully embolized), then the capillary forces would reduce the emboli to smaller initial volumes at 121 kPa. In this case, the Ideal Gas Law predicts smaller initial emboli than shown in Figure 3B; the emboli would fill only about 42% of the volume of the tracheids after flooding.
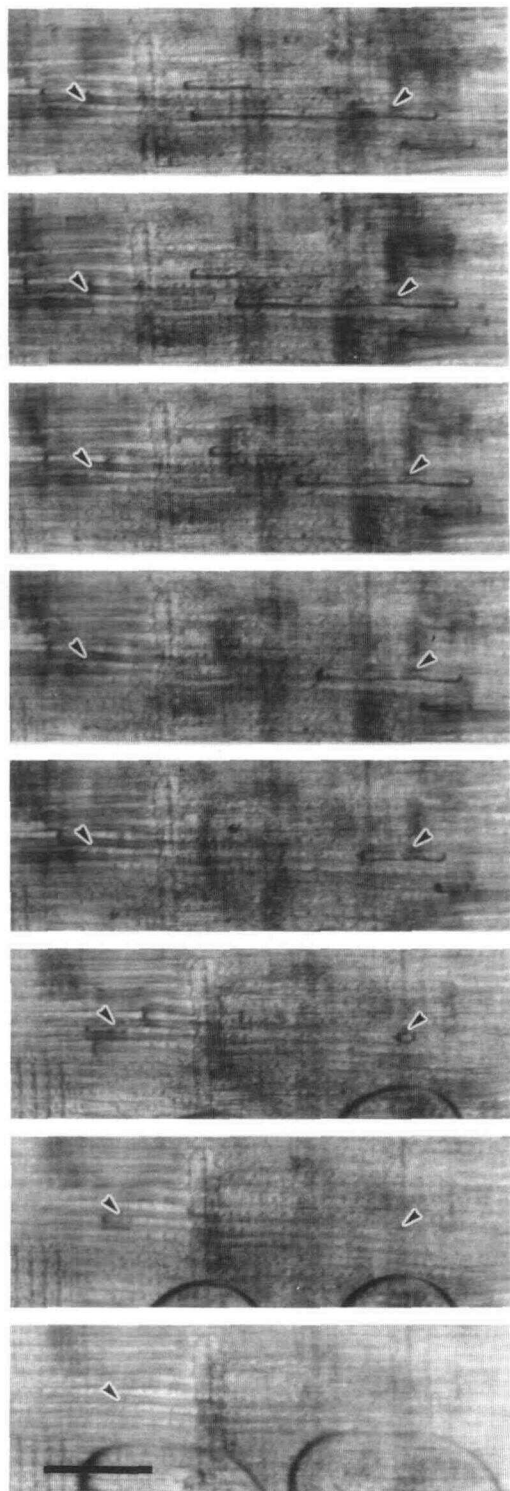
Figure 2. Collapse of emboli. This series shows a tissue sample as it rehydrates. It was rewetted with water after dehydration. The sequence begins after rewetting. Emboli (two are indicated with arrows) shrink and collapse through the series as time progresses. As the water content increases, the tissue becomes more translucent. The tissue is covered with a coverslip in this figure to improve visibility of the emboli during photographic reproduction. The sequence is typical of experimental dehydrations (without cover-
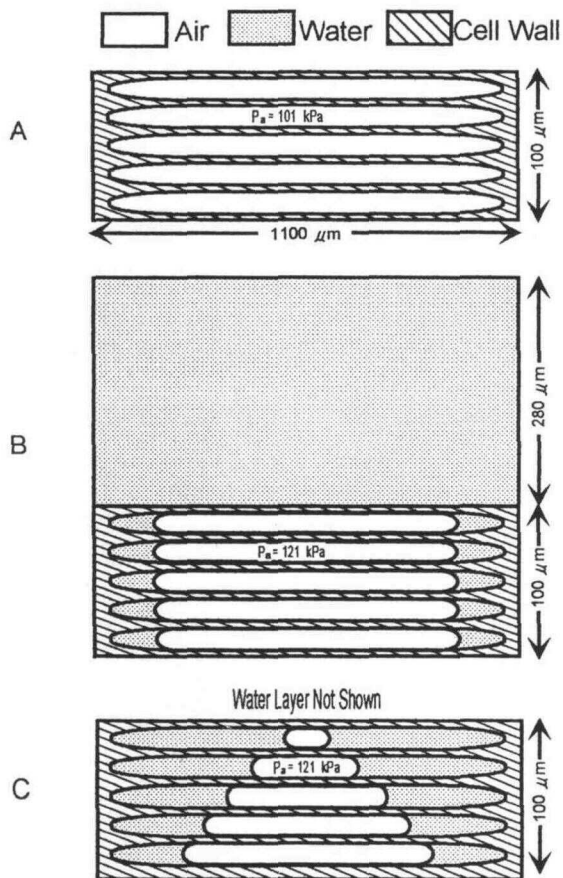
Figure 3. Placement of tracheids in the theoretical model. The diagram (not to scale) shows the symmetrical model of the placement of tracheids used in the theoretical calculation of embolus collapse. This is an edge view of a radial section about 100 μm thick (y axis), 4 mm long (x axis), and 1 mm deep (axis perpendicular to the drawing). The reader is to imagine additional rows of tracheids to the right and left. A, Air-filled tracheids at atmospheric pressure. B, Air emboli collapsed by surface tension immediately after flooding with a 280-μm layer of water. C, State of emboli partway though the collapse. See text for more details.

All collapse calculations used diffusion and solubility parameters reported in Tyree and Yang (1992). At 25°C the following values were used: diffusion coefficient of air = 1.95 $\times$ 10^{-9} and 0.942 $\times$ 10^{-9} m^2 s^{-1} in the water layer (D_a) and in the wood (D_w), respectively; solubility coefficient from Henry's Law = 7.75 $\times$ 10^{-6} mol m^{-3} Pa^{-1}.

RESULTS

Experimental Observations

The rehydration time (= the time from rewetting until the last embolus collapsed in the field of view) was a function of

slips) of 5 min, where all tracheids have embolized just before rewetting. A typical rehydration sequence of this type would span about 20 min. Scale = 0.2 mm.
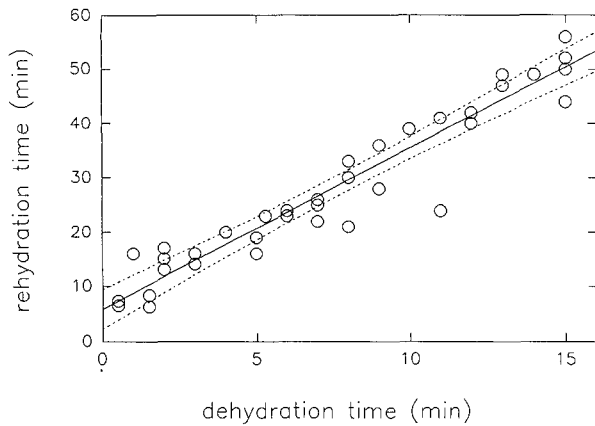
Figure 4. Linear relationship of rehydration time versus dehydration time for dehydrations up to 15 min (900 s). The linear regression line is straight and the 99% confidence interval is curved.

the time of dehydration (= the time from observation of formation of the first emboli until rewetting) when we dehydrated then rehydrated longitudinal tissue sections of *T. occidentalis* stemwood. Upon rewetting, the emboli abruptly shrank to smaller volumes, as would be expected from the Ideal Gas Law and the capillary equation, then the embolus volumes gradually shrank to zero (Fig. 2). The rehydration time was an approximately linear function of the dehydration time for dehydrations of 0.5 to 15 min (Fig. 4). To estimate the rehydration time of emboli immediately after their formation, the data were fitted by a linear regression; the straight line in Figure 4 is the regression line and the curved lines are the 99% confidence intervals. The y intercept is 6 min for an embolus to collapse when rewetted immediately after its appearance. For dehydration times from 15 to 80 min, the rehydration time asymptotically approached a maximum value of 70 min for collapse of the last embolus in the field of view (Fig. 5).
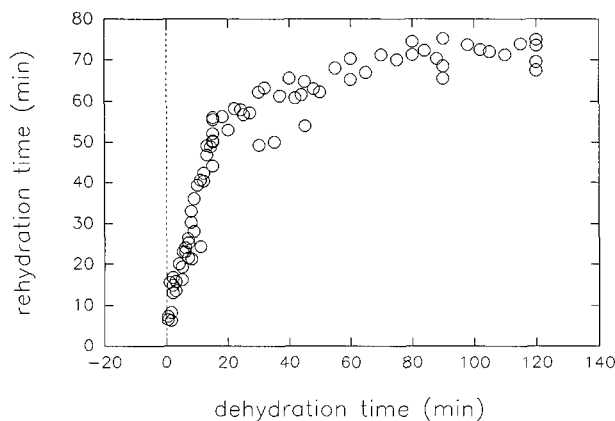
Figure 5. The relationship between rehydration time and dehydration time for excised tissue.

Diffusion Model Predictions

Theoretical time for embolus collapse was calculated for wood samples five tracheids thick, i.e. 100 μm thick in comparison with the actual mean thickness of 106 μm of the experimental samples. This approximation was needed because the finite elements in the computer simulation were cubes with sides of 20 μm (= average tracheid diameter). We also computed collapse times for samples four tracheids thick (80 μm) because the time for collapse should be approximately proportional to the total mass of air contained in emboli. With maximum-length dehydrations the estimated number of intact embolized tracheids in each sample is 250, but the actual number of air-filled tracheids was not known. During sectioning some tracheids on the upper and lower surfaces may be cut open parallel to the longitudinal axis. Upon flooding of the samples, the bubbles in the tracheids cut along the upper surface may have been dislodged. Thus, in the worst case, the samples may contain as little as four layers of embolized tracheids if all upper tracheids were cut open and none of the lowermost tracheids were cut open. In a visual check of transverse sections, the samples typically had four or five layers of tracheids.

The theoretical time for embolus collapse is shown in Table I for temperatures of 25 and 30°C. For the calculations at 30°C, we assumed that the diffusion coefficients were about 13% higher and that the Henry's Law solubility coefficient for air in water was about 3% lower. The theoretical times ranged from 66 to 90 min for computations in which the tracheids were assumed to contain air at atmospheric pressure (101 kPa) just prior to flooding. Figure 6 is a plot of the theoretical time for collapse versus the initial partial pressure of air in the tracheids at the time water was added. These calculations demonstrate that the theoretical time for collapse is a function of the mass of air initially present.

DISCUSSION

Regardless of the mechanism of formation, there are three possibilities for the composition of emboli upon formation: (a) emboli are 100% air or some other gas or mixture of nonaqueous gases; (b) emboli are 100% water vapor; and (c) emboli are some combination of water vapor and other gases.
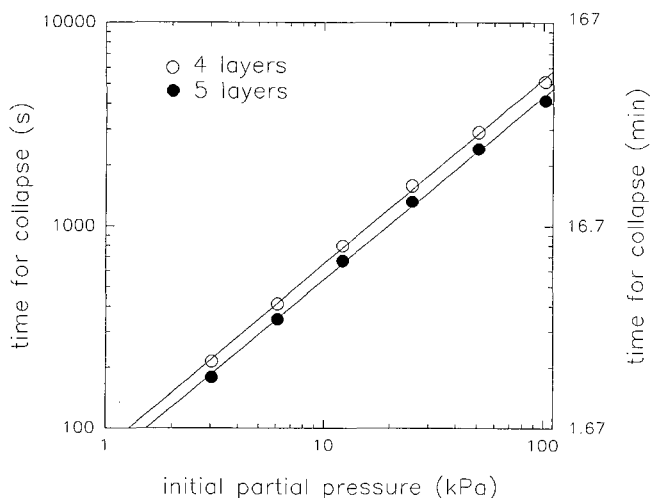
Table I. *Theoretical times for embolus collapse*

The following parameters were used: finite element grid size = tracheid diameter = 20 μm; tracheid lumen diameter = 15 μm; fraction of wood cross-section occupied by tracheid lumina = 0.56; tracheid length = 1100 μm; overlying water layer thickness = 280 μm. N, Number of layers of tracheids filled with air. T, Temperature. The theoretical time for embolus collapse decreases about 2% per °C increase in temperature. The reader may thus estimate times for other temperatures using the 2% factor.

T	N	Collapse Time
°C		s (min)
25	5	5379 (90)
25	4	4346 (72)
30	5	4898 (82)
30	4	3959 (66)

In the case where emboli are 100% air upon formation, the rate of embolus collapse will not depend on the dehydration time, and the curve of rehydration time versus dehydration time should be a line with slope = 0 and a positive y intercept. The bubble is at saturation and longer dehydrations will not increase the amount of air nor will it increase the rehydration time. In the case where the gas within the embolus is 100% water vapor, the curve should have a positive y intercept near zero and positive slope until the dehydration time is long enough to allow air to diffuse into the embolus and reach saturation. At that dehydration time, the curve should flatten to a slope of zero. When there is some air within the embolus at formation, the curve should be intermediate between the previous two cases. The portion of the curve with positive slope should be shorter because less time is required for the air diffusing into the embolus to reach saturation. Since the time for embolus collapse in our experiments is a function of the time of dehydration (Figs. 4 and 5), emboli must be composed partly of water vapor when initially formed.

We assume that the initial instantaneous shrinking of emboli upon rewetting is a combination of water vapor condensation and volume reduction of embolus air following Boyle's Law as the partial pressure of embolus air increases. The y intercept in Figure 4 indicates that 6 min were required for emboli to collapse at time zero, i.e. the instant when emboli first appeared in the field of view. The y intercept might be the time required to collapse the air drawn in as the initial air seed. A collapse time of 6 min corresponds to an initial average partial pressure of 6 kPa (5% air; see the theoretical data in Fig. 6). The calculations in Figure 6 were based on the assumption that all tracheids were embolized and contain 6 kPa at time zero, even though we cannot conclude that all tracheids contain 6 kPa of air immediately after air seeding. Other calculations (not shown) revealed that if only one-fourth of the tracheids were embolized initially and each contained air at 24 kPa pressure, then approximately the same amount of time would be needed for collapse.

The theoretical time for embolus collapse in fully embolized tracheids (66–90 min) was close to the observed value (70 min). This agreement lends support to the correctness of the underlying assumptions in the model. The model is based on the following assumptions: (a) the rate of embolus dissolution is controlled by diffusion according to Fick's Law; (b) the equilibrium concentration of gasses in water is determined by Henry's Law at all air-water interfaces; (c) the equilibrium solubility concentrations are determined only by the partial pressure of the gases in the gas phase and not directly by the hydrostatic pressure of the liquid phases; and (d) the gas pressure of a tracheid embolus is determined by the surface tension of water (as given by the capillary equation). One of the biggest uncertainties in the model is the value to use for D_w. The average D_a of oxygen and nitrogen is 1.95×10^{-9} m^2 s^{-1} (Tyree and Yang, 1992), but, to our knowledge, D_w never has been measured in wood. The value used here is the value estimated by Tyree and Yang (1992) for maple wood; they assumed that D_w would be less than the value in pure water because of the volume fraction of solids in wood. They estimated D_w from the Bruggeman equation that relates diffusion coefficients in media consisting of a mixture of solids and water. More than half of the rate limitation for collapse of emboli was due to the resistance to diffusion of the overlying layer of water (280 μm thick), but the theoretical time for collapse is partly influenced by the value of D_w; for instance, if D_w is given a value equal to that of pure water, then the computed time for collapse is reduced by about 38% of the values in Table I, giving a range of times of 42 to 57 min.

The experiments described in this paper were for an artificial situation, i.e. the behavior of emboli in excised tissue under controlled laboratory conditions with an overlying layer of water. This type of experiment is important to confirm the existing theory of the physics controlling embolus collapse. Although emboli in intact plants should follow the same physical laws, there are important differences between our experimental conditions and the conditions that may occur in real plants. In more realistic situations there may be a mixture of embolized xylem elements interspersed with nonembolized cells. The geometry of diffusion will more commonly be cylindrical for embolus collapse in stems and will include the bark as a barrier to diffusion. The fluid pressure in the xylem, P_x, may not be atmospheric (hydrostatic forces will usually result in subatmospheric fluid pressures). Generally, there will not be an overlying layer of pure water beyond the stem or a completely impermeable barrier to one side (here, the glass microscope slide).

Yang and Tyree (1992) reported the equation for the more general case for the time of embolus collapse in a fully embolized stem of diameter D_s. Due to typesetting errors the equation was reproduced incorrectly in the original publication. The time for all emboli to collapse, t measured in s, is given by:

$$t = \alpha \, (1.95 \times 10^5/D_w) \, (r_{cs} + 0.046)$$

$$\cdot \, [9.71 \times 10^{-6} + 1/(\Psi_x + 4\tau/D)] \, D_s^{1.86} \quad (2)$$

where r_{cs} is the volume fraction of wood initially embolized at a partial pressure of 101 kPa and α is a temperature

Figure 6. Theoretical relationship between time for total collapse of emboli and the partial pressure of air in the emboli at the time water is added.

coefficient, which is equal to 1 at 25°C. Equation 2 is valid only when $\Psi_x > -4\tau/D$. The model predicts no collapse of emboli when $\Psi_x < -4\tau/D$. For *T. occidentalis*, the critical pressure for collapse is $\Psi_x = -20$ kPa. It is not clear how often a *T. occidentalis* branch might be at such a high Ψ_x. Even if a tree were growing in soil at 0 kPa water potential, gravitational effects would make $\Psi_x < -20$ kPa for any branch more than 2 m above ground. In some species, Ψ_x values between 100 and 300 kPa were observed because of root pressure (Ewers and Tyree, 1994). Tyree and Yang (1992) showed that Ψ_x values < -6 kPa prevented emboli collapse in *Acer saccharum*, but Pickard (1989) suggested that rapid changes in shoot temperature between day and night might be sufficient to increase Ψ_x enough to collapse emboli in intact trees. Experiments have yet to be done to confirm this hypothesis.

Our model of the physics of bubble collapse received strong circumstantial support from the accuracy with which it predicted the observed time for bubbles to disappear from the field of view in wood sections. However, the agreement might be a lucky coincidence. Therefore, it is appropriate to examine evidence for alternative mechanisms for bubble collapse. Several theories of refilling have been investigated by Borghetti et al. (1991), who concluded that there is no evidence for active secretions by ray parenchyma (the so-called "vital theory" of refilling), and that temperature changes could not induce sufficient condensation to account for the quantities of water involved in their limited experimental conditions. Borghetti et al. suggested that tracheids may be chemically active and that this, in combination with surface tension forces exerted by the water menisci at the ends of the embolized tracheids, may be sufficient to induce redissolution of gases when water potential rises above the value at which cavitation is caused. The nature of the "chemical activity" of the tracheid walls was never defined in physical terms, making these suggestions unconvincing.

In an important subsequent study, Edwards et al. (1994) designed experiments to investigate the relationship between water content and stem hydraulic conductance and looked specifically for unexplained cases of bubble collapse under negative pressure. Balances were used to measure water flow into and out of *Pinus sylvestris* stem segments under a pressure difference of 20 kPa while maintaining negative pressures down to −40 kPa. Experiments were conducted on stem segments 100 to 200 mm long, 5 to 25 mm in diameter. Evaporation from the stem surfaces was prevented by placing the segments in tight-fitting, polyethylene tube sleeves. Water flow through the segments gave a measure of hydraulic conductance and accumulative differences in flow in versus flow out gave the net water uptake, which was equated with the volume of bubble displaced. These experiments were very similar to those of Tyree and Yang (1992) except that greater care was taken to prevent evaporation from the stem segments. Bubble collapse was observed with and without net flow through the segments and with and without de-aeration of perfusate solution.

Tyree and Yang (1992) argued that bubble collapse should occur as long as $\Psi_x > -4\tau/D$ and demonstrated that bubble collapse ceased in maple when Ψ_x was more negative than $-4\tau/D$. In *P. sylvestris* the tracheid diameters were 20 to 30

μm, so collapse should cease when $\Psi_x < -10$ to -15 kPa. Although Edwards et al. (1994) observed bubble collapse at $\Psi_x = -40$ kPa, there is no reason to alter our understanding of the physics of bubble collapse because these observations were made when stems were perfused with de-aerated water. The authors correctly observed that Henry's Law would predict that the air content of emboli should fall to zero when stems are perfused with air-free water (and radial diffusion of air, which would re-aerate the water, is prevented). Under these conditions a pressure difference was established between the air-free emboli and the source reservoir of water on the balance that could support water to a height of 10 m, which is equivalent to a tension of about 100 kPa. When capillarity and pressure difference are taken into account, bubble collapse should not stop until Ψ_x falls below -110 to -115 kPa.

The results of Edwards et al. (1994) provide support for our existing hypotheses, but under artificial conditions not likely to be encountered in nature because water moving from the soil to stems of trees is likely to be nearly air saturated. Edwards et al. (1994) demonstrated that embolus collapse was much reduced when stems were perfused with aerated water at −40 kPa. There are other reports of partial bubble collapse under apparent tension (e.g. Salleo and LoGullo, 1989). These interesting experiments should be repeated to better define the biophysical conditions during the partial reversal of emboli. We tentatively presume that these reports may be explained by the mechanisms detailed by Pickard (1989).

In summary, our results support the air-seeding hypothesis, in that emboli formed by air seeding are initially composed largely of water vapor and a small percentage of air. The air concentration of the emboli increases until air saturation at atmospheric pressure plus the pressure due to the bubbles' surface tension. Upon rewetting, the emboli collapse in a manner consistent with air dissolution as described by Fick's Law and the equilibrium concentration of gases in water as described by Henry's Law.

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