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A method for measuring hydraulic conductivity and embolism in xylem

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Abstract. Hydraulic conductivity of the xylem is computed as the quotient of mass flow rate and pressure gradient. Measurements on excised plant stems can be difficult to interpret because of time-dependent reductions in flow rate, and because of variable degrees of embolism. Using *Acer saccharum* Marsh. stems, we found that certain perfusing solutions including dilute fixatives (e.g. 0.05% formaldehyde) and acids with pH below 3 (e.g. 10 mol m^{-3} oxalic) prevent long-term decline in conductivity. Xylem embolism can be quantified by expressing the initial conductivity as a percentage of the maximum obtained after flow-impeding air emboli have been removed by repeated high-pressure (175 kPa) flushes. Correlation between microbial contamination and declining conductivity suggests that long-term (>4 h) declines are caused by microbial growth within the vessels. Unpredictable trends in short-term (<4 h) measurements may be caused by movements of air emboli in vessels and/or particulate matter.

Key-words: xylem hydraulic conductivity; xylem cavitation; xylem embolism; sugar maple; *Acer saccharum*.

Introduction

Measurement of xylem hydraulic conductivity has formed the basis for studies on fluid mechanics of xylem conduits (e.g. Giordano, Salleo & Wanderlingh, 1978; Siau, 1984; Jeje, 1985; Calkin, Gibson & Nobel, 1986), distribution and origin of resistances and pressure gradients in the water transport system (e.g. Dimond, 1966; Fiscus, Parsons & Alberte, 1973; Zimmermann, 1978; Tyree *et al.*, 1983; Ewers, 1985), pathogen modes of action (e.g. Dimond, 1970; Suhayda & Goodman, 1981; Van Alfen *et al.*, 1983; Newbanks, Bosch & Zimmermann, 1983), and methods of wood preservation (e.g. Anderson, Gortner & Schmitz, 1941). Hydraulic conductivity is typically measured on excised xylem samples, i.e. either dowels split from the wood or lengths of the plant axis. These are attached to a hydraulic system for measuring the pressure difference (ΔP) of a fluid (usually water) across the sample and the mass flow rate ($\dot{m}$) through the sample. The pressure–flow relationship has been expressed in a variety of ways, the simplest being hydraulic conductivity (L), where $L = \dot{m}/\Delta P$. Conductivity can be compared between samples of various lengths (l) by substituting pressure gradient ($\Delta P/l$) for pressure difference; variation in cross-sectional area of samples can be factored out by expressing conductivity per unit area ('specific conductivity').

Despite the simplicity and utility of such measurements, results have been ambiguous for two reasons. The first is that conductivity of a sample often declines with time. This decline, which can begin within the first minutes of measurement (Zimmermann, 1978), has been observed for xylem of several species under a variety of experimental conditions. Explanations include blockage by air coming out of fluid within the sample (Kelso, Gertjejansen & Hossfeld, 1963); narrowing of vessel diameters due to swelling of surrounding tissue (Jeje, 1986); electro-osmosis (Buckman, Schmitz & Gortner, 1935); particulate clogging (Krier, 1951); and swelling of intervascular pit membranes (Zimmermann, 1978). The second and less obvious problem with conductivity measurements is the potential presence of naturally occurring air-filled tracheids or vessels. These 'emboli' arise from water stress induced cavitation in the transpiration stream, and they cause variable degrees of 'embolism', or blockage of flow (Sperry, 1986); winter freezing of xylem sap may also create emboli (Zimmermann & Brown, 1971). Unless this blockage is quantified, the interpretation of a conductivity measurement is limited.

In this report, we describe a method for measuring xylem conductivity that prevents long-term decline, and allows the initial conductivity of a plant axis to be expressed as a percentage of the maximum value obtained after the removal of embolism. As a means of studying xylem cavitation the method has the advantage of being simple, and informative of its most immediate physiological consequence: increased resistance to water flow. Although this method was developed primarily with sugar maple (*Acer saccharum* Marsh.), we have used it successfully with

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grapevines (Sperry, Holbrook & Tyree, 1987) and are currently applying it to other tree species including mangroves and conifers.

Measurement techniques

Plant material

Axis segments must be collected and prepared in such a way as to minimize the introduction of additional air bubbles into the xylem. For this reason, the initial segment cut from the plant must be long enough so that few if any vessels at the cut ends extend into a central portion reserved for the conductivity measurement. Vessel lengths were measured according to the methods of Zimmermann & Jeje (1981). For *A. saccharum* branches between 0.4 and 0.7 cm in diameter, we found that none of the vessels extended beyond 15 cm, so we chose this as our 'buffer' length on either side of a central conductivity portion that ranged between 10 and 15 cm. In the field, stem segments were immediately wrapped in plastic bags to prevent drying and brought to the laboratory where they were immersed in tap water and soaked for a minimum of 15 min. Even extended soaking did not dissolve emboli in *A. saccharum*, as indicated by preliminary experiments in which hydraulic conductivity was found not to change significantly after embolized segments had been soaked overnight. Keeping the segment submerged, lengths of a few centimetres were snipped off either end repeatedly with sharp anvil clippers until only the previously-marked central portion was left. Gaskets cut from rubber tubing were slipped over either end to provide a leak-proof fitting of the segment to the conductivity apparatus. Both ends of the segment were then trimmed with a fresh razor blade and it was rapidly fitted to the apparatus.

Conductivity apparatus and measurement

Figure 1 illustrates an apparatus designed to measure conductivity in stem segments using gravity-induced flow with pressure differences across the segment of 1 to 6 kPa. Perfusing solution was stored in a supply tank (Fig. 1a) made from PVC pipe capped at both ends. This solution must be one of those found to prevent long-term decline in conductivity (see below). Pressurizing the tank with compressed air (Fig. 1b) forces solution to pass through an in-line membrane filter holder with a 0.22 μm filter of 47 mm diameter (Fig. 1c). The solution was routed via a three-way stopcock (Fig. 1d) into a secondary supply reservoir consisting of a graduated cylinder closed with a vented rubber stopper (Fig. 1e). Adjusting the stopcock isolated the secondary reservoir from the pressurized solution and allowed flow by gravity from this reservoir through the stem (Fig. 1f) and into a drain reservoir on an electronic balance (Fig. 1g).

A computer in communication with the balance was programed to output the conductivity taking into account the following variables: (i) changing pressure head as reservoir levels changed; (ii) evaporation from the balance reservoir; (iii) displacement of the pipette conducting solution into the balance reservoir; (iv) length of the segment; and (v) temperature of the perfusing solution. Conductivity changed by approximately 2.3% per °C due to viscosity changes, so our current program corrected all measurements to 20 °C. Long-term continuous readings remain subject to temperature-induced fluctuations, but subsequent to this study our apparatus was modified to include automatic monitoring of solution temperature. Conductivity was calculated every 30 s together with a running mean of the previous ten 30 s readings. The first running mean to deviate from any previous trend was taken as the conductivity for the segment, e.g. if means were increasing, the first mean to stay the same or decrease was used. An analysis of error indicated that the coefficient of variation (approximately 0.3 to 5% for high and low conductivities, respectively) might be accounted for by the measurement resolution of the balance.

Quantifying embolism

The degree of embolism in a stem segment is estimated by its initial conductivity as a percentage of the maximum obtained after removal of emboli (Fig. 2). In a previous investigation, emboli in palm (*Rhapis excelsa*) xylem were removed by pressurizing the sample underwater (Sperry, 1986); when this technique was used on sugar maple, we found that new emboli were created by air coming out of solution on depressurization. Our preferred method

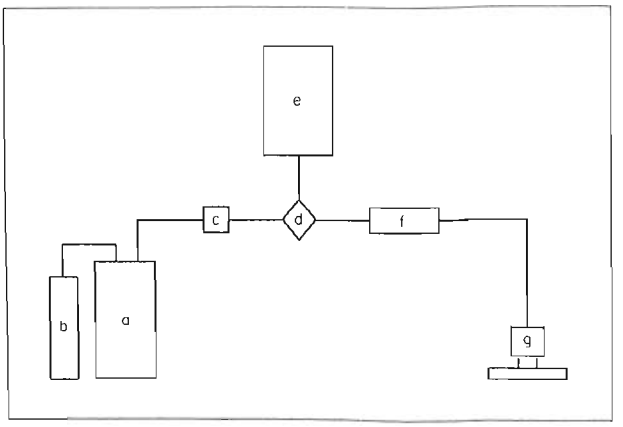

Figure 1. Schematic representation of the apparatus for measuring hydraulic conductivity in xylem of stem segments: (a), supply tank containing perfusing solution; (b), compressed air tank with regulator; (c), filter; (d), three-way stopcock; (e), secondary supply reservoir; (f), stem segment; g, drain reservoir on electronic balance. Line connecting air and solution tank is a pressure hose; other joining lines represent solution-filled clear tubing. Balance is interfaced with a computer program to calculate hydraulic conductivity.

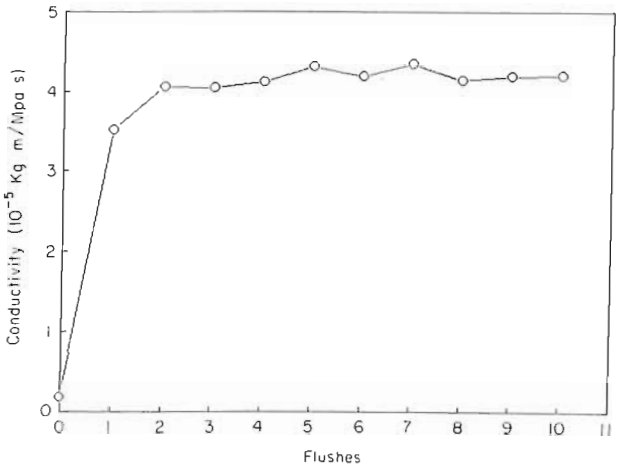

Figure 2. Effect of repeated high-pressure (175 kPa) perfusions, or 'flushes', on hydraulic conductivity of a sugar maple stem (< 1 cm diameter); prior to measurement the stem had been dehydrated to −4.5 MPa. The initial conductivity of the stem after dehydration (at flush 0) was 5% of maximum obtained after five flushes; the increase is attributed to removal of air emboli from the vessels.

is to 'flush' the stem with perfusing solution at higher pressure which dissolves and expels the emboli.

Maximum conductivity was achieved in the following manner. Once the conductivity of a segment had been measured, the segment was flushed by applying a regulated pressure of 175 kPa to the supply tank (Fig. 1a) and directing solution to the stem, bypassing the secondary supply reservoir. The efflux from the stem was routed away from the balance reservoir to prevent overflowing. After at least 20 min of flushing, the pressure was released and conductivity of the segment was measured as before. Alternation between flushing and measuring was continued until conductivity stopped increasing (Fig. 2).

To confirm that flushing removes emboli, we perfused pairs of contiguous, equal-length segments with safranin dye (0.1%). One of the pair was perfused without conductivity measurement, and the other was perfused after being flushed to its maximum conductivity. In all segments the dye was perfused by suction pressure of 10 kPa for 20 min. Figure 3(a) is a transverse view of a stem stained without being flushed; the absence of complete staining indicates numerous non-conducting vessels. This staining pattern reflects a 47% reduction in conductivity below maximum as measured in the adjacent segment. Figure 3(b) is the adjacent segment stained at its maximum conductivity; the xylem is completely stained indicating that flushing restored conduction in all vessels.

When a partially stained stem such as that in Fig. 3(a) was sectioned longitudinally at a thickness approximately twice that of the average vessel diameter (70 μm), portions of vessels remained intact and emboli could be observed. Sections through unstained portions of stems revealed air bubbles in

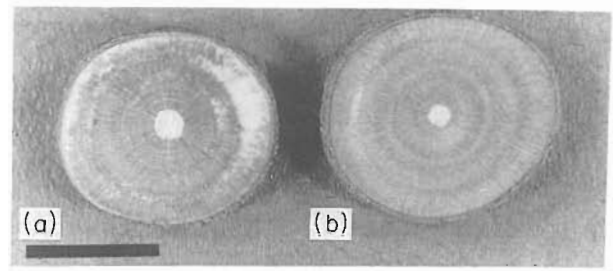

Figure 3. Result of safranin dye perfusions of two contiguous segments from a sugar maple stem (bar is 1 cm): (a) not flushed; (b) flushed to maximum conductivity. Incomplete staining of (a) represents 47% reduction in conductivity by emboli as measured on the adjacent segment. Complete staining in (b) indicates flushing removes flow-impeding emboli.

every vessel (Fig. 4(a)), whereas bubbles were absent in sections from stems that had been flushed to maximum conductivity (Fig. 4(b)). Flushing also filled the normally air-filled fibres of maple with water compare (Figs 4(a) and 4(b)). Apparently, water-filled fibres do not conduct measurable amounts of water because we often encountered stems whose initial conductivity was 100% of the value obtained after flushing, and the fibres were not stained by dye perfusions.

Investigation of decline in conductivity

In order to accurately measure conductivity of segments after repeated flushes, we had to determine why conductivity of continuously perfused stem segments tend to decline, and how to prevent it. Figure 5 shows the response of *A. saccharum* stem segments to continuous perfusion with a variety of

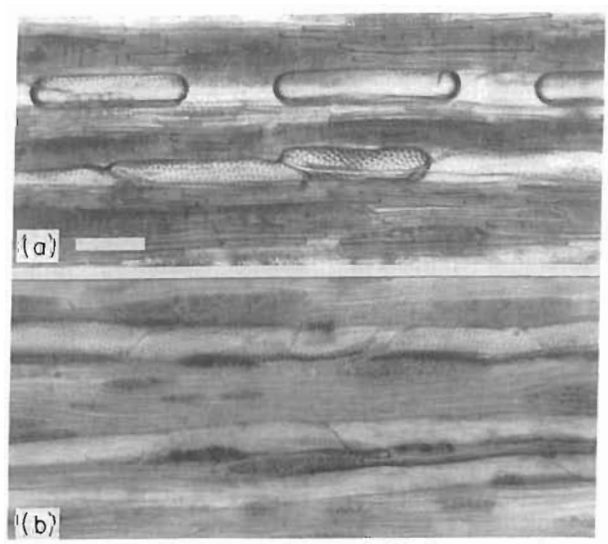

Figure 4. Longitudinal sections (70 μm thick) of sugar maple stems (bar is 100 μm): (a) non-stained area of stem perfused with safranin dye without having been flushed, note emboli in vessels; (b) stem flushed to maximum conductivity, all vessels are water-filled as are the normally air-filled fibres

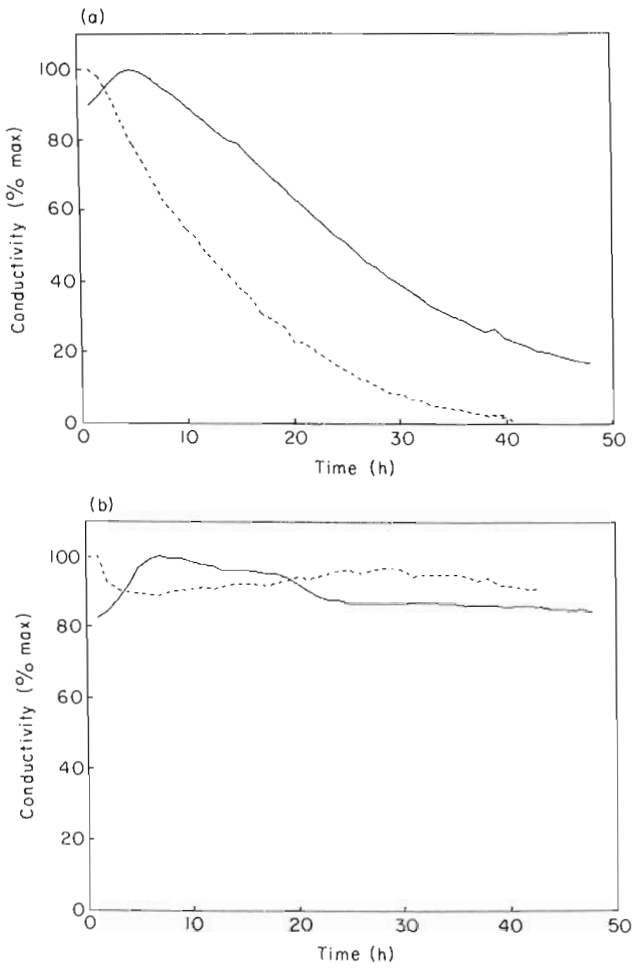

Figure 5. Time-dependent changes in hydraulic conductivity of sugar maple stems perfused with: (a) solutions that fail to prevent long-term decreases in conductivity, e.g. deionized water (———) and 10 mol m^{-3} sodium chloride (– – – –); (b) solutions which prevent long-term decreases in conductivity, e.g. 0.05% formaldehyde (———) and 10 mol m^{-3} oxalic acid (– – – –). Initial increases in conductivity, as depicted in stems perfused with deionized water and formaldehyde, are common and probably result from the rearrangement and/or dissolution of emboli within xylem conduits. The initial drop in conductivity within the oxalic acid-perfused stem was most likely the result of gas bubbles formed at the stem surface by residual sodium hypochlorite used in a previous rinse of the tubing.

solutions. Within the first several hours there was no predictable trend regardless of solution; values increased, decreased, or remained constant. If a segment had been flushed to remove emboli prior to perfusion, this variation was largely eliminated and initial conductivity was constant.

The effect of the perfusing solution became evident after 4 to 10 h; solutions resulting in long-term decline included: deionized water (Fig. 5(a)), 10 mol m^{-3} NaCl (Fig. 5(a)), mixed salts (1 mol m^{-3} NaCl, 0.5 mol m^{-3} $CaCl_2$, 0.2 mol m^{-3} KCl), maple sap, and citric acid (10 mol m^{-3} pH 4)). After prolonged perfusion with these solutions, conductivity essentially ceased. Solutions that prevented decline included: formaldehyde (0.05% and 0.5%; Fig. 5(b)), gluteraldehyde (0.05%), citric acid (10 mol m^{-3}, pH < 3.0), and oxalic acid (10 mol m^{-3}, pH 1.3 to 2.4; Fig. 5(b)). Stems perfused with these solutions were measured for up to 2 weeks without showing decline. Fluctuations in long-term measurements with these solutions could be largely accounted for by changes in temperature.

Within the first 2 or 3 d of perfusion, decline in conductivity occurred primarily towards the influx end of segments. Segments perfused for 3 d with distilled water and showing a 4-fold increase in resistance (the reciprocal of conductivity) were cut into equal thirds and each third remeasured; 47% of the total resistance occurred in the influx third. In control segments showing no previous decline in conductivity, each third contributed very close to 33% of the total, indicating equal resistance along the segment. In other experiments, we found that cutting as little as 1 cm from the influx end caused stems at reduced conductivity to increase substantially. These results suggest that declining conductivity is caused by particles carried into the stem with solution and trapped within the vessels at the influx end. This view is supported by the observation that a decrease in conductivity did not consistently occur when stems were attached to the measuring apparatus and flow-through between measurements was prevented by eliminating the gravity head, suggesting that particles were prevented from entering the stem in significant numbers. When perfusions were continued for a week or more, increased resistance spread throughout the segment indicating a gradual downstream progression of occlusion.

Observations indicated that the occluding material was easily dislodged; if a stem at reduced conductivity was detached from the apparatus and then simply replaced in its original orientation, its conductivity temporarily increased. The same response was elicited by squeezing tubing adjacent to the stem without removing it. Presumably, the rapid changes in magnitude and direction of pressure gradients that attended the removal and replacement of the stem, or the squeezing of tubing, was sufficient to shift material in the vessels.

Microscopic observations indicated that this occluding material was microbes. Figure 6 is an extreme example where microbes, (perhaps yeast), have completely occluded a vessel in a stem that had been perfused with deionized water for 10 d and conductivity had ceased. For studies of shorter duration, it was much more difficult to determine with confidence if the vessels were infected; often a stem with reduced conductivity looked anatomically identical to a stem functioning at full capacity. This is probably because initial blockage occurred at intervascular pit membranes.

More convincing evidence for microbial infection came from studies in which micro-organisms were isolated from stem sections. Segments, approxi-

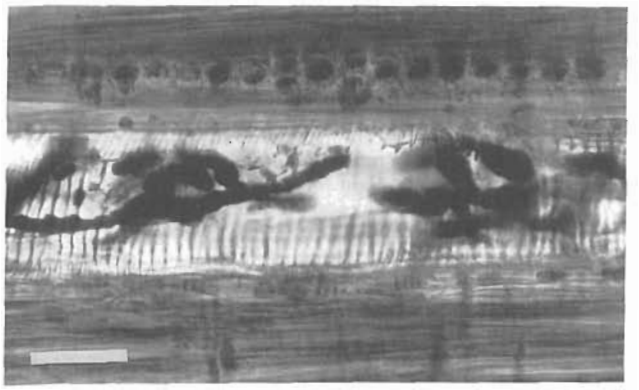

Figure 6. Microbe (perhaps yeast) within sugar maple vessel after stem sample had been perfused for 10 d with deionized water (bar is approximately 15 μm). Section was stained with periodic acid-Schiff's reagent.

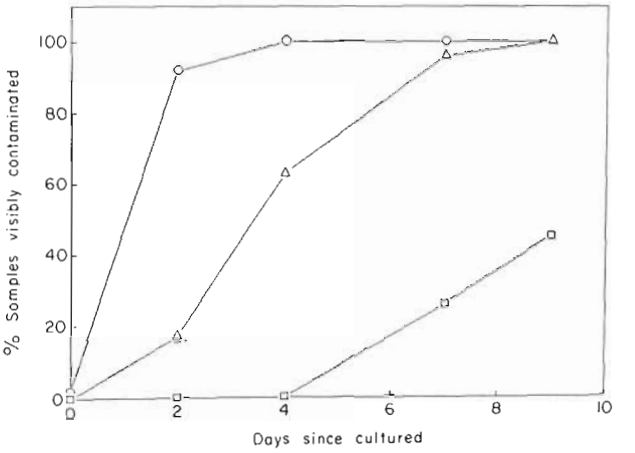

Figure 7. Micro-organism contamination in splinters cut from sugar maple stem segments and cultured on malt agar. Deionized water and formaldehyde stems had been perfused for 3 d before culturing; controls were cultured immediately after field collection. Percentages based on 53 deionized-water splinters (○), 70 formaldehyde (0.05%) splinters (△), and 31 control splinters (□). Results are expressed as the percentage of splinters possessing microbes visible with a dissecting microscope.

mately 15 cm long, were surface sterilized, cut into thirds, and splinters split from each third were placed in slits cut in malt agar (1.5% w/v). Figure 7 shows microbial growth (fungal and bacterial) in three types of segments: (i) stems perfused with deionized water and reduced to below 10% initial conductivity; (ii) stems perfused with 0.05% formaldehyde and showing 90% or greater initial conductivity; and (iii) non-perfused stems taken directly from a tree. Pooling results for influx, middle and efflux samples, the deionized-water perfused stem supported the most microbial growth, whereas growth from the formaldehyde perfused stem was much slower. The control stem had the lowest infection percentage. In each of the formaldehyde and deionized-water perfused stems, the influx and efflux portion of the stem had more rapid and complete colony growth than did the middle portion. Apparently, formaldehyde prevents the decline in conductivity by arresting microbial development rather than killing the organisms. This explains why when a formaldehyde-perfused stem was subsequently perfused with deionized water, conductivity declined; the microbes were still present and able to increase in numbers in the absence of formaldehyde.

Discussion

Previous investigators of the decline in xylem conductivity have failed to distinguish long-term ($\geqslant$4 h) decline, which we attribute to microbial clogging, from initial short-term trends which our results show to be unpredictable. These short-term fluctuations may have been due to air emboli and/or particulate matter, because they were not evident when flushed stems were measured. Emboli could cause conductivity to increase by gradually dissolving, or to decrease by coalescing to form larger bubbles. Particulate matter would cause a decrease until it was filtered out at vessel ends. Flushing the stem could conceivably do this for a finite amount of particles such as could originate at the cut surface of the stem, or be introduced as the stem was fitted to the tubing. Of course, if a solution was used that did not inhibit microbes, microbially-related particles would continue to accumulate in the vessels indefinitely; the same would be true if the solution was not filtered prior to perfusion allowing all manner of particles in addition to microbes to enter the stem.

Given the inconsistency of initial trends in conductivity, it is easy to misinterpret results based on single experiments. Zimmermann (1978) reported that 10 to 100 mol m^{-3} salt solutions prevented decreasing conductivity encountered when using distilled water. Our results revealed no consistent difference between the two types of solution; both ultimately caused decline (Fig. 5(a)).

The primary site of infection in microbially clogged stems was probably the cut surface of the stem and adjacent bark. In addition to organisms already on the bark, potential sources of contamination during segment preparation included hands, tap water, and air in the laboratory. Once attached to the apparatus segments could be infected by permanent microbial colonies established in the tubing downstream from the filter, although this was occasionally sterilized by a sodium hypochlorite rinse (5% w/v). Once the microbes were in the vessels, nutrients from the stem would have favoured growth. Microbes potentially could have spread further along the stem, by decomposing or (in spore stage) by passing through pit membranes (Dimond, 1970); this would explain why in stems perfused for a week or more, occlusion was evident throughout stem segments.

The role of micro-organisms in the stoppage of xylem flow has long been recognized by researchers in the maple syrup industry. Tap holes will stop flowing if there is excessive contamination by microbes, and paraformaldehyde tablets in the hole have been used to inhibit contamination as well as the tree's wound response in order to maintain flow (Sheneman *et al.*, 1959).

Our explanation for declining conductivity differs from previous ones, some of which if true would have had significant consequences for current models of xylem transport. For instance, Jeje (1986) has proposed that the decrease in conductivity is due to decreasing vessel diameters caused by swelling of the wood during conductivity measurements. He extrapolates this to intact trees, speculating that conductivity of the xylem would fluctuate between minimum values during periods of favourable water balance to maximum values during drought. Several of our experiments are inconsistent with this hypothesis: (i) flow through the stem segment was required to consistently produce a decline in conductivity; (ii) when flow was induced the decline in conductivity continued until flow stopped; (iii) in stems with stoppage of flow the vessels were occluded with micro-organisms; and (iv) decline was prevented by using dilute solutions of fixatives (formaldehyde, gluteraldehyde) and solutions with a pH below 3.

Although it has always been assumed that the consequence of xylem sap cavitation is an impediment to water flow, by resolving the problem of declining conductivity we now have a simple method for evaluating this assumption. We are currently using this technique to investigate the seasonal occurrence of embolism in several tree species, and to discover the relationship between embolism, xylem pressure potential, and xylem anatomy.

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