Plant, Cell and Environment (1991) **14**, 357–369

The refilling of embolized xylem in *Pinus sylvestris* L.

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Received 17 July 1990; received in revised form 22 October 1990; accepted for publication 7 November 1990

Abstract. The hypothesis that ray parenchyma cells are actively involved in the refilling of embolized xylem of *Pinus sylvestris* L. was tested by killing the ray parenchyma and comparing rehydration of killed stems with that of control material. Killing of ray parenchyma was achieved using hot water or sodium azide. In most experiments, the available water for refilling was at negative water potential. Experiments were done on three kinds of plant material: small branch segments, potted seedlings and small potted trees. In all experiments, there was no indication that the azide-killed xylem was slower to refill than the control material and it was concluded that the parenchyma has no role in the refilling process, which therefore must be purely physical or physico-chemical. Stems treated with hot water did not refill; we suggest that this may be caused by high temperatures decreasing the water permeability of the tracheid wall. The refilling of small branch segments may be explained by surface tension forces (capillary action), which inside the tracheid lumen may lower the water potential down to −9.7 kPa; this may be enough to draw in water from the available water that in experiment one was at −2 kPa. In the case of seedlings or saplings, capillary action cannot explain refilling, because the xylem water potentials were always lower than those estimated from tracheid radii. Condensation of water during diurnal cycles of warming and cooling is also unlikely to contribute to refilling significantly. To account for refilling in these cases, it is supposed that the tracheid wall may be chemically active and able to lower the water potential below the value expected by capillarity.

Key-words: Pinus sylvestris; Pinaceae; xylem; tracheid; embolism; refilling; parenchyma; capillary force.

Introduction

A common view of the xylem as a pathway for water transport is that it is a *vulnerable pipeline* (Milburn, 1979). According to this view, when plants are actively transpiring the water columns come under great tension which may exceed their tensile strength, leading to breakage. The resulting embolized tracheid is no longer available for transport, unless it can be refilled.

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The water content of the xylem of conifers is known to fluctuate diurnally and seasonally, to decline in drought and increase when water is supplied (Chalk & Bigg, 1956; Roberts, 1976; Waring & Running, 1978; Waring, Whitehead & Jarvis, 1979; Peña & Grace, 1986). Although one part of the fluctuation in water content is believed to be caused by changes in the turgor of the ray parenchyma cells, and another part by changes in the volume of capillary water held in crevices between tracheids (Zimmermann, 1983), it is widely held that much of the change results from reversible cavitation of xylem lumens. That is to say, the tensile strength of water is often insufficient to withstand the tensile forces, and tracheids empty when their water cavitates. However, when water becomes available again, they have the capacity to refill. The evidence for such cavitation comes mainly from the use of acoustic sensors (Milburn & Johnson, 1966). For instance, when water potential falls below a threshold value of −0.5 MPa in *Pinus sylvestris* ultrasonic acoustic emissions begin (Peña & Grace, 1986). Similar thresholds occur in other conifers (Dixon, Grace & Tyree, 1984). The critical evidence that acoustic emissions are diagnostic for cavitations is reviewed elsewhere (Tyree & Sperry, 1989).

The refilling process is very poorly understood, not well characterized and scarcely discussed in major reviews (Pickard, 1981; Zimmermann, 1983; Tyree & Sperry, 1989). Most authorities consider that refilling will occur only when gas inside each tracheid comes under a positive pressure, causing it to dissolve (Milburn, 1979; Zimmermann, 1983). In angiosperms, these conditions may be achieved as a result of root pressure (Sperry *et al.*, 1987).

It has been suggested that the ray parenchyma of the xylem has a role in maintaining the water balance of the stem. Anatomical studies show that in conifers each tracheid is in contact with the medullary ray tissue (Carlquist, 1975), so that entry of water into a tracheid may be influenced by metabolic activity of the associated parenchyma cells (Wodzicki & Brown, 1970; Braun, 1983). We may call this the *vital theory* of refilling. It has never been clear how the parenchyma cells could

perform this role; whether by extruding water directly or by secreting ions and thus causing a water potential gradient on a microscale. Evidence for the vital theory comes from Wodzicki & Brown (1970). They found that treatments designed to kill the xylem parenchyma reduced the uptake and transport of water by stem segments in several angiosperms and in one gymnosperm.

In the present work, the intention was to test the hypothesis that living parenchyma cells are required for refilling. To do this, stem segments or young trees were first water stressed until they exhibited a reduction in their xylem water content accompanied by acoustic emissions. After this, water was supplied and the pattern of rehydration of killed material was compared with that of the controls. Killing was achieved with hot water or appropriate concentrations of sodium azide. Another aim of the present work was to characterize the refilling process: how long does it take, and can it occur when the available water is at negative water potential?

Materials and methods

Three experiments were performed, during August and September 1989, using three different kinds of plant material: (1) small branch segments, (2) potted seedlings, and (3) small potted trees. All material was *Pinus sylvestris* L., of Scottish provenance.

Killing the xylem parenchyma

Segments (50–60 mm long) were stripped of their bark and perfused under gravity with 5 or 10 mol m^{-3} sodium azide solutions: azide is known to be an effective metabolic poison, blocking phosphorylation at 1 mol m^{-3} (Dawson *et al.*, 1986). As controls, segments perfused with 5 or 10 mol m^{-3} KNO_3 solutions were used. In the perfusion, the solution was made to flow through the branch segment by connecting the segment to a plastic tube and feeding the solution from a header tank at 1 m above the segment. Immersion in hot water at 70 or at 100 °C was tested as an alternative method of killing.

When seedlings were used (experiment 2), a treatment with hot water was applied; a portion of the main stem, 60 mm long, was enclosed in a rubber collar, connected by nylon tube to a thermostatic bath, and water at 70 °C was pumped through the collar for 5 min.

When small trees were used (experiment 3), the stems of the trees were cut at the base and their ends were immersed in a 10 mol m^{-3} azide solution; 10 mol m^{-3} KNO_3 was used for the controls.

The effectiveness of these treatments in killing the tissue was determined by measuring the respiration rate of treated and control materials, using an infrared gas analyser, in absolute mode (Li-6002, Li-Cor Inc., Lincoln, Nebraska, U.S.A.).

Measurement of wood volume fractions

The volume fraction of water (V_w) of debarked samples was calculated knowing their fresh (W_f), dry weight (W_d) and volume (V_f) as:

$$V_w = (W_f - W_d)/(\rho_w V_f) \quad (1)$$

where ρ_w is the density of water. Weights were measured to the nearest 0.1 mg; dry weight was measured after 48 h in an oven at 80 °C. The volume was measured as immersed weight in distilled water, on the basis of Archimedes' principle. The volume fraction of solids (V_s) was estimated from the dry weight by assuming the density of solids (ρ_s) to be constant at 1530 kg m^{-3} (Siau, 1971):

$$V_s = W_d/(\rho_s V_f) \quad (2)$$

The volume fraction of gas was calculated by difference (Whitehead & Jarvis, 1981):

$$V_g = 1 - V_s - V_w \quad (3)$$

The variation of water volume fractions in a dehydrating, debarked segment is mostly the result of variation in the water content of the tracheids. Indeed, ray parenchyma cells only occupy 6% of the total volume of conifer wood (Panshin & De Zeeuw, 1980).

Measurement of ultrasound acoustic emissions

Ultrasonic acoustic emissions (UAE) from dehydrating wood segments or from the stems of potted plants were recorded using an ultrasonic transducer (PAC I15I, Physical Acoustic Corporation, Princeton, NJ, U.S.A); UAE were amplified 75 dB (model 4615 Drought Stress Monitor, Physical Acoustic Corporation).

Segments were debarked and the transducer was clamped in the centre of the segment. For work on whole plants in experiments two and three, the stems were prepared by removing a small portion ($\simeq$100 mm^2) of the bark, to expose the xylem in the area where the transducer was applied. The exposed surface was coated with silicon grease to prevent water loss from the tissue. The acoustic contact between the transducer and the sample was improved by using an ultrasound transmitting gel.

Experiment 1: branch segments

Several segments, 50–60 mm long and 4–6 mm in diameter, were cut from the lateral branches of a mature open-grown Scots pine tree located on the Edinburgh University campus. They were enclosed in plastic bags and immediately taken to the laboratory.

Thirty debarked segments underwent the following treatments: (a) 1 h perfusion with sodium azide 5 mol m^{-3}; (b) 1 h perfusion with KNO_3 5 mol m^{-3}; (c) immersion in water at 100 °C for 10 min. Ten segments were used for each treatment. To prepare cavitated materials, segments were weighed and dehydrated on

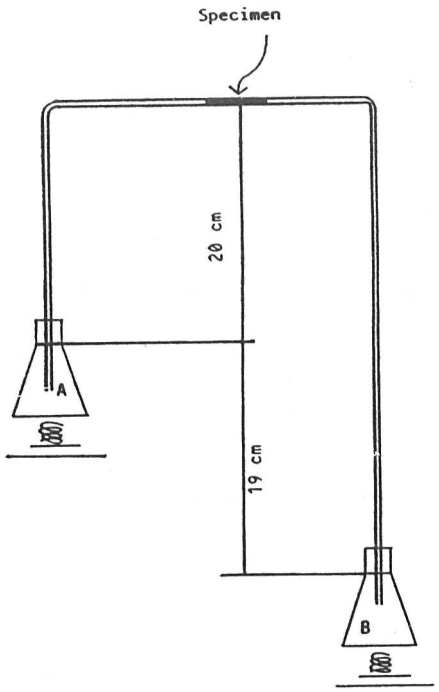

Figure 1. Apparatus used in experiment 1; 25-cm^3 flasks (A & B) were continuously weighed on two analytical balances, at different heights, and horizontal branch segments were kept 20cm above the upper flask level. Evaporation from the flasks was negligible with respect to water fluxes between the flask and the segment.

the laboratory bench until their weight had fallen to 75–80 per cent of the initial weight. Afterwards, for the rehydration period the samples were coated with 'Parafilm' and held vertically, with their exposed lower end recut under water and immersed to a depth of a few millimeters in degassed distilled water which had been passed through a 0.1 μm filter. Degassing was done by exposure of freshly distilled water to a vacuum line whilst subjected to ultrasonic excitation. Samples were weighed after 0.5, 1.5, 2.5, 3.5, 6.5, 24.5, 45.5 and 71.5 h from resupply of water. At the end, the volume and the dry weight of each sample was determined.

Another part of this experiment was intended to enable the water potential of the supply to be varied. Ten debarked segments which had been perfused overnight with sodium azide (10 mol m^{-3}) and eight debarked segments perfused overnight with KNO_3 (10 mol m^{-3}, controls), were dehydrated over the laboratory bench; groups of treated and control segments were submitted to different periods of dehydration in order to obtain different levels of xylem embolism. The segments were then weighed, their ends shaved under water, and mounted on rubber tubes connected with two small flasks at different heights (Fig. 1). Segments were covered with 'Parafilm' and kept horizontally 20 cm above the upper flasks. In this way, the water for rehydration was under tension equal to a potential of −2 kPa. As permeating liquid, degassed distilled water, filtered to 0.1 μm, was used. The experiments were performed in a controlled temperature room and the temperature was regularly recorded to obtain the appropriate viscosity values. The flasks were on two analytical balances and inflow and outflow rates were measured gravimetrically. Readings were taken every 1–2 min during the first 30 min, and 5–10 min subsequently. Experiments with a duration up to 900 min were done and large time gaps between readings occurred in these cases. The length and diameter of the segments were determined to the nearest 0.01 mm, using a digital vernier caliper.

Finally UAE readings were repeated over 3-min periods, at irregular intervals, for 30 h, on each of four debarked, untreated, segments dehydrating in air. At the same time, the segment diameter was measured at four fixed positions on each sample, to the nearest 0.01 mm and the fresh weight was determined.

The volume and the dry weight of all samples were determined at the end of the experiments.

The variation in permeability of stem segments was estimated by fitting the following model to the data using least-squares optimization procedures:

$$P = \alpha(1-\beta e^{-t\gamma}) \quad (4)$$

where P is the permeability, calculated by Darcy's law from the outflow rates (Edwards & Jarvis, 1982), α is the water saturated permeability (the asymptotic permeability), β is the difference between asymptotic and initial permeability, t is the elapsed time, and γ is the time constant.

Experiment 2: seedlings

Two hundred, 3-year-old potted seedlings of Scots pine, grown in a peat and sand mixture in the nursery and selected for uniformity (debarked stem diameter = 6–8 mm) were taken to the glasshouse; water was withheld from 100 seedlings between 8 and 28 August; the remaining 100 were kept well watered (the so-called never-stressed plants).

On 11, 15, 17, 21, 22, 23 and 28 August, six seedlings were randomly sampled from the water-stressed plot and xylem water potential was measured, at 0800 h, on three excised needle-fascicles per seedling, using the pressure chamber. On 11, 15, 17, 21 and 23 August, daily transpiration rates of another six plants from the same plot were measured by weighing the pots which were enclosed in plastic bags to prevent water loss from the soil. On 11 and 23 August, both water potential and transpiration measurements were carried out throughout the day, about every 2 h.

On 23 and 25 August, UAE from the lower stem of six water-stressed seedlings were repeatedly recorded over successive 5-min periods; on 11, 15 and 21 August, UAE were recorded continuously from one seedling over several hours.

On 28 August, the previously described hot water treatment was applied to the stems of half of the water-stressed seedlings (treated plants); immediately

afterwards all the seedlings were copiously rewatered. On the following days, dawn pressure potential and daily transpiration rates of treated and control plants were measured.

The volume fraction of water was determined before the drought period and 4, 24, 48 and 100 h from rewatering, on six treated, six control and, irregularly, on six never-stressed plants; 50–60 mm long-samples were taken from the treated portion of the stem from each treated seedling and from similar positions in the control and the never-stressed plants.

Osmotic potential of xylem sap was measured with a freezing osmometer (Osmomat 030, Gonotech, GmbH, Berlin, Germany). On 28 August, a few drops of xylem sap were extracted, using the pressure chamber, from the stems of three water-stressed, three control (4 h from rewatering) and three never-stressed plants. Measurements were repeated 216 h after rewatering, on three control plants.

Leaf area was measured at the end of the experiment with an area meter (Li-3000, Li-Cor Inc., Lincoln, Nebraska, U.S.A.).

Experiment 3: saplings

Six potted, 7-year-old pine trees (underbark stem diameter = 40–50 mm, plant height = 2–3 m) were taken to the glasshouse and uprooted to achieve a water stressed condition within a few days. After 6 d, these trees were resupplied with water by excising the roots under running water and putting the cut stems in buckets containing 10 mol m^{-3} sodium azide or KNO_3 (three trees per treatment). UAE and stem wood density were measured throughout the experiment. Xylem pressure potential was determined by sampling six needle-fascicles from lower branches in the canopy.

Stem wood density was determined non-destructively using the attenuation of a collimated beam of gamma radiation (Edwards & Jarvis, 1981). The source was americium 241. Permanent holders were clamped on each tree to accommodate the source and detector with a fixed geometry, enabling comparable measurements to be made on successive occasions (Brough, Jones & Grace, 1986). The 8-mm diameter beam passed through the stem, and the gamma radiation was sensed by a crystal scintillation detector connected to a portable ratemeter (PSR8, Nuclear Enterprises Ltd, Edinburgh, U.K.). Where the beam traversed the stem, 'windows' were made by cutting away patches of bark so that all attenuation was by the xylem rather than the bark tissues. To avoid evaporation, the exposed bark was smeared with petroleum jelly and covered with PVC tape.

The attenuation of the beam was related to the mass in the beam's path by calibrating the device with water-filled spectrophotometer cells. Knowing the path length, it was thus possible to estimate the stem density. This was finally related to the water volume fraction by calibration against pieces of the stem whose water content was determined gravimetrically.

Results

The effectiveness of various methods of killing xylem parenchyma was examined by measuring the respiration rate after treatment (Table 1). Perfusion of azide was most effective at 10 mol m^{-3}, which reduced the respiration rate to 16% of the control value. Boiling reduced the rate even further, to 10%. Even when the parenchyma is completely killed, some CO_2 is likely to be given off for some time by diffusion, and so the efflux rates are likely to overestimate the current respiration rates. Perhaps this explains why the rates appear much lower when measured 2 and 20 h after treatment. The azide technique is undoubtedly a less reliable way to kill the tissue than boiling, presumably because embolized regions of the stem segments do not receive full dosage when the segment is perfused. Nevertheless, the 10 mol m^{-3} azide treatment achieved an adequate kill and as the work progressed it became the preferred treatment.

Experiment 1

The first experiment was to investigate dehydration and refilling in small segments of xylem obtained from branches of a mature Scots pine tree.

When such segments, which had been peeled of their bark, were allowed to dehydrate on the bench, a characteristic pattern of acoustic emissions was observed (Fig. 2a). There was a lag of about 1.5 h before the onset of activity. Then, there was a gradual increase in the rate of emission until a maximum rate of 107 counts s^{-1} was recorded after 11.5 h. Thereafter, the rate declined until very few counts were recorded after 32 h (Fig. 2a). The same data may be plotted with the water volume fraction as the abscissa (Fig. 2b). Fully hydrated segments had a water volume fraction of 0.66–0.70. Acoustic emissions started when the volume fraction fell below 0.55. A small amount of water remained when the acoustic emissions had stopped, but this volume fraction was only 0.05 (Fig. 2b).

In the course of dehydration, there was a reduction in diameter of the stem segment (Fig. 3). This reduction was, as a first approximation, a linear function of the water volume fraction in the range 0.25 to 0.7. This shrinkage represents a 6% reduction in the volume of the system as a whole, whereas the volume fraction of gas increased by 58% (Fig. 3).

Table 1. Effectiveness of killing treatments, assessed by measuring the respiration rate as CO_2 efflux from the treated stem segments (plunged in hot water or perfused with sodium azide). Respiration is expressed as percent of control. There were at least five replicates in each trial

Treatment	Respiration rate (%)
Control, a fresh specimen	100
Perfused with 5 mol m^{-3} azide	52
Perfused with 10 mol m^{-3} azide	16
100 °C for 3 min	10
100 °C for 3 min, after 2 h	3
70 °C for 3 min	13
70 °C for 3 min, after 20 h	6

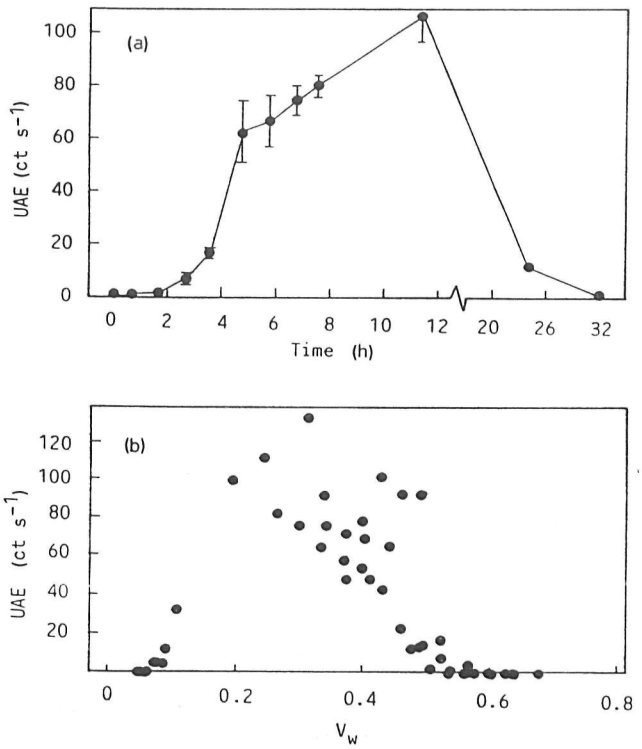

Figure 2. Experiment 1. Ultrasound acoustic emissions (UAE) from segments dehydrating in air: (a) UAE as a function of time; and (b) UAE as a function of water volume fraction, V_w. Vertical bars are standard errors, $n = 3$.

Rehydration of the segments with their bases immersed in degassed, distilled water also followed a characteristic pattern (Fig. 4). Initially, the partially dehydrated material contained a water volume fraction of 0.46–0.48. The control and azide-treated segments rehydrated over 71.5 h, the two curves being statistically indistinguishable (Fig. 4). However, the boiled stems rehydrated at the same rate initially, but then at a somewhat lower rate, so that after 71.5 h they had a water fraction of only 0.61, this being significantly lower than their initial water volume (0.67). In contrast, the control and azide-treated segments at 71.5 h contained more water than they had done initially. The same experiment was repeated using *Pinus nigra*, with essentially the same result (data not shown here). Also, an experiment on Scots pine was done using water both at 70 and 100 °C to kill the parenchyma. The rehydration pattern was very similar regardless of the killing temperature, and the water content after 74 h was less than the initial water content (results not shown here).

When segments which had been partially dehydrated to give a water volume fraction of 0.49 were connected to a water supply at each end, as indicated in Fig. 1, it was possible to record precisely any movement of water between flasks and stem. Initially, there was a rapid flow of water from the upper flask A to the segment, accompanied by a lesser flow from flask B (Fig. 5a, b). Over 40 min, the inflow from flask A declined very rapidly, but continued slowly until the experiment was terminated. Flow from the segment to flask B became positive at 40 min in the case of the azide-treated case shown in Fig. 5b. However, overall, the control and the azide-treated material behaved in the same way. The pattern of refilling, calculated from the inflows and outflows, is the same for the control and azide-treated segments (Fig. 5c). Segments started at a gas volume fraction of 0.31–0.32 which, after 80 min, had recovered to 0.25. Although Fig. 5 reports the result from two segments only, the experiment was carried out on 10 azide-treated segments and eight controls, at a range of initial water volume fractions. Another comparison is shown in Fig. 6 a–c. Here, the initial gas volume fraction was 0.41 for the control and the azide-treated segment. This experiment lasted 800 min, by which time refilling was nearly complete (the gas volume fraction was less than 0.1). As before, the inflow exceeded the outflow throughout the experiment, and the difference between the treatments in the course of refilling was small. For the entire data set, comprising 18 segments, the mean net uptake (obtained from the algebraic sum of inflows and outflows) was not significantly different between the treatments: over the first 10 min, the water uptake by the controls was 1.19 mg min^{-1} with a standard error of 0.74 and by the azide-treated segments 1.29 with a standard error of 0.63.

This type of experiment was also carried out by putting both flasks at the same level so that there was no pressure gradient between the ends of the segments. Under these conditions, rehydration still occured in both treatments (Fig. 7).

The experiments with the two flasks differ from the initial experiments, in which segments were merely stood in water, in one important respect; they show that refilling can occur even though the source of water is at a

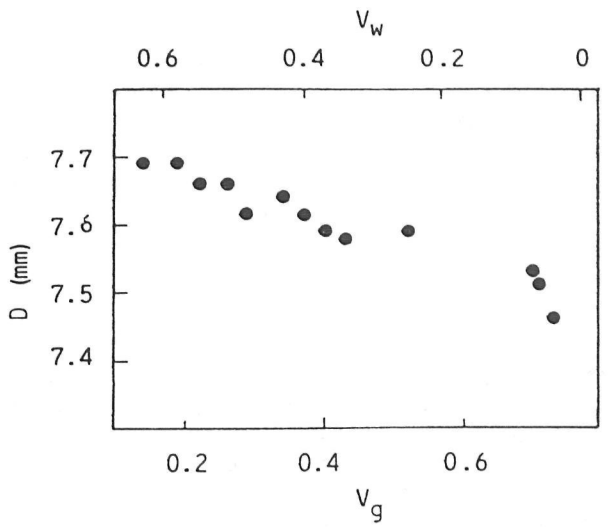

Figure 3. Experiment 1. Segment diameter (D) as a function of gas (V_g) and water (V_w) volume fraction. Each point is the mean of four branch segments.

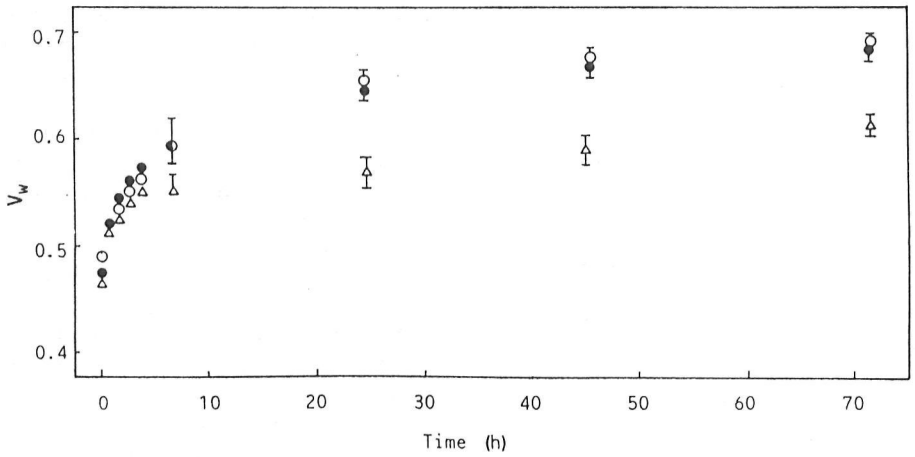

Figure 4. Experiment 1. Variation of the water volume fraction (V_w) during the rehydration phase of embolized segments. Open circles are KNO_3-perfused segments; solid circles are azide-perfused segments; open triangles are boiled segments. Vertical bars are standard errors, $n = 10$.

negative pressure rather than at a small positive hydrostatic pressure, relative to the cut surface of the stem.

The data on water outflow from these segments can, at least in principle, be used to calculate permeabilities (Edwards & Jarvis, 1982). One might suppose that the permeability would be low in embolized, dehydrated, material, and gradually increase as tracheids become refilled and consequently functional. Such 'ideal' behaviour occurred in only nine cases. 'Non-ideal' behaviour may be caused by properties of the wood itself (e.g. refilling of tracheids may be very patchy, leading to erratic data), or by technical difficulties with the experiment. These could include an effect of transient pressure on connecting the pipe to the segment, or a leak at any point in the system. It is not possible to distinguish between these alternatives. One example of 'ideal behaviour' is given in Fig. 8. The coefficients in this instance are all significantly greater than zero ($p < 0.01$), and an analysis of variance yielded an *F*-ratio of 251, indicating that the model fits the data very well. However, when all the data for the 'ideal' cases were assembled, the estimated parameters were found to be variable (Table 2), and there was no significant difference between the treatments. The mean saturated permeability was 0.12×10^{-12} and 0.13×10^{-12} m^2 in the controls and azide-treatment, respectively.

Experiment 2

In this experiment, attention was focused on intact 3-year-old potted plants. The object was to compare the rate of xylem refilling between controls and plants whose xylem had been killed by heat treatment at 70°C for 5 min. Over 20 d of drought, the transpiration rate fell, the acoustic emission rate increased to 1.9 counts s^{-1} and the xylem water potential, measured at dawn on needles, declined to −2.1 MPa (Fig. 9). Diurnal trends in transpiration rate, acoustic emissions and water potentials were obtained on days 3 and 15. These data (not presented) show that, by day 15, the acoustic emissions from the droughted plants persisted at 0.5 counts s^{-1} from 0800 to 2000 h, whilst the well-watered plants produced a maximum of only 0.03 counts s^{-1}. At this time, the needle-water potential was less than −1.2 MPa all day, with a minimum of −1.7 MPa. When the plants were rewatered, the transpiration rate increased over a period of 4 d, the acoustic emission rate became zero and the xylem water potential at dawn increased to −0.5 MPa in the controls and −0.6 MPa in the case of the heat-treated plants. The difference in the xylem water potential was not statistically significant.

Table 2. Experiment 1. Estimated parameters for eqn 1 (see text) fitted to the permeability values of 10 control and eight azide-perfused segments; α is the saturated (asymptotic) permeability (in units of 10^{-12} m^2), β is the difference between asymptotic and initial permeability (in units of 10^{-12} m^2), and γ is a dimensionless time constant

N	α	β	γ
Azide perfused			
1	0.063	0.540	0.059
2	0.110	0.320	0.140
3	0.042	4.230	0.022
4	0.290	0.470	0.042
5	−0.060	3.840	−0.010
6	0.100	−0.970	0.210
7	0.160	0.640	−0.001
8	0.580	0.920	−0.010
9	0.170	−0.110	−0.020
10	0.251	3.180	0.023
Controls			
1	0.230	0.890	0.007
2	0.120	1.120	0.020
3	0.020	3.170	0.010
4	−0.130	0.790	−0.002
5	0.102	208.660	0.820
6	0.110	−1.430	1.010
7	0.250	−0.280	0.003
8	0.230	−0.340	0.002

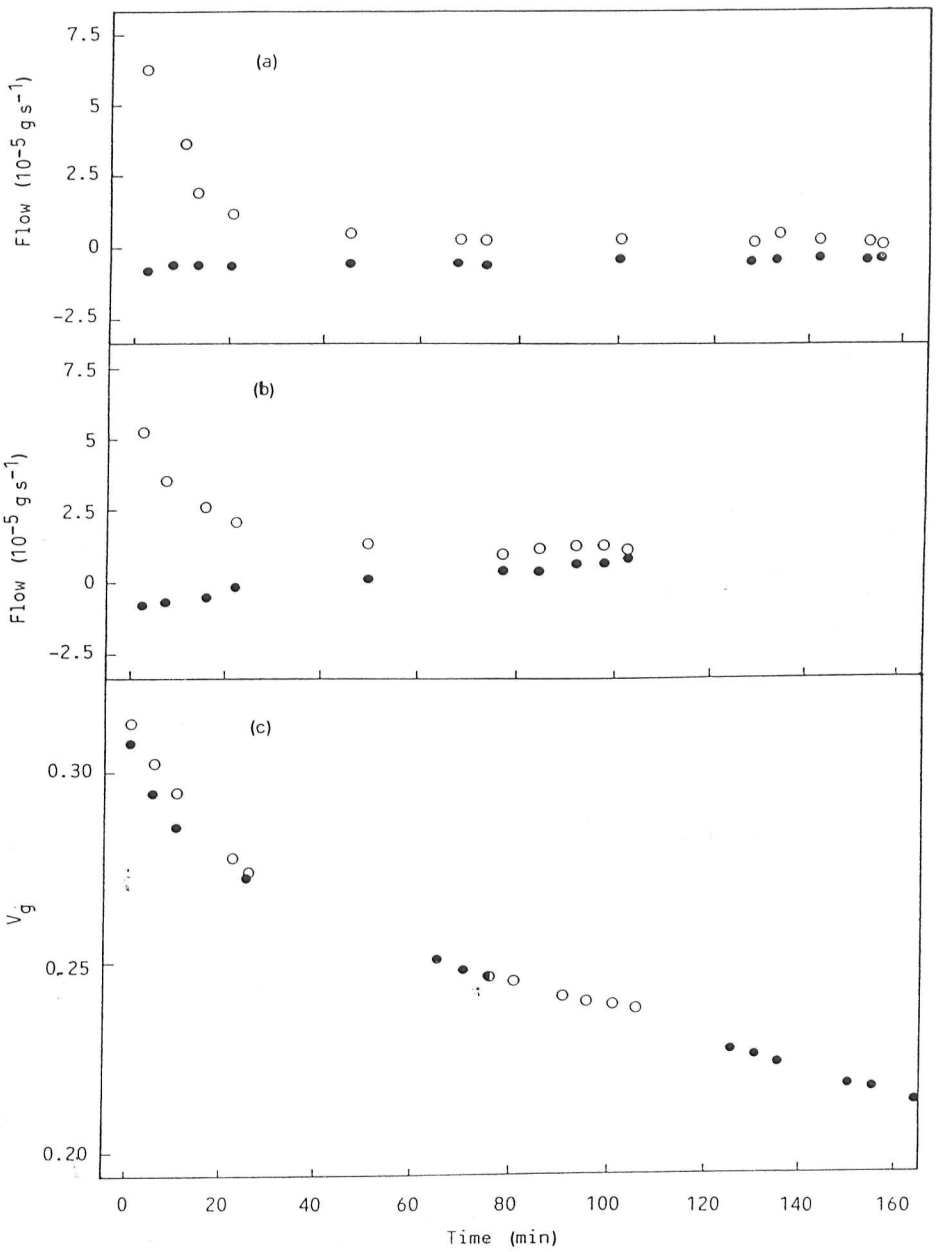

Figure 5. Experiment 1: (a) flow of water between the flask A and the segment (open circles), or between the segment and the flask B (solid circles), in the case of the KNO_3-perfused segment; (b) flow of water between the flask A and the segment (open circles), or between the segment and the flask B (solid circles), in the case of the azide-perfused stem (the sign convention is that the A to B flows were considered positive); and (c) variation of gas volume fractions (V_g) when segments were connected to flasks, as shown in Fig. 1 (open circles are KNO_3-perfused segments; solid circles are azide-perfused segments).

The water volume fractions of the xylem was 0.69 at the start of the experiment when the plants were defined as well-watered, but after water had been withheld for 20 d, the fraction had fallen to 0.61 (Fig. 9). The controls refilled in one day, even though the xylem water potentials were always negative. The xylem which had been heat-treated had a water volume fraction as low as 0.48, showing that the heat treatment had, incidentally, caused embolism. None the less, the heat-treated xylem showed some recovery (Fig. 9).

Osmotic potential of the xylem sap was never less than −0.12 MPa (Table 3). There was a decline in osmotic potentials over the period during which water was withheld, and an increase during the recovery period.

Experiment 3

This experiment involved larger, 7-year-old trees, and the volume fraction of water in the xylem was inferred from measurements made with the gamma probe.

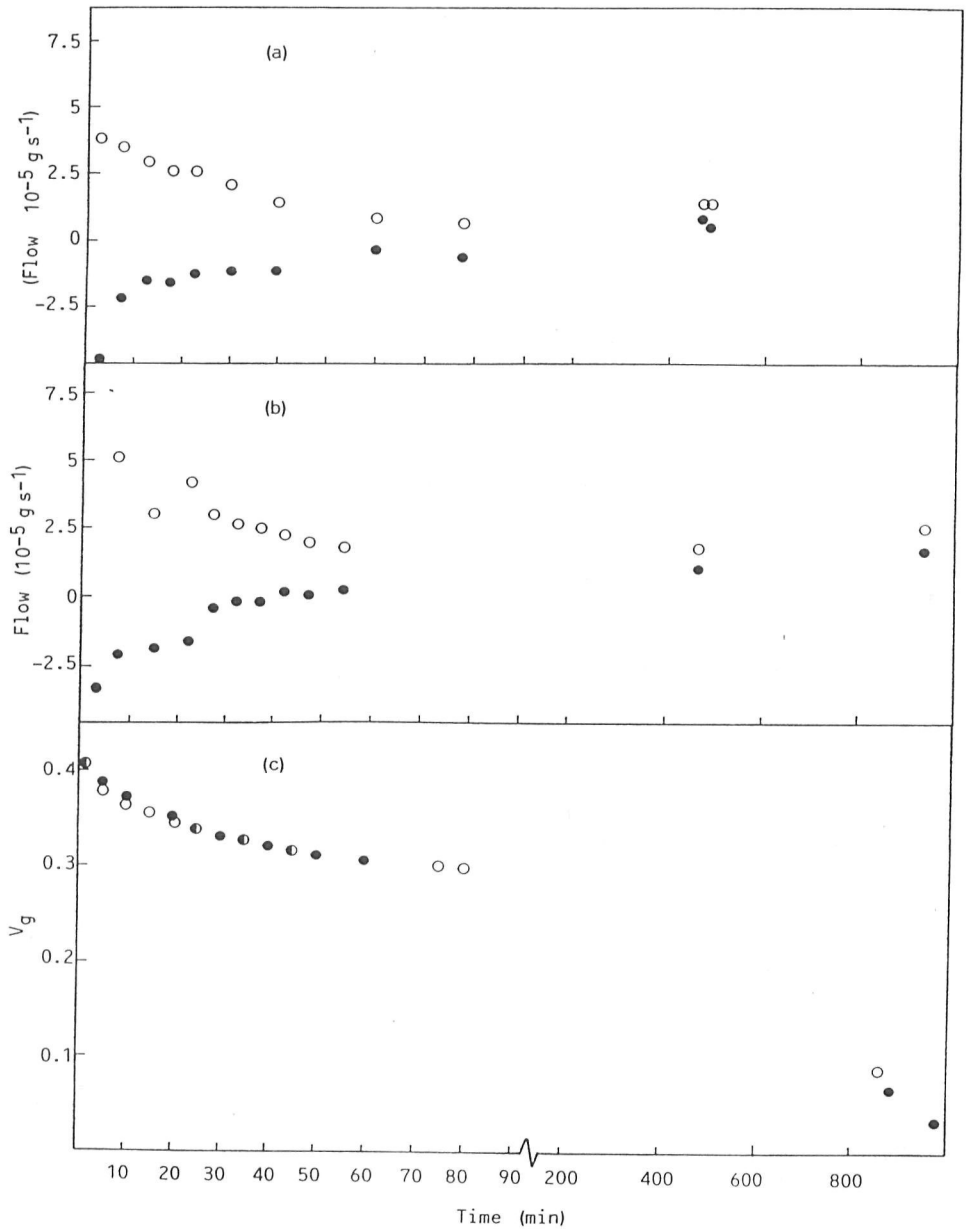

Figure 6. Experiment 1: (a) flow of water between the flask A and the segment (open circles), or between the segment and the flask B (solid circles), in the case of the KNO_3-perfused segment; (b) flow of water between the flask A and the segment (open circles), or between the segment and the flask B (solid circles), in the case of the azide-perfused stem (the sign convention is that the A to B flows were considered positive); and (c) variation of gas volume fractions (V_g) when segments were connected to flasks, as shown in Fig. 1 (open circles are KNO_3-perfused segments; solid circles are azide-perfused segments).

The water volume fraction of the xylem declined over 5 d from 0.64 to 0.50. In this period, the water potential declined from −0.5 MPa to as low as −2.6 MPa (Fig. 10). Acoustic emissions were recorded on several plants during the drying period, a single sensor being attached to an individual tree over periods of one day. All plants tested displayed acoustic emissions during this drying period. The typical patten of acoustic emissions began at 0900 h and increased rapidly during the morning. The maximum recorded on any plant was 42 counts s^{-1} on day 5 of drought. To illustrate this, Fig. 11 shows the signals from one plant over a 2-d period.

After resupplying the plants with sodium azide or potassium nitrate solutions (10 mol m^{-3}), their initial water volume fraction recovered to the initial value over a period of 24 h (Fig. 10). The water potentials of the leaves recovered also, but within 8 h of rewatering. The plants treated with azide recovered at the same rate as the controls.

Discussion

Refilling occurs in dead material

In experiments of this kind, it may be very difficult to kill

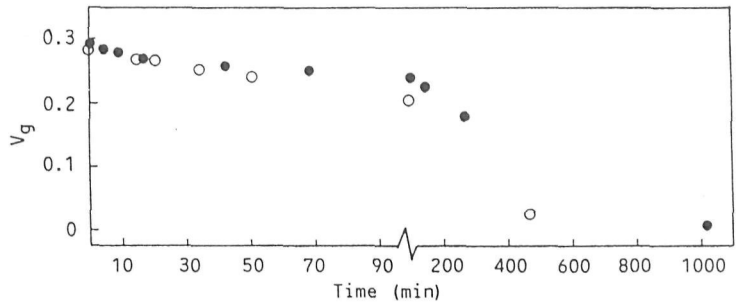

Figure 7. Experiment 1. Variation of gas volume fractions (V_g) when segments were connected to flasks at the same level. Open circles are KNO_3-perfused segments; solid circles are azide-perfused segments.

the xylem parenchyma completely, especially in intact plants when the intention is to keep the rest of the plants alive. An azide solution, applied to the cut stem, will ultimately reach the foliage where it may cause transpiration to decline. In the present case, however, the 10 mol m^{-3} azide treatment and, to lesser extent, the 5 mol m^{-3} treatment seem to have been effective in killing the xylem parenchyma, as judged from the respiration data, without side effects. The hot water treatment had the unfortunate side-effect of causing embolisms. This is evident from Fig. 9, where the hot water-treated plants had a much lower water volume fraction than the controls. Presumably, the increase in water vapour pressure, consequent on the temperature rise, caused bubbles of vapour to form. Under tension, very small bubbles would grow rapidly to fill the tracheids.

The absence of refilling in heat-treated xylem in experiments 1 and 2 was conspicuous. It is not known how the microstructure of tracheid wall may be influenced by high temperatures. Polysaccharides may have been hydrolysed, modifying the structure, decreasing wall permeability and thus impairing refilling. The fact that refilling was prevented by heat treatment in both segments and intact plants suggests that the same mechanism might be involved in both cases.

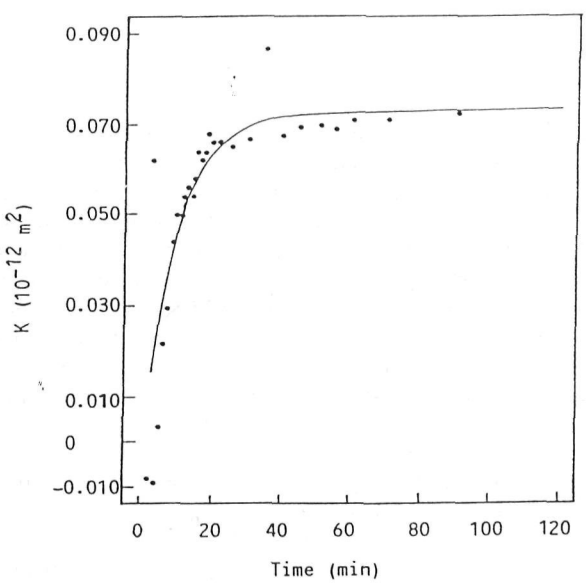

Figure 8. Experiment 1. Variation in permeability (K) as a function of time in one untreated stem segment. The fitted curve (see model in the text) is superimposed on the experimental points.

Table 3. Experiment 2. Osmotic potential, π, (MPa) of xylem sap extracted from the seedlings. W indicate the never-stressed, plants, D, the droughted plants before rewatering, W_4 and W_{216} the control plants after 4 and 216 h from rewatering, respectively; SE is the standard error, $n = 3$

	W	D	W_4	W_{216}
π	0.072	0.117	0.112	0.085
SE	0.009	0.011	0.022	0.001

The main concern in this work was to compare refilling in killed xylem with that in live material, using both branch segments and stems of young trees after treating the tissue with azide. In most cases, the xylem rehydrated over a period of 1–3 d. In the process of rehydration, the volume fraction of water increased and the volume fraction of gas fell. Thus, much of the water taken up must have entered embolized cells and any gas in them must have dissolved in the water. Therefore, it is possible to speak unequivocally of refilling, as refilling must have constituted much, probably most, of the rehydration.

There was no indication that azide-killed xylem was slower to refill than live material in both branch segments and small trees. Therefore, it can be concluded that the parenchyma had no role in the refilling process, and that the process must have been purely physical or physico-chemical at the negative pressures present in the water supply in these experiments.

Refilling mechanisms

The possibility that capillary action can account for the refilling will now be considered. The gas in an embolized tracheid may be considered to be an elongated bubble, with a radius dependent on the geometry of the tracheid. When the residual water is present only at the ends of the tracheids, the radius of the water-gas interface will be small. The relationship between water potential and

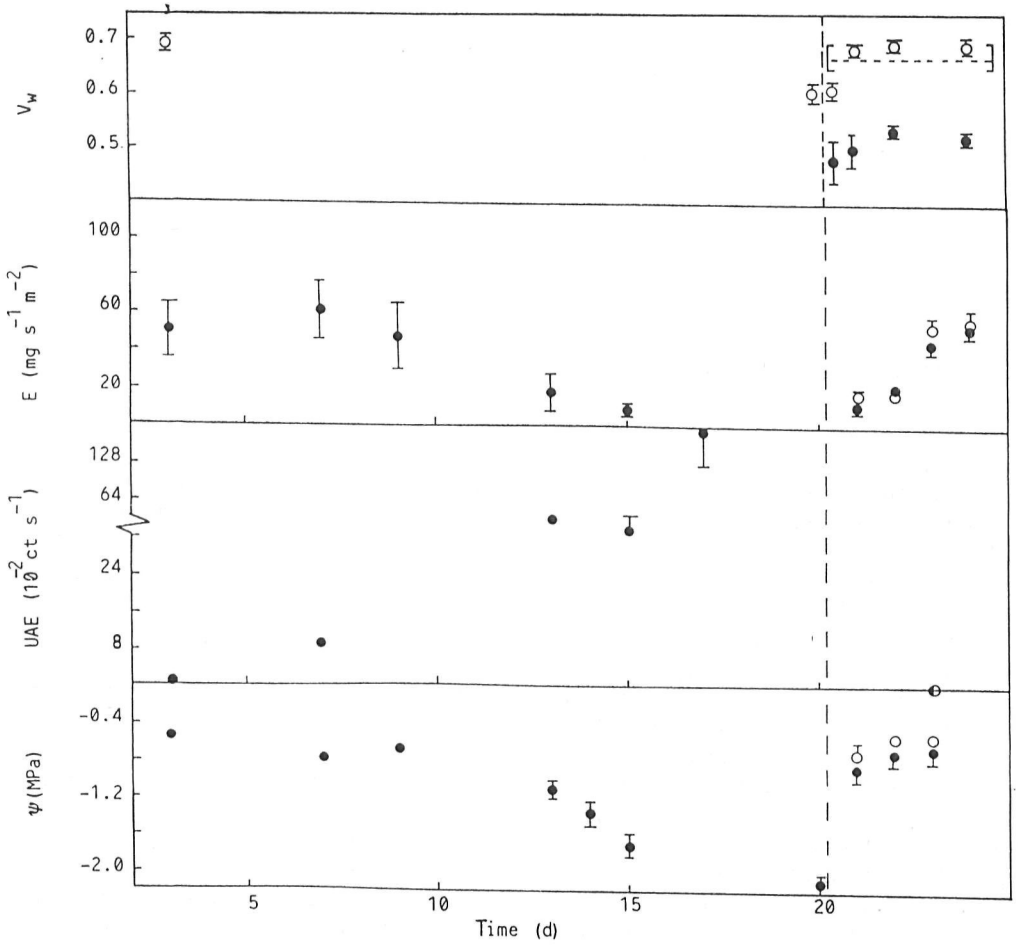

Figure 9. Experiment 2. Variation of stem water volume fraction (V_w), transpiration rate (E), ultrasound acoustic emssion (UAE) and dawn xylem water potential (ψ) during the drought and rehydration periods. The vertical dashed line indicates time of application of the heat treatment followed by rewatering on 28 August; after rewatering, open circles indicate the control plants, whereas solid circles indicate the heat-treated plants; the horizontal dashed line, within brackets, indicates the mean value of stem water volume fraction of never-stressed plants; vertical bars are standard errors, $n = 6$.

radius is well established. From the equation used by Skaar (1972), the water potential (ψ) of this residual water, assuming it to be pure water, can be estimated as:

$$\psi = -2\sigma/r \qquad (5)$$

where σ is the surface tension of water (7.3×10^{-2} Pa m at 20°C) and r is the radius of the water-gas interface. The radius of the tracheid lumen was 15 μm, from which we expect a water potential of −9.7 kPa. At the ends of cut segments, this suction would draw water from the available free water, which in experiment one, where water was taken up from flasks at a lower height than the segment, was at −2 kPa. Water can pass freely through tracheid walls as long as a potential gradient exists, according to permeability measurements made elsewhere (Palin & Petty, 1981; Petty & Palin, 1983). Thus, the segment might continue to absorb water, as those tracheids which are water-filled would be under tension and would absorb water until they reached a potential equal to that of the supply water. As water was sucked into an embolized tracheid by the pressure across the water-gas interface, the gas inside would come under positive pressure and consequently would dissolve. There have been few measurements of the gas composition of live wood but from available data it seems to be CO_2 and O_2-enriched air (Sperry *et al.*, 1987). When cavitation first occurred, the embolism would have contained water vapour in equilibrium with the water potential of liquid water in the walls, at the bordered pits and in the tapered ends of the tracheids. Gas pressure would be very low. The carbon dioxide derived from parenchyma and cambial respiration would then have diffused in followed by air from outside which would occupy the cavitated tracheids. The constituent gases of air do not all dissolve equally readily in water, nitrogen being the least soluble (Table 4). Small bubbles might remain as water was sucked in, making the tracheid liable to recavitation, but very small bubbles would have

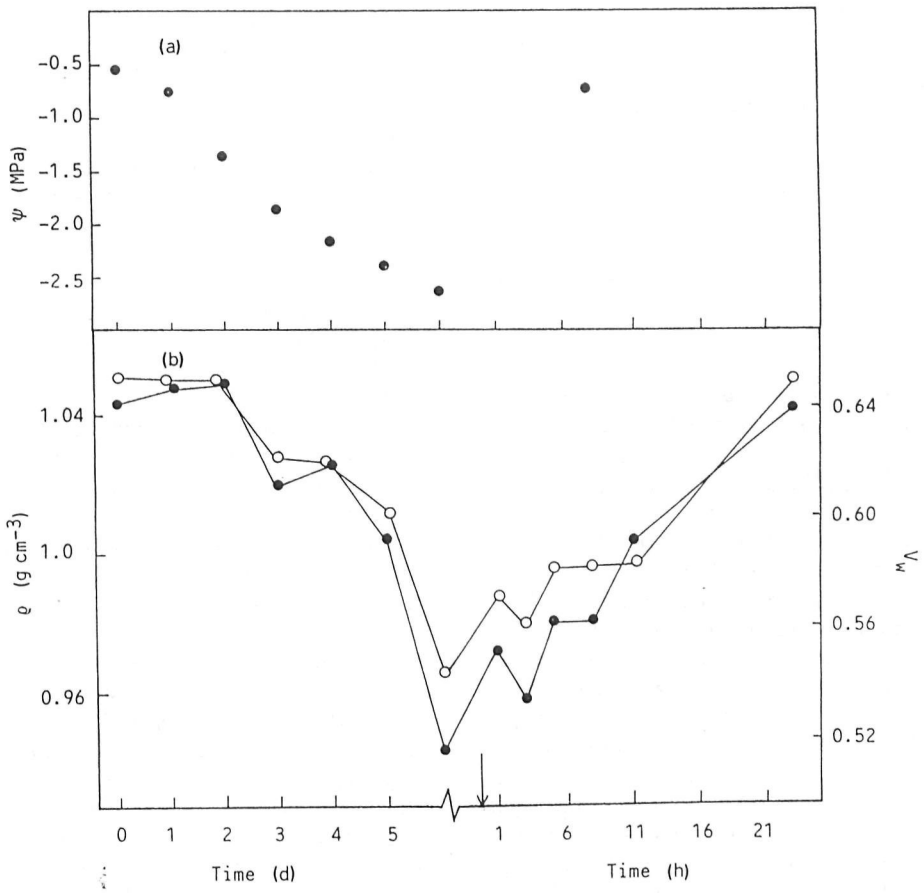

Figure 10. Experiment 3: (a) Variation of xylem water potential (ψ) of trees during the drought phase and after resupply of water (solid circles are the mean for all trees); and (b) variation of stem wood density (ρ) and stem water volume fraction (V_w), during the drought phase and after resupply of water (open circles indicate the control plants, solid circles the azide treated ones). The arrow indicates when water was resupplied: note the change of scale at this time. There are no significant differences between the rates of recovery and final water contents of treated and control plants.

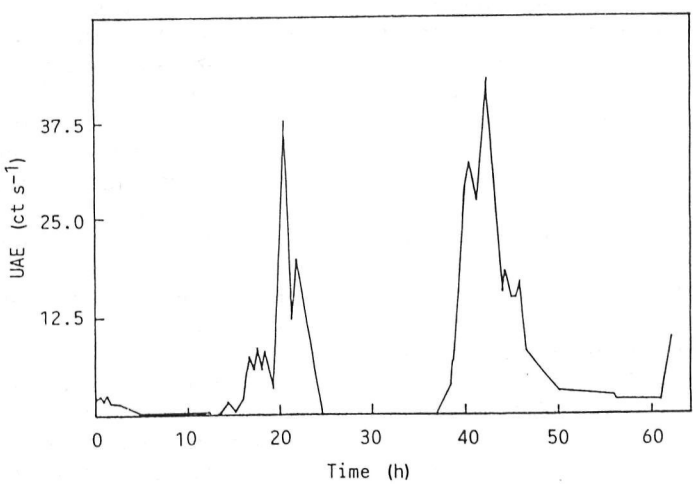

Figure 11. Experiment 3. Ultrasound acoustic emissions (UAE) from a water-stressed tree over a 2-d period. Time 0 corresponds to 1730h of 15 September.

Table 4. Gas solubilities at different temperatures (m^3 of gas in m^3 of water) when pressure of gas plus water vapour is 101.3 kN m^{-2} (from Kaye & Labey, 1973)

Gas	Temperature (°C)		
	10	20	30
Argon	0.041	0.032	0.028
CO_2	1.163	0.848	0.652
N_2	0.018	0.015	0.013
O_2	0.037	0.030	0.026

an extremely high pressure inside them, progressively forcing the gas into solution as the bubbles become smaller.

This hypothesis of refilling by capillarity explains rehydration in the segments from experiment one, where the tensions in the applied water were small. However, it does not easily explain refilling in the whole-tree experiments, where during refilling the twig xylem water potentials were much lower than those calculated across the bubble interface in the cavitated tracheids. The generation of more negative water potentials by capillarity requires gas-water interfaces with a smaller radius. These will occur if the interfaces in the tracheid lumens have withdrawn into the tapered ends of the tracheids. It is also possible that such interfaces occur in crevices between tracheids, although anatomical data on this point seem to be lacking. Another source of negative water potential is the presence of dissolved salts in the xylem water. In this work, solute potentials of expressed sap was never less than −120 kPa. Thus, the capillarity and osmotic potential together are unlikely to produce a potential much less than −130 kPa, once the water-gas interface has recovered to the extent that it occupies the main lumen of the tracheids.

Waring *et al.* (1979) and Edwards & Jarvis (1982) described relationships between xylem water content and water potential in *P. sylvestris, Pinus contorta* and *Picea sitchensis*. Two techniques were used, *viz.* forcing water from wood with a pressure chamber or allowing wood to equilibrate with osmotica. Using the latter method with *P. sylvestris,* Waring *et al.* (1979) found that a 50% reduction in relative water content was associated with a potential of −100 kPa. Such 'pressure-volume' curves suggest that the reductions in water content in the current experiment would have produced negative potentials in the tissue, that facilitate water uptake. It is presumed that most of the negative potential would originate from capillary forces as water-gas menisci withdraw into smaller and smaller corners of the tracheids. Thus, gas in refilling would come under pressure as water entered tracheids, and this gas would dissolve.

However, severe difficulty with this hypothesis of refilling arises when the tracheid ends have filled and the water meniscus encounters the main part of the tracheid. The radius of a *P. sylvestris* tracheid is ≃15 μm and the water potential to be expected from equation 5 is then only −9.7 kPa. Yet we know from Waring *et al.* (1979) and Edwards & Jarvis (1982) that bulk water potentials in the range of water content that must apply during this stage of refilling are much lower. Moreover, it is known from field observations that rehydration of Scots pine may occur when early morning needle water potentials measured at the base of the canopy are as low as −0.7 MPa (see data for Julian days 152 and 153 in Waring *et al.*, 1979).

The possibility cannot be excluded that the xylem water potentials at the site where refilling was measured in the stems may have been rather higher than was measured in the twigs. It is, none the less, very unlikely that the stem water potential could have been high enough (≃−0.1 MPa) for refilling to have occurred by capillarity when the twig water potentials were in the range −2.6 to −0.5 MPa.

Another possible refilling mechanism is the condensation of water within the tracheid when the temperature falls at night. Cycles of daytime warming and nocturnal cooling might lead to evaporation of water from the (wet) walls to the void, followed by condensation and a consequent increase in the volume of liquid water. As this liquid water accumulates, the gas in the void would come under pressure, assisting it to dissolve. However, calculation of the quantity of water that could possibly be condensed from a water vapour-saturated cavity shows that one cooling cycle would contribute a droplet of only 0.01% of the volume of the cavity. Very many cooling cycles would thus be required. Refilling in experiment three occurred over one day, with only one cooling period. Published observations of rehydration in conifers suggest that refilling may occur over 3 d (Peña & Grace, 1986), 14 d (Borghetti & Vendramin, 1987), or 80 d (Waring & Running, 1978). Thus, it seems likely that condensation contributes only minutely to refilling. To sustain the process as continuous distillation would require a continuous energy source and sink at opposite ends of a catena of cells. This is perhaps possible in some restricted ecological conditions, when the plant stem receives a strongly unidirectional radiation flux.

Alternatively, where refilling occurs at xylem water potentials of less than −100 kPa, it may require that the wall of the tracheid is chemically active and lowers the potentials far below the value expected by capillarity. The surface of wood has gel-like properties, and attracts water, probably as a result of hydrogen bonding between H_2O and the hydroxyl groups which exist in the cellulose and hemicellulose fractions of the cell walls (Skaar, 1972). The surface potentials of plant walls are not well understood, and the electrical fields involved are thought to act over nanometer distances (Tyree & Karamanos, 1981). It would seem that the time is ripe to re-examine this question.

A further alternative has been suggested by Milburn (1979) that a common source of water for refilling could be 'reverse transpiration', the uptake of water from rain or dew through cracks in the leaf surface. In this case,

the gravitational potential would be analogous, and similar in magnitude, to that in root pressure (Sperry *et al.*, 1987). It is well known that pine fascicles have the ability to take up water from an uncuticularized zone at the needle base and this may often happen in the natural environment (Leyton & Juniper, 1963), but could not have occurred in the conditions of the experiment.

Acknowledgments

The experimental work was conducted at the Department of Forestry and Natural Resources, University of Edinburgh, Edinburgh, U.K. M. Borghetti and A. Raschi were supported by a CNR-NATO senior fellowship and by a grant from the British Council, respectively. W.R.N. Edwards was supported by DSIR, New Zealand.

We thank Paul Van Gardingen for assistance in measuring respiration rates of stem segments.

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