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# Mechanisms of Long-Distance Water Transport in Plants: A Re-Examination of Some Paradigms in the Light of New Evidence

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# Mechanisms of long-distance water transport in plants: a re-examination of some paradigms in the light of new evidence

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[Plates 1 and 2]

## SUMMARY

According to the widely accepted Cohesion Theory, water is pulled by transpiration from the roots through the xylem to the leaves.

It is believed that this process results in the development of large tensions (negative pressures) in the xylem. In this chapter we re-examine some of the indirect methods that were used to support the formulation of this theory. We conclude that because of ambiguities inherent in the interpretation of the results obtained by these approaches the evidence in support of the Cohesion Theory is not conclusive. Direct measurements of xylem pressure in herbaceous plants and tall trees have yielded values of tensions that are inconsistent with the Cohesion Theory. In the light of the data from the xylem pressure probe and nuclear magnetic resonance (NMR)-imaging, we believe that several forces may be responsible for long-distance water transport in plants. These include tension, osmotic pressure, capillary and air–water interfacial forces.

## 1. INTRODUCTION

The problem of the ascent of water in vascular plants, particularly tall trees, has attracted the attention of botanists for more that a century. Several theories explaining the ascent of sap have been proposed over the years (Askenasy 1895; Zimmermann & Brown 1980). In modern textbooks it is assumed that transpiration pulls water from the roots to the leaves to heights greater than could be achieved by use of a vacuum pump. This implies that the water in the lumen of the relatively large xylem vessels must be under considerable tension (negative pressure), at least in plants taller than the height of a water column that can counterbalance the pressure of the atmosphere (i.e. 10 m). The tension gradient in a standing (continuous) vertical water column should thus be at least 0.01 MPa $m^{-1}$ in a non-transpiring tree (e.g. before sunrise). If transpiration occurs, a substantial steepening of the gradient should be expected because of the high resistances to water flow.

The tensile strength of water is sufficiently high to meet the demands encountered in pulling the water to the top of the tallest tree. However, when the so-called Cohesion Theory was introduced by Dixon & Joly (1984) many investigators in the field did not believe that it fully explained the ascent of water (and solutes). These doubts arose because:

(i) the existence of gradients of negative pressure in the water-conducting vessels for any length of time had never been demonstrated directly; and
(ii) it was hard to visualize how continuous water flow could occur in the postulated metastable state for long periods of time because of the vulnerability of the xylem to cavitation and embolism.

The xylem pressure probe developed recently by Zimmermann and co-workers (Balling *et al.* 1988; Zimmermann 1989; Balling & Zimmermann 1990; Benkert *et al.* 1991) allows the direct measurement of sub-atmospheric and negative pressures in the xylem of intact plants. Many of the data obtained by means of this technique appeared impossible to fit into the standard framework of plant water relations and suggested that the traditional view of water ascent may require revision. This has revived a rigorous and very controversial debate about the mechanisms of water ascent (Passioura 1991).

In an effort to overcome this impasse in our understanding of plant–water relations, and mechanisms achieving ascent of sap, we will critically analyse the information which can be obtained from currently available indirect experimental techniques for determination of 'xylem tension'. We will then discuss

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recent data on xylem pressure and water distribution in tissues, measure on intact herbaceous plants and tall trees, using the xylem pressure probe and high resolution nuclear magnetic resonance (NMR)-imaging, respectively. This approach is intended to provide the necessary background to evaluate data and conclusions published in the plant–water relations literature, particularly with regard to the use of traditional indirect methods for estimating xylem pressures in intact plants. Finally, we will discuss processes which may be involved in water ascent against gravity. These processes include water movement in capillaries, osmotically induced and transpiration-driven water flow as well as *Marangoni* streaming, a powerful and hitherto unconsidered process for water ascent in large vessels.

## 2. CLASSICAL AND CURRENT METHODS

The various approaches developed in the past for estimating the high xylem tensions postulated by the Cohesion Theory suffer from the disadvantage of being indirect; they depend on certain assumptions which are difficult to verify. The most important methods are: (i) the 'vacuum pump – leafy twig' experiment of Renner (1912, 1925); (ii) the pressure bomb technique (Scholander *et al.* 1965); (iii) the 'root pressurization' technique (Passioura & Munns 1984); (iv) psychrometry (Brown & Tanner 1981; Turner *et al.* 1984).

In Renner's approach, a constriction is placed near the base of an excised twig. Xylem tension is then calculated from the change in the rate of water uptake into the twig which occurs when the leaves are replaced by a vacuum pump (Renner 1925). It was assumed that the vacuum pump imposed an absolute pressure of +0.009 MPa on the vessels of the twig. Using this technique, Renner and other workers of his time (Nordhausen 1919; Ursprung & Blum 1916), estimated absolute xylem pressures down to a minimum of −0.9 MPa. Their values were usually in the negative range, but positive values between zero and atmospheric were also recorded.

Renner's approach is useful only if the cross-sectional architecture of the conducting pathway remains unchanged when the leaves are replaced by a vacuum pump. This is because only the velocity of flow in individual vessels, not the total flow rate, is coupled to the driving pressure gradient. If some vessels become blocked by Renner's treatment, then the results will overestimate xylem pressures *in vivo*.

Indeed, recent measurements of flow velocity in xylem vessels by injection of dye through the xylem pressure probe have clearly shown (Benkert *et al.* 1991; see below) that in some plants, and in twigs excised from tall trees, the flow velocity was often much higher in leafless twigs with a vacuum pump attached than in the corresponding excised leafy twigs (Zimmermann *et al.* 1993*a*).

With the pressure bomb technique, the 'balancing' chamber pressure required to force sap to the protruding cut end of an enclosed and pressurised leaf or twig is measured. This is assumed to be equal to the tension which existed in the vessels prior to excision. The pressure bomb technique is currently the standard method for exploring the water relations of plants in both laboratory and field. Bomb overpressures between 1 and 12 MPa are reportedly required to reach balancing pressure in most leafy tissues (Lange *et al.* 1972; Turner *et al.* 1984). Despite the fact that these values are an order of magnitude higher than those from Renner-type experiments, little effort has been made to test the validity of the pressure bomb technique.

Several workers have used the pressure bomb to assess the vertical gradients of pressure which exist in the xylem of tall trees. Although the gradients reported in some of these studies are cited as being consistent with the Cohesion Theory, the gradients observed in others were often less steep than predicted by the Cohesion Theory. In one study, Tobiessen *et al.* (1971) concluded on the basis of their measurements in a giant sequoia tree that the pressure bomb technique and/or the Cohesion Theory must be called into question. Similarly, our pressure bomb measurements performed on twigs taken from heights of up to 35 m on the tropical tree *Anacardium excelsum* (Panama) in the early afternoon on a dry day during the rainy season, were also inconsistent with the Cohesion Theory. A vertical tension gradient was not detected and the absolute pressure value of about −0.3 MPa measured at 35 m would not be sufficient to overcome frictional resistance and to supply the transpiring leaves at this height with water from the roots. In contrast, during the preceding dry season balancing pressures of the order of 1.5 MPa were recorded for excised twigs of the same *A. excelsum* tree (F. C. Meinzer, unpublished observations). Similar or even higher balancing pressures up to 2.5 MPa were also recorded for twigs taken from *Salix fragilis* (Germany), *Fagus sylvatica* (Germany) and an *Argyrodendron peralatum* tree (Queensland, Australia) at heights up to 35 m (Zimmermann *et al.* 1993*a*, unpublished data). Although these trees had been subjected to prolonged drought, xylem pressure probe recordings in their vessels revealed absolute values and diurnal changes in tension of the order of about 0.1 MPa, comparable with those measured in *A. excelsum* during the rainy season (see below).

This large discrepancy between pressure bomb and xylem probe estimates of tension in droughted plants suggests that the water content of the leaves, and not simply xylem tension, plays a role in determining the bomb overpressure required to force sap through the protruding cut surface of the excised twig. High resolution $^{1}$H-NMR-imaging of the leaves taken from *A. excelsum* during the rainy season (Zimmermann *et al.* 1993*b*) revealed signal intensities throughout the cross-section which were comparable to those of pure water (plate 1, figure 1). This indicated that the relative water content of the tissue was extremely high. By contrast, NMR-microscopy of leaves of trees subjected to severe drought revealed that large amounts of air were present throughout the tissue (plate 1, figure 2).

The percolation theory (Kaye 1989) predicts that




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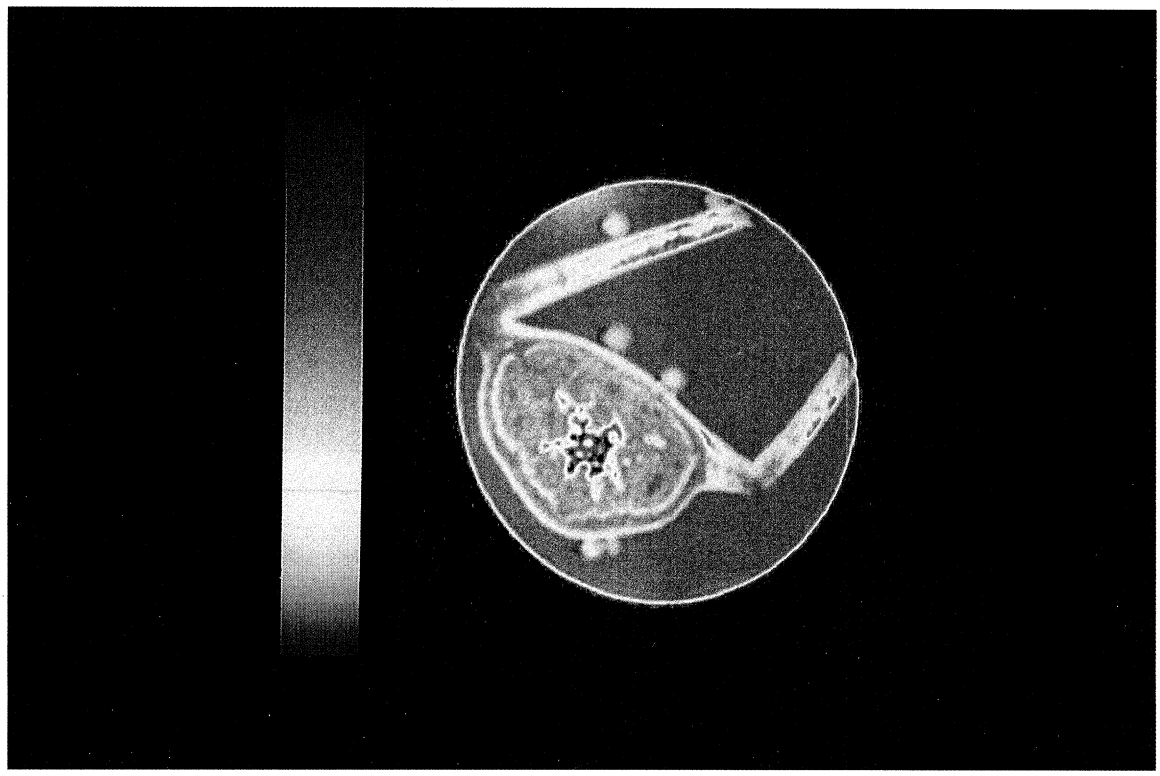

Figure 1. Typical cross-sectional $^{1}$H-spin-echo NMR image of the region close to the midrib of a leaf of an *Anacardium excelsum* tree growing near Panama City, Panama. The leaf was excised at 35 m height during the rainy season. Slice thickness of projection was 400 μm, field of view 5 mm × 5 mm, magnetic field strength 11.7 T, the magnetic gradient up to 350 mT m$^{-1}$ and the matrix 128 pixels × 128 pixels. The spin echo time was 3.4 ms. The column shows on a linear scale the percentage of water concentration in the tissue (red = 0% and violet = 100%). Note that with the exception of the pith region (which contains air) the tissue is well supplied with water. The average signal intensity is as high as in the surrounding water (E. Kuchenbrod, A. Haase, R. Benkert, F. Meinzer & U. Zimmermann, unpublished data).

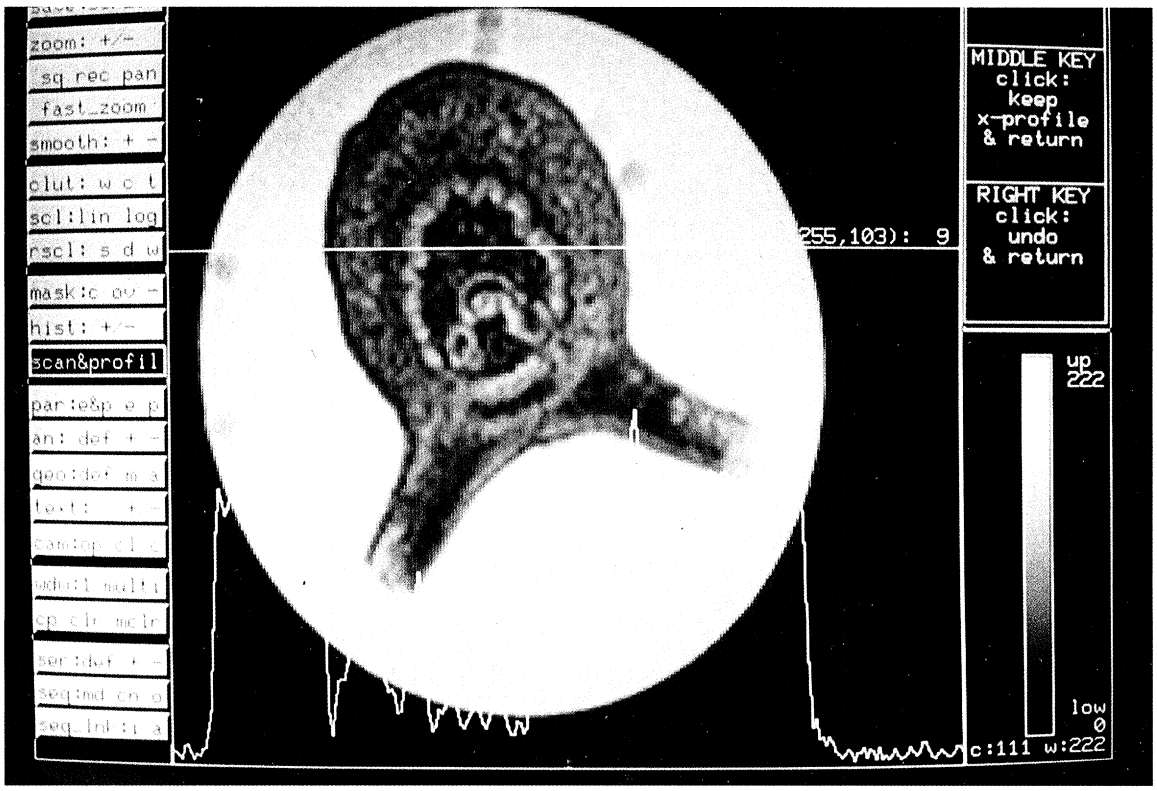


Figure 2. Typical $^{1}$H-spin-echo NMR image and signal intensity profile of a cross-section of an excised leaf of *Argyrodendron peralatum* (Queensland, Australia, leaf cut at 32 m height). The tree had been subjected to prolonged drought. Experimental conditions were as in figure 1 (E. Kuchenbrod, A. Haase, R. Benkert, H. Schneider, G. Zimmermann & U. Zimmermann, unpublished data). It is evident that the signal intensity throughout the tissue is very low indicating the presence of a substantial amount of air and correspondingly a relatively low water content. Xylem pressure probe measurements confirmed this result (see figure 8).

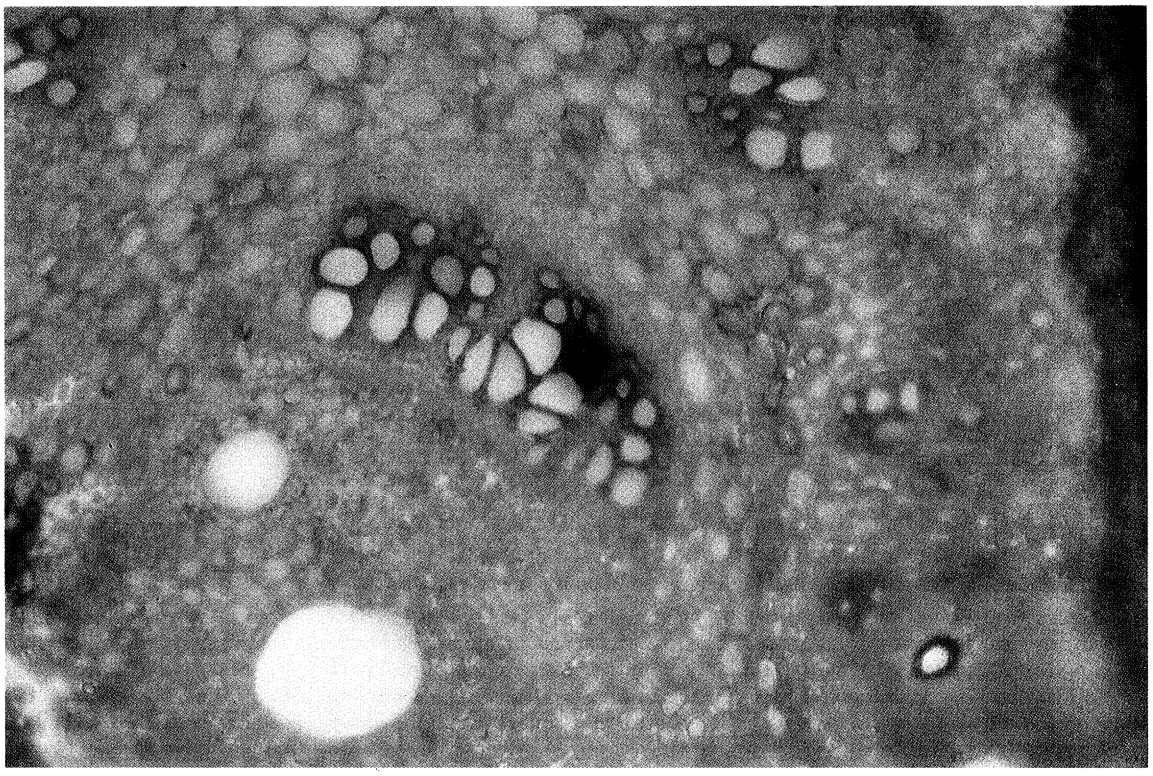

Figure 9. Insertion of the microcapillary of the xylem pressure probe into a xylem vessel of a leaf of *Anacardium excelsum* (close to the midrib). The tip of the microcapillary was filled with Indian ink. Note that the dye was sucked into only one of the vessels (R. Benkert, F. Meinzer & U. Zimmermann, unpublished data).



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liquid cannot enter the air-filled pores of a porous structure until a threshold pressure is applied. If the wall material is partly hydrophobic (as is the case for the lignified xylem vessels), threshold values of the order of 0.16 to 1.6 MPa are expected (pore radii between 1 and 0.1 μm, respectively).

There are additional reasons why the presence of air-filled spaces and pores in the tissue prevent or attenuate the propagation of externally applied pressure. For example, if we consider two compartments joined by capillaries, then pressure propagation occurs very rapidly if the system is completely filled with water. However, if the second compartment contains air, pressure cannot increase until a substantial mass flow through the capillary from the water-filled compartment has taken place. According to the Hagen-Poiseuille equation and to xylem pressure probe measurements on microcapillary systems (see below) this requires at least 7–10 min (radius of the vessels less than 5 μm). This is much longer than the period normally used to determine the balancing pressure for an excised plant sample assuming that the bomb pressure is increased at a rate of 0.3 MPa $min^{-1}$.

Furthermore, let us assume that an air-filled space in the tissue has a volume, $V_1$, at the original pressure, $P_{int}$, in the tissue. After excision of the leaf, the cut end is at atmospheric pressure. Upon application of a bomb pressure, $\Delta P$ (equal to the original pressure difference between xylem and environment), the air-filled space will assume a volume $V_2$ (once equilibrium has been established):

$$V_2 = V_1\ P_{int}/(P_{int} + \Delta P), \qquad (1)$$

where $P_2 = P_{int} + \Delta P$. This means that, despite the application of the original pressure difference, $\Delta P$, the xylem sap will not assume the same height as before excision. Higher bomb pressures must be applied and the tissue must be compressed further in order to bring the water back to the cut surface.

In agreement with these considerations, pressure probe measurements in the xylem of intact tobacco plants and in excised leaves have shown that, up to a certain threshold value of about 0.25 MPa, ambient overpressure is not transferred to the tiny xylem vessel compartment within the period of time of pressure application (Balling & Zimmermann 1990; figure 3). The pressure recorded in the whole tissue (by sealing the probe to the cut end of the petiole) was less than that applied and increased with applied pressure in a ratio of 1:1 only above the threshold value. In contrast, after vacuum pre-infiltration with water, changes in bomb overpressure were instantaneously recorded in a ratio 1:1 both in the leaf tissue and the vessels (Balling & Zimmermann 1990).

Similarly, pressurisation of submerged roots in intact plants or leafy shoots severed from roots yielded an immediate pressure response in the vessels at low bomb pressures because of the hydraulic continuity between the xylem and the external solution (Zimmermann *et al.* 1991). In the case of the roots, however, the pressure response was less than 1:1

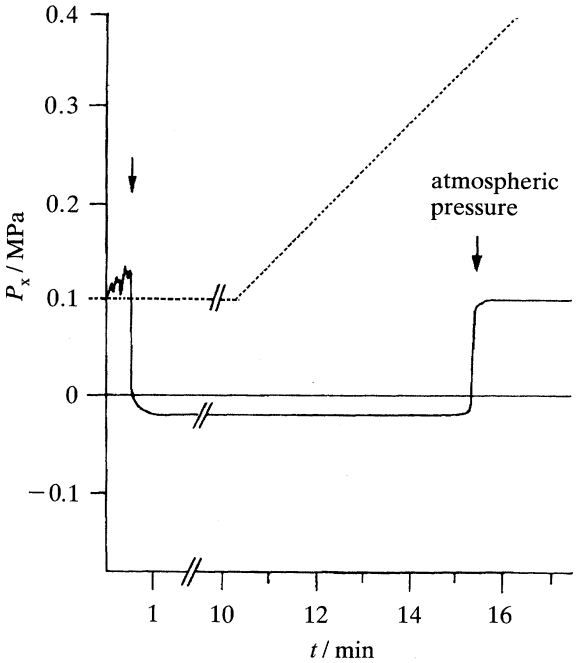


Figure 3. Kinetics of pressurization of an excised tobacco leaf in a Scholander bomb (dotted line) and simultaneous recording of the response in xylem pressure (solid line) in the portion of the petiole exposed to atmospheric pressure. The downward pointing arrow indicates penetration of a xylem vessel in which the initial absolute pressure was slightly negative. About 10 min after penetration of the probe the bomb pressure was continuously increased. The xylem pressure remained constant until the overpressure in the bomb exceeded 0.25 MPa (relative to atmospheric pressure). Above this value the xylem pressure increased within about 30 to 50 s to atmospheric. At this moment liquid appeared at the cut surface. (Redrawn from Balling & Zimmermann 1990.)

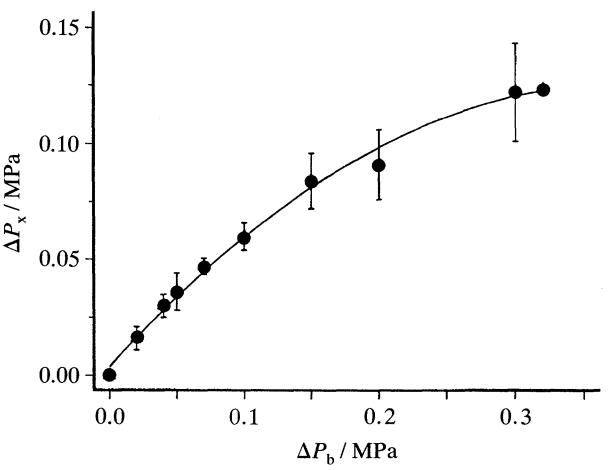


Figure 4. Step-wise pressurization of water-immersed roots of a tobacco plant in a Scholander bomb. The xylem pressure probe was inserted into a vessel of the stem outside the bomb. The end-value of the increment in xylem pressure, $\Delta P_x$, in response to the overpressure in the bomb, $\Delta P_b$, followed a sigmoid curve. Note that an overpressure of about 0.30 MPa was needed to change the xylem pressure by about 0.1 MPa. (Redrawn from Zimmermann *et al.* 1991.)




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(figure 4). This is expected because of the high (frictional) root resistance.

A related observation is that of Renner who demonstrated that rapid drying of tissue at the cut end of twigs excised in air had a tremendous effect on the magnitude of the negative xylem pressure value estimated from the leaf–vacuum method. From the foregoing considerations it can be predicted that balancing pressures for excised twigs should be reduced dramatically by cutting and maintaining them under water. In *Coffea arabica*, for example, the balancing pressure of excised twigs recut under water and pressurized in a water-filled chamber was even consistent with the absolute sub-atmospheric pressure of about +0.06 MPa determined with the xylem pressure probe (U. Zimmermann, unpublished observations). Balancing pressures for twigs excised and pressurized according to conventional pressure bomb protocol differed from those determined with the xylem probe by more than 0.3 MPa.

These observations and considerations indicate that air-filled spaces interfere with the propagation of pressure across the tissue. The compression of the leafy tissue by the bomb overpressure creates additional resistances which prevent short-term pressure equilibration between the vessel compartment and the surrounding tissue. The excised leaf apparently reacts as a simple hydraulic system only when its water content is high (and its compressibility is low).

A frequent assumption in Scholander bomb experiments is that when a leaf is first excised, the 'high' internal tensions cause water in the xylem to recede from the cut end (although some authors assume that the air–water interface may not recede beyond the first vessel–vessel junction). High-resolution $^{1}$H-NMR-imaging of tissue a few millimetres from the cut ends of excised tobacco leaves (Zimmermann *et al.* 1991) revealed that this assumption is not valid. The signal intensity profile across the petiole indicated that most of the vessels were full of water.

In light of the above considerations we think that at the moment, on balance, it is logical to question the data obtained with the Scholander bomb technique, in particular because there are no clear-cut model experiments which convincingly demonstrate that the balancing pressure is numerically equal to the original tension in the vessels.

The root pressurization technique (Passioura & Munns 1984) is a highly sophisticated variant of the Scholander bomb. It allows (almost) intact plants to be used. Plants are grown with their roots in sand inside steel pots. The pots are enclosed in a pressure chamber, with the shoot of the plant protruding. Nutrient solution can be supplied to the roots via a port in the lid of the pot. The entire water-immersed root system can thus be pressurized with a mixture of compressed air and nitrogen. The midrib near the tip of one leaf is exposed by trimming the leaf to a point, using a sharp razor blade. A piece of fine nylon tubing (0.5 mm i.d.) is placed over the exposed midrib, and the pressure in the root chamber is increased until water forms a meniscus inside this tube. Chamber pressure is then regulated to achieve 'balance', i.e. to maintain the meniscus stationary inside the tube at a given location. The pressure of water in the midrib is thus clamped at +0.1 MPa (=atmospheric).

At normal transpiration rates, the balancing pressure for well-watered herbaceous plants is about 0.3 MPa. If transpiration changes, the meniscus starts to move in the capillary and the balancing pressure is adjusted accordingly in a very short time in order to keep the meniscus in the original position.

Under certain conditions a linear dependence between the transpiration rate and the balancing pressure is found. For roots immersed in nutrient solution a nonlinear dependence between both parameters exists, and hysteresis phenomena occur when transpiration is decreased and the balancing pressure is correspondingly reduced. Xylem pressure probe measurements suggest (R. Benkert, unpublished data) that the hysteresis is probably attributable to the release of gas bubbles in the portion of the shoot outside the bomb, i.e. the plant is faced with a problem which is well-known from diving as 'the bends'.

As in the Scholander bomb technique, the 'balancing pressure' applied to the root system at a given transpiration rate is assumed to be numerically equal to the tension which existed in the xylem prior to pressurization (Passioura & Munns 1984).

However, this interpretation has to be questioned because in a pressure-clamped system (Wendler & Zimmermann 1982) the outflow of water (i.e. loss by transpiration in the root pressurization method) is measured against inflow of water (created by the balancing pressure) which must be equal at any time. Therefore, extrapolation from the balancing pressure to the original xylem tension is possible only if the root resistance and the osmotic pressure in the tissue and in the external medium (which must be equally overcompensated by the bomb pressure) are taken into account. Concurrent xylem probe and (short-term) bomb measurements on intact tobacco plants with their roots immersed in water have shown (Zimmermann *et al.* 1991; see also figure 4) that about 0.3 MPa external pressure must be applied to the roots to change the actual xylem tension by about 0.1 MPa. The hydraulic and osmotic resistances (which may be altered by root pressurization because of the compression of the air-filled spaces) must be known before the xylem tension can be calculated from the balancing pressure. If we assume that the pressure drop across the (osmotic) resistances in a root of a plant is of the order of 0.2 MPa (Passioura 1991) it can be easily shown that the root pressurization method and the xylem pressure probe yield comparable values for xylem pressure (see below).

With psychrometric methods (Brown & Tanner 1981; Turner *et al.* 1984) the water potential is measured by determining the chemical potential of water vapour in equilibrium with water in the tissue. The physical basis of this technique is clearly defined. However, xylem water cannot be accessed directly. Instead, its hydrostatic pressure is estimated from the water potential of vapour in equilibrium with sur-




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rounding tissues. This approach requires three assumptions if xylem pressure is to be estimated: (i) the water potential of the vessel lumen must be in equilibrium with that of the surrounding tissue; (ii) the osmotic pressure of water in the apoplast of the surrounding tissue must be negligible; and (iii) there must be no effective osmotic gradients between the xylem lumen and the apoplast of the surrounding tissue, either because solute levels throughout the apoplast are negligible, or because the reflection coefficient of the pathway is small. At present, it is not clear to what extent all these assumptions are valid.

In addition, *in situ* psychrometers have limited use because of difficulties with sealing, calibration and temperature and chemical water potential equilibration with the leaf. In view of these uncertainties, psychrometric estimates of xylem water pressure must be treated with extreme caution.

## 3. XYLEM PRESSURE PROBE

Xylem pressure can be measured directly within an individual vessel by using the xylem pressure probe. This probe was developed from the cell turgor pressure probe (figure 5). The xylem probe is filled with degassed water instead of oil (Zimmermann 1989) and it incorporates a water-compatible pressure transducer. The microcapillary of the probe is driven manually or electrically into the tissue. Penetration is stopped immediately when the transducer registers a pressure below atmospheric. Xylem pressure probe measurements in herbaceous plants and twigs of tall trees (under *in situ* conditions) have shown (Zimmermann *et al.* 1993*a*) that the xylem pressure attains subatmospheric or slightly negative values (down to about −0.2 MPa). These values are much smaller in magnitude than those measured with the indirect methods discussed above, but comparable to those obtained by the vacuum–leaf technique (see also below).

Experiments with model systems have shown that the probe will provide accurate measurement of negative pressures (if they are present) in systems which are either in equilibrium or in a (quasi-) stationary state.

In the Hepp-type bio-osmometer of Balling *et al.* (1988) an isolated vascular bundle of a leaf of *Plantago major* (or a pressure transducer) was sealed to one end of the glass capillary. The capillary was filled with degassed water and connected with the other end to a small rigid, water-filled chamber. The chamber and the glass capillary were separated by a semipermeable membrane supported on a horizontal metal grid. The chamber compartment was open to the atmosphere. Addition of sufficient osmoticum (more than 40 mOsm) to this compartment caused pressure in the

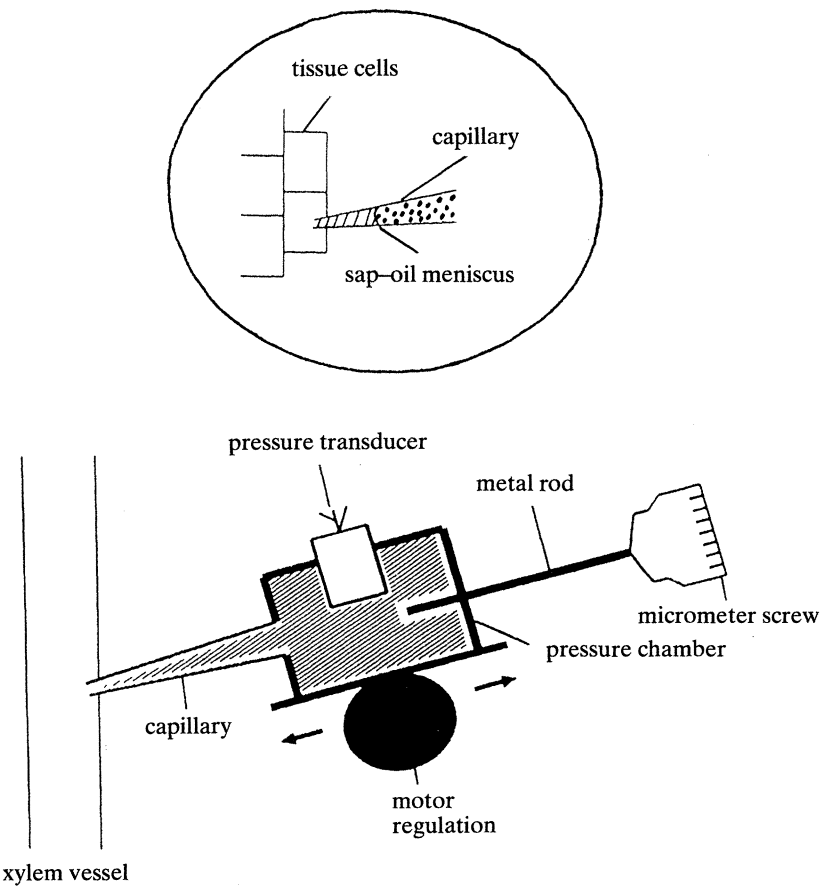


Figure 5. Schematic diagram of the xylem pressure probe and the cell turgor pressure probe (inset). In contrast to the cell turgor pressure probe, the xylem pressure probe is filled with de-gassed water and contains a special, water wettable pressure transducer. By displacement of the metal rot, volume increments can be injected into a punctured vessel to induce transient changes in xylem pressure (see figure 7).




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glass capillary (and the vascular bundle) to decrease below zero (absolute). The negative pressures generated in this way could be measured inside vessels of the vascular bundle by means of the pressure probe. Pressures down to −0.7 MPa could be established in the glass capillary of the osmometer without the attached vascular bundles.

However, such negative pressures (which represent a metastable state) could be recorded for only a few seconds to minutes because of spontaneous cavitation which increased dramatically with increase in negative pressure. When cavitation occurred the pressure within the system rose very rapidly to the saturation vapour pressure of water (+0.023 MPa at 20°C). Leaks also occurred frequently, probably because of vibrations in the environment. These were distinct from cavitations in that the pressure increased further, usually to atmospheric.

When the vascular bundles of *P. major* were attached to the one end of the glass capillary, insertion of the pressure probe into a xylem vessel after pre-establishment of a certain negative pressure did not lead to a change of the original value. However, at negative pressures below about −0.2 MPa in the vessel or glass capillary system, air was frequently sucked in from the tissue (see the discussion above of pressure propagation in the pressure bomb experiments).

Negative pressures were also measured with the xylem pressure probe in sections of tobacco stem (Balling & Zimmermann 1990). These sections were excised under water and clamped at each end by screw-clamps. When bathed in PEG solutions of a range of osmotic pressures, the sections yielded xylem pressures which decreased linearly with increasing PEG osmolality (slope 0.1 MPa per 40 mOsmoles $kg^{-1}$ water). This relationship extended down to xylem pressures of about −0.4 MPa.

Transient negative pressures were also measured (H. Schneider, unpublished data) when the tip of the probe was inserted into highly viscous solutions of certain polymers (2% DNA, polysaccharides secreted from seeds of *Lepidum sativum*). This occurs because the outward diffusion of water across the narrow tip of the probe capillary (about 10 µm o.d.) will be significantly faster than the inward diffusion of polymer. There will therefore be a transient net loss of water from the capillary with associated decrease in hydrostatic pressure within the probe. Mass flow of the viscous polymer solution into the tip is so slow that sub-atmospheric or slightly negative pressures can be developed.

Consistent with these findings, sub-atmospheric and somewhat negative absolute pressures have been recorded in the vicinity of polysaccharide-secreting glands and of resin ducts in various transpiring plants and trees (U. Zimmermann, unpublished data).

The xylem pressure probe also measured sub-atmospheric and negative pressures from drying capillary matrices such as tissue paper and string (figure 6). These materials are derived from the plant cell wall and should therefore be useful model systems (Zimmermann *et al.* 1993*a*). Negative (absolute) pressures down to −0.4 MPa could be measured, and the measured pressure was dependent on the water status of the material. Stable recordings could be held for extended periods – at least in the underpressure range. When negative pressures were recorded, the pressure decreased continuously until terminated by cavitation (to the vapour pressure) followed by slowly leakage to atmospheric pressure. The cavitation event was clearly audible (by ear) as a 'click'. These continuous decreases in pressure can be explained if it is assumed that the material is gradually drying out and its water content declining. However, such an effect would also be expected even if the material were not drying, and its water content were constant. This is because the mass flow of water of neighbouring tissues from the matrix directly under the tip is slow compared with the hydraulic capacity of the probe itself. According to the Hagen–Poiseuille equation the flow velocity in such small capillaries will be extremely low (see above), and it will decrease with the second power of the capillary radius as pressure falls. The various model experiments described above demonstrate that negative pressures, if they are present, will be detected by the xylem pressure probe.

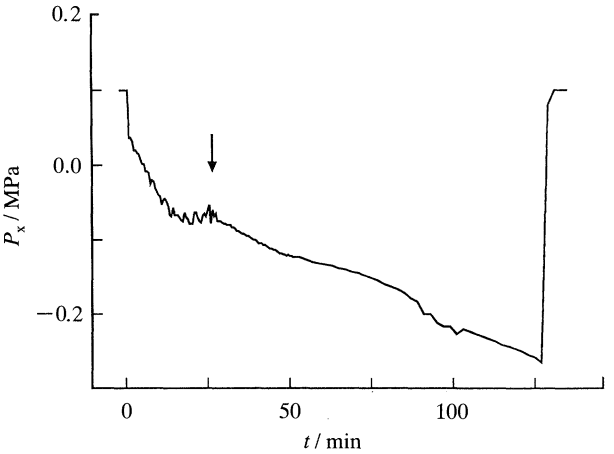


Figure 6. The microcapillary of the xylem pressure probe was advanced into a wetted parcel string (3 mm o.d.). Initially, this recording was noisy, not unlike some recordings from twigs *in situ* (see figure 10). When the draft from a nearby open window was eliminated (arrow) the recordings became much smoother. Cavitation followed by a leak occurred at an absolute pressure of −0.27 MPa. (Redrawn from Zimmermann *et al.* 1993*a*.)

The results of these experiments are also interesting from another standpoint. When the microcapillary of the xylem pressure probe is advanced into plants (e.g. in the Hepp-type bio-osmometer or in a tobacco stem) stable sub-atmospheric or negative pressures are recorded within two seconds. In contrast, if the probe is directed into a capillary model system or a viscous solution, a slow-kinetic response is observed and stable pressures may not be reached for many minutes.

This difference confirms that measurements in plants usually involve xylem vessels. If the probe tip were located elsewhere during these measurements, for example in cell and xylem wall capillaries, then the resistance to water flow around the tip would lead to




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slow-kinetic responses, as observed in the model systems.

Xylem pressure probe measurements from some plant tissues, especially twigs from tall trees, occasionally exhibit both fast- and slow-kinetics responses (figures 7 and 8). The slow-kinetics responses probably reflect recordings from very tiny xylem vessels (less than 5 μm), and from microcapillary regions of cell wall, respectively (Zimmermann *et al.* 1993*a*).

In tissues of well-watered plants the pressures recorded from microcapillary (slow-kinetic) regions within the vascular tissue may also reflect xylem tension. However, after a severe drought or when the humidity is low and the temperature high, the microcapillary regions may contain very little water. Under these conditions, insertion of the probe may provide a significant additional source of water for the microcapillary region in a transpiring tissue, so that a gradually increasing tension is created in the probe in a manner analogous to the filter paper and string experiment. Consistent with this explanation, after cavitation (at pressures below about −0.1 MPa) followed by leaks, negative pressures in these microcapillary regions were often re-established after the

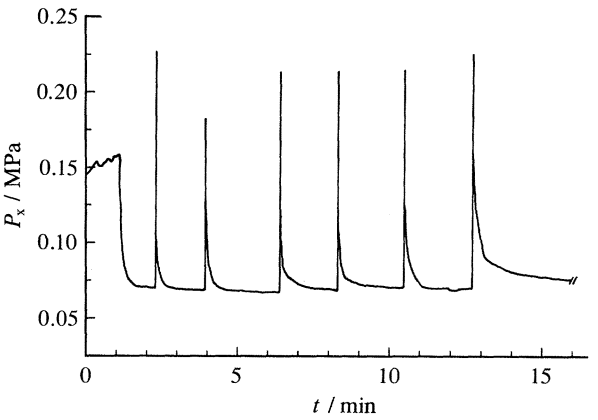


Figure 7. *In situ* xylem pressure recording in a one-year old beech twig (located at a height of 17 m) after insertion of the xylem pressure probe into a vessel (arrow). Within a short time a stable underpressure was attained. Then successively larger volume increments were injected into the vessel from the probe (by appropriate displacement of the metal rod). In each case, the original sub-atmospheric pressure was rapidly re-established. Note that the pressures are given in absolute values. (Redrawn from Zimmermann *et al.* 1993*a*.)

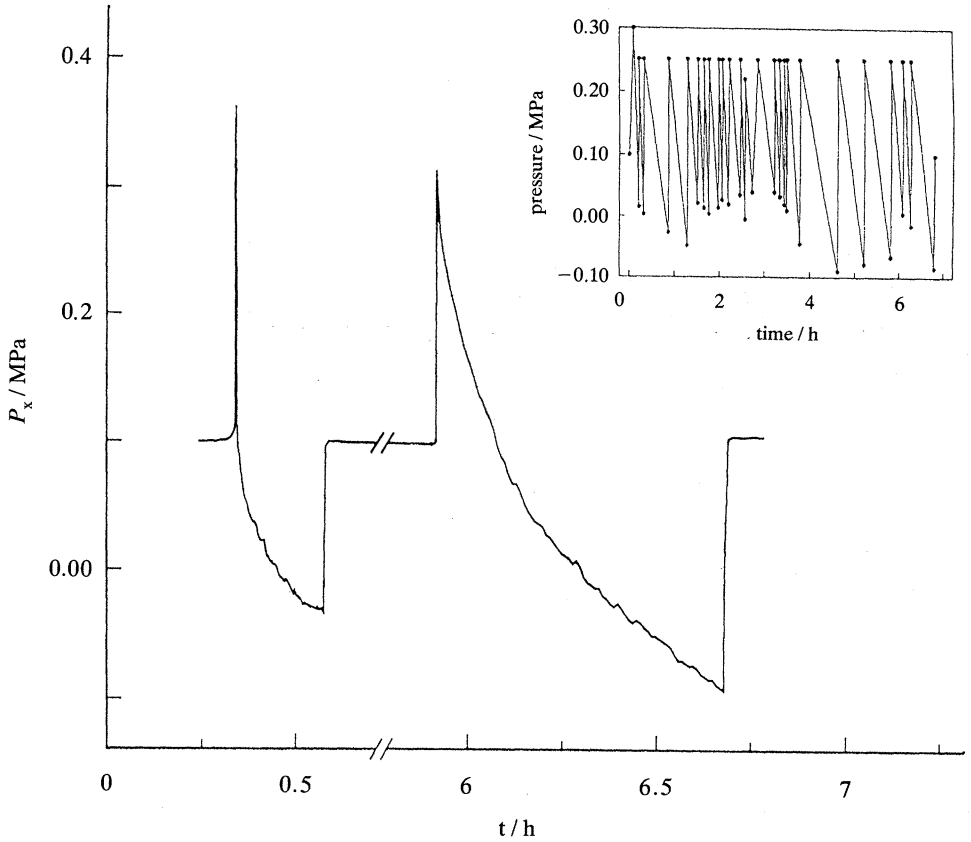


Figure 8. The xylem pressure probe was advanced into the microcapillary region (close to the midrib) of an excised leaf of an *Argyrodendron peralatum* tree (under laboratory conditions). The leaf belonged to the same batch of leaves on which NMR-image analysis was performed (plate 1, figure 2). A slow-kinetic type of pressure response was recorded in this considerably dehydrated leaf. The pressure decreased continuously down to a negative value of about −0.05 MPa. At this pressure, cavitation followed by a leak occurred with a rapid return to atmospheric pressure. Repeated injection of volume pulses (as schematically shown in the inset over a period of 7 h) caused slow-kinetic transient returns of the pressure to negative values, followed by a leak with recovery to atmospheric pressure. During this period, the pressure at which cavitation occurred assumed more and more negative values as indicated by the last recording (G. Zimmermann, H. Schneider, R. Benkert, S. T. Turton & U. Zimmermann, unpublished data). Similar results were obtained under *in situ* conditions on twigs of various tree species.




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injection of water pulses from the probe into the microcapillary region (figure 8).

## 4. FLOW VELOCITY

It is conceivable that xylem vessels penetrated by the capillary of the pressure probe become hydraulically isolated from the remainder of the xylem. However, measurements of dye movement from the penetrated vessel have shown (Benkert *et al.* 1991) that such isolation does not occur. In these experiments the microcapillary of the probe was loaded with a solution of dye before penetration. On initial penetration, the pressure of water in the xylem was below that in the probe capillary. Some dye was therefore sucked into the vessel from the capillary. Under conditions of reduced transpiration, or when Indian ink or dyes of high molecular mass (fluorescein-labelled dextran) were used, most of the injected dye is retained within the lumen of the punctured vessel (plate 2, figure 9). However, dyes of low molecular mass (e.g. fluorescein) were transported with the transpiration stream from injection sites in the lower stem to the upper part of the plant. In transverse sections taken from the stem at increasing distances above the site of injection, the dye was visible in progressively more vessels. These observations indicate that the punctured vessel was not disconnected from the remainder of the xylem network. The order of magnitude of the flow velocity was also estimated using this approach. The vertical displacement of dye over a set time interval was estimated as the distance from the injection point to the furthest stem section in which the dye could be seen. In tobacco plants the average flow velocity was about 0.3 mm $s^{-1}$ (Benkert *et al.* 1991). This was one order of magnitude smaller than the value calculated from the observed pressure gradient and the dimensions of the xylem vessels, according to the Hagen–Poiseuille equation. However, the flow velocity determined by dye injection agreed well with that obtained by the heat pulse technique for herbs (Zimmermann & Brown 1980). The difference between the values determined experimentally and those expected from theory is not very surprising because the Hagen–Poiseuille equation holds only for a cylindrical tube. However, for one-year old twigs of a willow tree very close agreement was observed between the measured and theoretically calculated values (about 5 mm $s^{-1}$; see Zimmermann *et al.* 1993*a*). The values were also consistent with those determined for this species using the heat-pulse technique (Zimmermann & Brown 1980).

These measurements prove that the punctured vessel had not become blocked or isolated. This was also apparent from the rapid equilibration of pressure which occurred when pulses or water were injected into a punctured vessel (figure 7). Taking these results together we can conclude that the punctured vessel is in direct hydraulic communication with the remainder of the xylem network.

Further progress in the determination of flow velocities in intact plants under various environmental conditions can be expected from NMR imaging measurements. It has been known for nearly four decades (Torrey 1956) that the intensity and time-dependent phase of an NMR signal is influenced by microscopic (molecular self diffusion) and macroscopic (flow) motions of water molecules. Today, flow NMR imaging is a frequently used technique in medical NMR applications. Studies on plants (and animals) have shown that flow velocities can be quantified in intervals between 50 μm $s^{-1}$ to 1 m $s^{-1}$ (Kuchenbrod *et al.* 1993).

## 5. CHANGES IN XYLEM PRESSURE

Xylem probe measurements in some rapidly transpiring plants (e.g. sunflower and sugar cane) and in twigs on transpiring tall trees (willow, beech, tropical trees) have shown that pressure in xylem vessels follows a characteristic pattern during the day. A typical example is shown in figure 10. Soon after daybreak (06.00 to 07.00 h or after lights went on in the glasshouse) xylem pressures were generally near atmospheric (about +0.08 to +0.09 MPa or higher). At this time of the day, tissue regions of lower pressures could not be found despite many insertions of the pressure probe into various parts of plants and twigs. As the day progressed, temperature rose and humidity decreased causing an increase in the rate of transpiration which was reflected in lower xylem pressures. By about 08.00 h average xylem pressures had decreased to about +0.04 to +0.06 MPa. This was found in all trees (and other species mentioned above), regardless of height on the plant.

During the next few hours, further 'erratic' decreases in xylem pressure were observed (depending

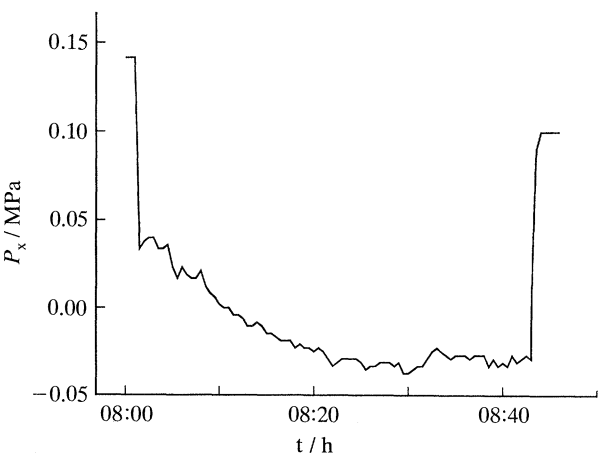


Figure 10. *In situ* pressure probe recordings in a xylem vessel of a transpiring leaf of *Anacardium excelsum* at 35 m height (near Panama City, see figure 1). The penetration of the vessel can be detected by the rapid drop of the pressure from above-atmospheric to an sub-atmospheric value. The measurement shown in this figure started 2 h after daybreak. It is evident that, on average, the pressure dropped over the next 45 min. Cavitation and a leak, which occurred at an absolute pressure value of about −0.03 MPa, were induced by vibrations of the twig. Note the 'erratic fluctuations' of the xylem pressure during the timecourse of the development of tension (Redrawn from Zimmermann *et al.* 1993*b*.)




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strongly on environmental conditions). The pressure in the xylem often recovered slightly between successive pressure drops (figure 10). The general decrease in xylem pressure towards noon probably reflected increasing transpiration rates over this time. During *in situ* measurements on twigs of the tropical trees *Anarcardium excelsum* (in the rainy season) and *Argyrodendron peralatum* (in the dry season) it was observed that xylem pressure always decreased more strongly when a light breeze arose. It is clear that in these cases the breeze caused an increase in transpiration rate and, in turn, a decrease in xylem pressure.

By about 09.00 to 11.00 h pressures in the xylem reached zero or even became negative. However, on all days, the wind speed increased perceptibly around this time. During outdoor measurements this caused strong vibrations of the twigs and branches resulted in cavitations and leaks (figure 10). Vibrations were, however, not a problem during laboratory measurements on plants exposed to an air stream, and it was often possible to record the diurnal pattern of xylem pressure down to fairly large negative values. Under laboratory conditions, the 'erratic' fluctuation in xylem pressure was also observed to some extent. In the experiment on sunflower shown in figure 11 the pressure frequently moved back and forth between the positive and negative ranges. Over time, however, the average pressure continued to decrease and the duration of periods of near-constant pressure became longer. Eventually, between 11.00 and 12.00 h negative absolute pressures of about −0.15 MPa were reached. These pressures invariably terminated in cavitation. Just before cavitation, there was usually a period free of fluctuations during which pressure dropped continuously. Cavitation did not always end in leakage to atmospheric pressure. Often, pressure stabilised in the sub-atmospheric range (around +0.05 MPa), indicating that the punctured vessel contained small gas bubbles. Insertion of the probe into other vessels of the same plant or twigs on the trees around noon also showed values which were in the (positive) sub-atmospheric range.

Very recent xylem probe measurements on *A. excelsum* before midday at the beginning of the dry season revealed that in some vessels cavitation apparently occurred, but that in other xylem regions pressure increased from sub-atmospheric values to above-atmospheric values up to +0.2 MPa. Sub-atmospheric and even negative pressures were recorded in the same vessels again towards the afternoon and early evening (as in the other plants mentioned above).

A different pattern of changes in xylem pressure during the day was observed in plants (such as tobacco) which were well-watered and exposed to constant temperature (about 22°C) and relative humidity (about 40%). During the first two hours of the morning the xylem pressure was very sub-atmospheric as in the plants exposed to fluctuating conditions, but throughout the rest of the day xylem pressures were lower or even negative in plants held under constant conditions in the laboratory.

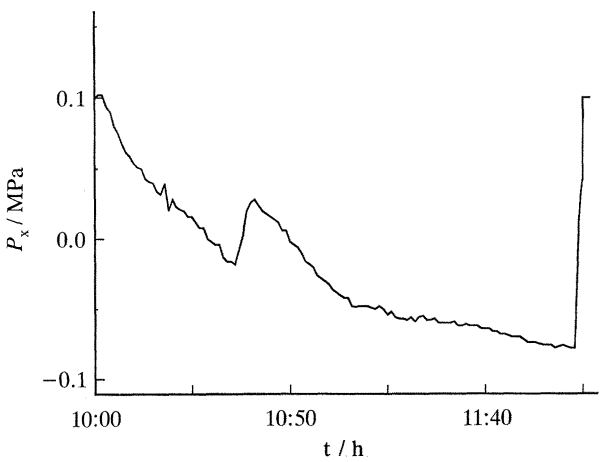


Figure 11. Xylem pressure recordings in the stem of an intact sunflower plant under laboratory conditions. The experiment started at 10.00 h (2 hours after light was switched on in the greenhouse). The plant was exposed to an air stream during the measurements. In a manner analogous to the pattern seen in figure 10, the xylem pressure decreased on average until cavitation occurred at about −0.08 MPa. Cavitation ended in leakage to atmospheric pressure. Note the 'erratic fluctuations' in xylem pressure. When a vacuum was reached (0.0 MPa), the pressure moved back and forth between the positive and negative pressure range (Redrawn from Zimmermann *et al.* 1993*b*.)

## 6. WHAT ARE THE FORCES RESPONSIBLE FOR ASCENT OF WATER?

### (*a*) *Capillary forces*

Xylem probe readings have shown that the average tension in the lumen of relatively large vessels is much less than expected from the Cohesion Theory. *In situ* measurements and model experiments suggest that relatively high tensions can be developed temporarily in the capillary network (less than 1 μm diameter) and microporous structures of herbaceous plants and trees. Water can be lifted as much as 100 m in microporous structures by capillary forces (Sachs 1887; Nobel 1983). However, the flow rates in these structural elements would be extremely small. This was the argument of Renner (1912, 1925) at the beginning of this century against the so-called 'Imbibition Theory' of Sachs (1887).

Even though the relative importance of the hydrated microcapillary system is difficult to determine (Borghetti *et al.* 1991; Tyree *et al.* 1991) it is reasonable to assume that these structural elements represent an essential reserve for the water demand of the tree, particularly after noon when transpiration declines and the conductivity of the large vessels is transiently reduced because of water vapour and air bubble formation. The experiments in which water volume increments were injected into the microcapillary region by use of the probe have clearly shown that the water transport properties of dried microcapillary elements can be easily regenerated provided that water is available. This can be accomplished by water uptake through the roots or alternatively by 'reverse




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transpiration' during the night (Milburn 1979) i.e. by capillary force-induced water uptake from rain or dew through cracks in the leaf surface. Even in periods of severe drought, as was the case during the course of our xylem pressure probe experiments on *A. peralatum* in Queensland, Australia, the amount of condensed water on the leaf surfaces (above a height of 25 m) in the early morning hours was substantial. The thick layers of leaf hairs seemed to be able to help conserve the condensed water (see also Jones 1992).

### (*b*) *Osmotic pressure and tension*

A second potential water reservoir consists of the turgescent living cells which are hydraulically connected to the microcapillary regions and to the large vessels. Transpiration-induced water loss from the large vessels and the resulting increase in tension will lead to (slightly delayed) water loss from the surrounding living cells. Turgor pressure declines and the unbalanced (effective) osmotic pressure increases. If the unbalanced osmotic pressure exceeds the negative pressure in the microcapillary system the water stored in these elements becomes available for the cells. The time course of the development of tension in the transpiring trees and plants reported here suggests an important role of living cells in water ascent in tall trees (see also Ursprung & Blum 1916; Nordhausen 1919). The tension typically increases progressively (in some cases associated with free movement of the pressure around zero, see figure 11) which seems to result from water flow between the xylem and cell compartments after disturbance of the stationary (equilibrium?) state. The relatively high frequency of 'erratic fluctuations' under *in situ* conditions also suggests that, under windy conditions, transient distortion of the twigs and branches of the tree may also impose local and temporary changes in xylem pressure.

The main pathways for water transport are the large xylem vessels which will function as conductance amplifiers. The average tension in these vessels (as measured by the xylem pressure probe) is not consistent with the assumption of the Cohesion Theory that water columns from the roots to the foliage are continuous. Our measurements of xylem tensions in trees have been done only in leafy branches, not main stems. Even though we cannot completely exclude the possibility that twigs have peculiar water conductance properties, it seems to be likely that the tension in the large vessels of the stems is of the same order as in the twigs. If this is the case, we have to postulate (in agreement with similar ideas of Sachs (1887)) that the large vessels are segmented in axial compartments of modest length bounded by solute-reflecting barriers. If compartmentation of the xylem exists, then water can be raised in smaller vertical steps and we would not expect to measure a gravitational pressure gradient in a tall tree. Axial barriers in the xylem have not been reported but they may be associated with tyloses, resins, polysaccharides or other blockages (Sachs 1887; Morse 1990). Occasional transverse, solute-impermeable barriers should not impose large resistances to axial water flow (Zimmermann & Brown 1980; Benkert *et al.* 1991). If the pressure in the large, segmented xylem vessels is supported by solute gradients, we must further postulate that solute-reflecting barriers also ensheath these vessels radially. Such 'sheaths' could be provided by the plasmalemmae of cells surrounding the xylem. To make the sheath continuous there would also have to be impermeable barriers between neighbouring cells of the sheath, perhaps analogous to the Casparian bands found in the endodermis of roots. Such concentric solute-reflecting sheaths have recently been found around the vascular bundles of maize (Canny 1990) and sugarcane (Jacobsen *et al.* 1992; Welbaum *et al.* 1992). In addition, it is difficult to envisage how positive 'root pressures' could be created within stems without membraneous barriers ensheathing the xylem (Schwenke & Wagner 1992).

According to these considerations, the large axial tension gradients postulated by the Cohesion Theory would be replaced by axial solute gradients within the xylem†.

Although the osmotic pressure of the xylem sap is assumed to be small, of the order of about 0.05 MPa, substantially higher values have been reported (e.g. Smith & Lüttge 1985). Furthermore, recent studies have provided evidence that standing osmotic gradients in organs of intact, transpiring plants (e.g. roots and stems) collapse rapidly when transpiration is interrupted upon excision of the organ (Zimmermann *et al.* 1992, unpublished data; Rygol *et al.* 1993). This rapid redistribution of solutes suggests that obtaining a representative sample of xylem sap may involve hitherto unrecognized technical difficulties. It is thus conceivable that the solute content of the large vessels in intact, transpiring trees and other plants is much higher than is generally supposed.

High osmotic pressures throughout the xylem sap would not necessarily be required to prevent large tensions from developing. Only local solute gradients situated across the ensheathing solute-reflecting barrier analogous to that envisaged for *Utricularia* bladder cells (Sydenham & Findlay 1975) and for 'apoplast canals' of roots (Katou & Furumoto 1986) would be needed. These local gradients would have to be maintained by active pumping or recovery of ions. Above-atmospheric pressures in the xylem regions of transpiring *A. excelsum* leaves (see previous section) are consistent with regulation of xylem pressure via exchange of solutes between the vessel lumen and the surrounding compartments.

### (*c*) *Interfacial forces*

The hypothesis of vertically segmented xylem compartments bounded by solute-reflecting barriers has more far-reaching implications if we include in our

† The liana experiment of Strasburger (1891) does not contradict the scenario described here for the ascent of water in tall trees. The lianas were, at maximum, 12 m in length. This would require a tension of about −0.12 MPa to hold the water column against gravity. This is within the range measured by the xylem pressure probe. In addition, capillary forces and the *Marangoni* streaming can still operate (see below).




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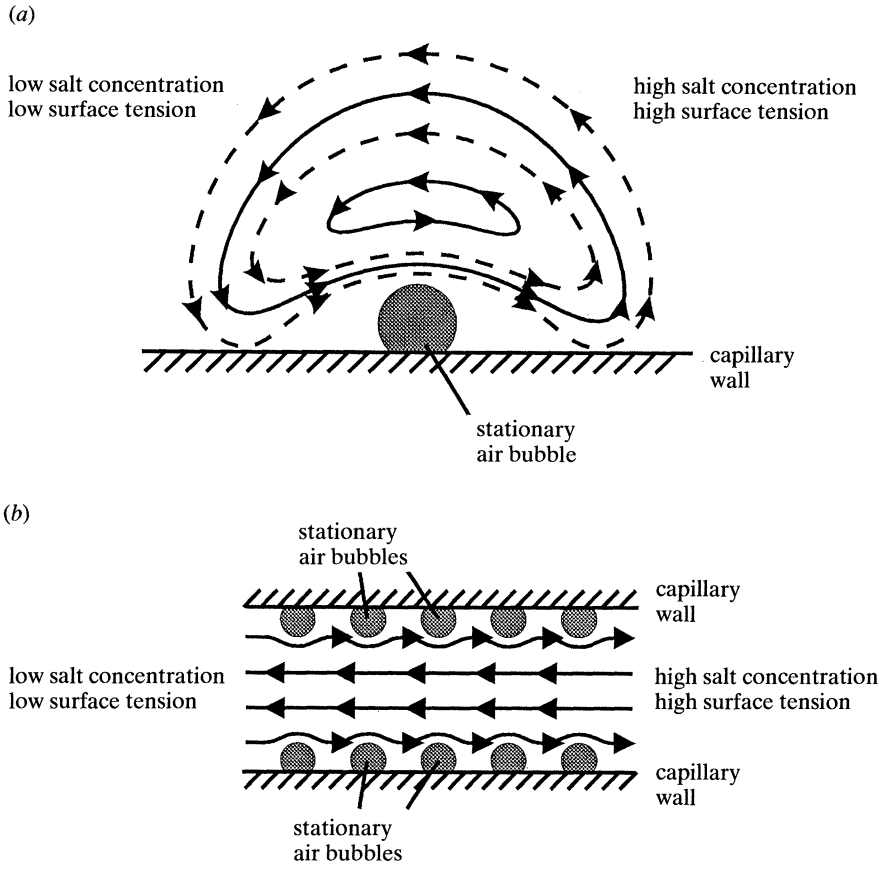


Figure 12. Schematic diagram of *Marangoni* flow induced by a gradient in interface tension along a liquid/gas interface. Flow profiles are shown for a gas bubble fixed in a solid matrix (*a*) and for a necklace of gas bubbles adhering to the inner wall of a capillary (*b*). Note that the surface tension of water increases with increasing salt concentration. In this case, the gradient in surface tension and the resulting interfacial liquid flow is directed to the solute-rich side, whereas the counterflow in the bulk solution is directed to the other side. For further explanation, see text.

discussion the presence of tiny gas bubbles adhering to the inner walls of the large vessels (Laschimke 1989). Even though some botanists (Zimmermann & Brown 1980) believe that the occurrence of gas bubbles (so-called Jamin's chains) is an artifact associated with the release of the 'high tensions' when the twig is cut, the xylem pressure probe measurements have given strong evidence for the presence of tiny air bubbles adhering to the walls of the xylem elements. These air bubbles (which occasionally are also observed on the inner walls of the pressure probe) cannot be dissolved by application of high pressure (however, see Tyree & Yang 1992). Consequently, if we assume that the walls of the xylem vessels (particularly the lignified regions) are covered by tiny air bubbles, we have to consider the possibility of interfacial (*Marangoni*) convection. *Marangoni* convection arises if a gradient in interface tension exists along a fluid–fluid or fluid–gas interface (figure 12). In contrast to free convection, *Marangoni* convection is gravity-independent and can, therefore, also operate under microgravity (Young *et al.* 1959; Langbein 1986; Langbein and Heide 1986).

The gradient $\nabla v$ in flow velocity induced along the surface by a gradient in interface tension $\nabla\sigma$ is given by:

$$\nabla v = \nabla\sigma/\eta, \tag{2}$$

where $\eta$ is the dynamic viscosity.

This stress tensor equation states that a gradient in interface tension creates a flow parallel to the gradient along the interface. The magnitude of flow depends on the dynamic viscosity. Since at the walls of the capillary the flow velocity must vanish, we obtain for the flow velocity along the liquid–gas interface:

$$v = \nabla\sigma/\eta \cdot d, \tag{3}$$

where $d$ = diameter of the capillary.

The surface tension of water increases with increasing salt concentration (or with decreasing temperature). The gradient in surface tension and the resulting interfacial fluid flow will, therefore, be directed to the solute-rich side (or the regions of lower temperature). This flow causes a hydrodynamic pressure increase on the respective side, and consequently, a counterflow of water in the bulk. The latter can be calculated from the continuity equation. (Note that a free droplet or bubble experiences a flow in the direction described along its surface, but itself moves to the opposite side, i.e. in the direction of decreasing interface tension. This is analogous to a swimmer who creates a

backward flow in order to move forward!). The created flows are shown for a gas bubble adhering to the wall of a capillary in figure 11*a*. In the case of a necklace of gas bubbles fixed to the wall of a capillary (figure 11*b*) we obtain similarly an interfacial flow from the region of low tension (i.e. low solute concentration) to the region of high tension (i.e. high solute concentration) and a flow within the centre of the capillary in the opposite direction. If water is withdrawn at one end of the capillary (e.g. because of evaporation) a net mass flow (against gravity) is created through the capillary.

*Marangoni* flows, in being interface-driven, will always dominate gravity-driven free convection, if small enough dimensions are considered. According to the above equations, the flow velocity induced increases with increasing capillary diameter. For vessels of 10 cm length (typical for diffuse-porous species; see Zimmermann & Jeje 1981) and diameters of 20 and 100 μm the flow velocity is in the order of 0.2 mm $s^{-1}$ and 1 mm $s^{-1}$, respectively, if the tension gradient $\nabla\sigma$ is assumed to be $10^{-3}$ N $m^{-1}$ (1 dyn $cm^{-1}$). These values are in the range measured both by the heat pulse and xylem pressure probe technique. Interfacial tension gradients of $10^{-3}$ N $m^{-1}$ can be established by local solute gradients in each vessel (see also above), by temperature gradients or by surface active compounds (such as proteins; see Biles & Abeles 1991).

Even though we do not know the xylem composition over the whole length of a stem the above discussion shows that fixed gas bubbles (or generally speaking, air-filled spaces in the tissue) may play an important role in xylem transport‡). It is quite conceivable that this mechanism is used by nature for water ascent during transpiration or for refilling of the vessels after severe water loss. Because increasing tensions are apparently developed during the morning hours, *Marangoni* streaming in the large vessels apparently cannot completely meet the instantaneous demand for replacement of transpirational water loss during the day.

At first sight it seems that the transient occurrence of negative pressures in the vessels is inconsistent with the postulated presence of fixed air bubbles. However, in model experiments it can be easily shown that adhered gas bubbles are swept along with water flow. Cavitation at relatively low negative pressures and formation of tiny stable gas (air) bubbles (adhered to the walls) would then be an absolutely necessary condition to re-create the *Marangoni* streaming for replacing the water loss. In other words, the occurrence of (modest) cavitation may not necessarily lead to a catastrophic dysfunction of the xylem (Tyree & Sperry 1989), but may represent a survival strategy for replacement of transpirational water loss.

‡ Air bubbles in water can be easily visualized using NMR imaging techniques. It is known that the magnetic susceptibility constant of water is different from that of air. This results in strong magnetic field inhomogenetics across the interface between water and air. This inhomogeneity is extended in large areas around air bubbles and reduces the NMR-signal intensity measured by NMR imaging. It can be expected that air bubbles with diameters of more than 5 μm are NMR visible (Kuchenbrod *et al.* 1993).

*Marangoni* streaming may also be a powerful mechanism for water uptake through the roots in halophytes (such as *Aster tripolium* and mangroves; see Zimmermann *et al.* 1992). The roots of these species contain more or less radial cell strands separated by large air-filled spaces. The tension difference between sea water and xylem water is about $2 \times 10^{-3}$ N $m^{-1}$. Thus *Marangoni* convection can occur quite easily at the apoplastic water–air interfaces.

## 7. CONCLUSIONS

Plant physiologists consider the xylem as a 'vulnerable pipeline' (Milburn 1979), i.e. as continuous water columns which are kept under very high tension. In the light of the pressure probe data and the above discussion we suggest that nature has developed different physical strategies for water transport against gravity, even under severe drought conditions. The diversity of the structural water-conducting and water-storing elements of a plant and tree supply the pre-requisite for the operation of pressure-, capillary forces- and interface-driven water transport against gravity. At high transpiration rates the creation of tensions modulated by stomatal regulation (Tyree & Sperry 1989) represents an additional force lifting water to the upper foliage.

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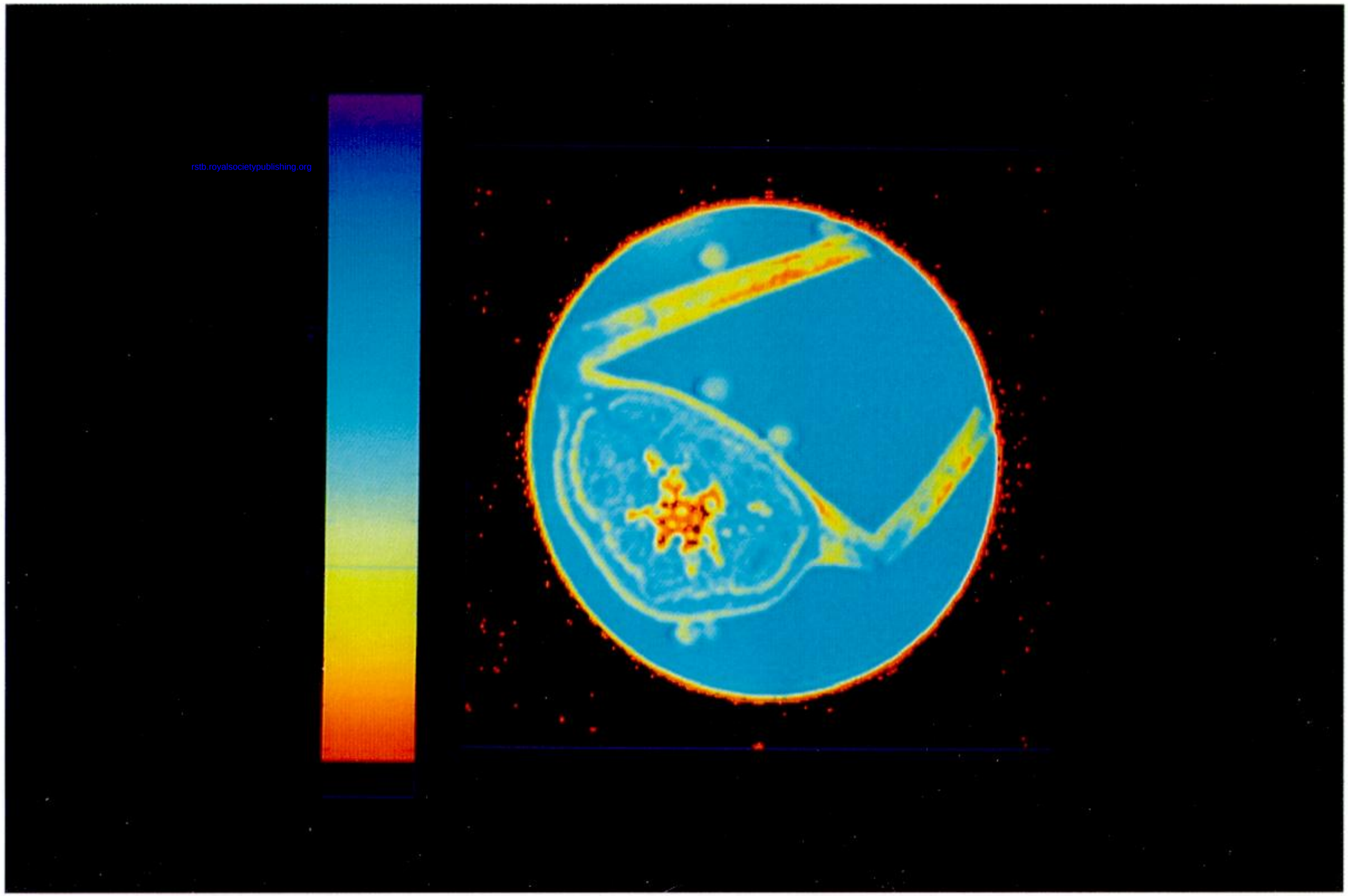
Plates 1 and 2 were printed by George Over Limited, London and Rugby.

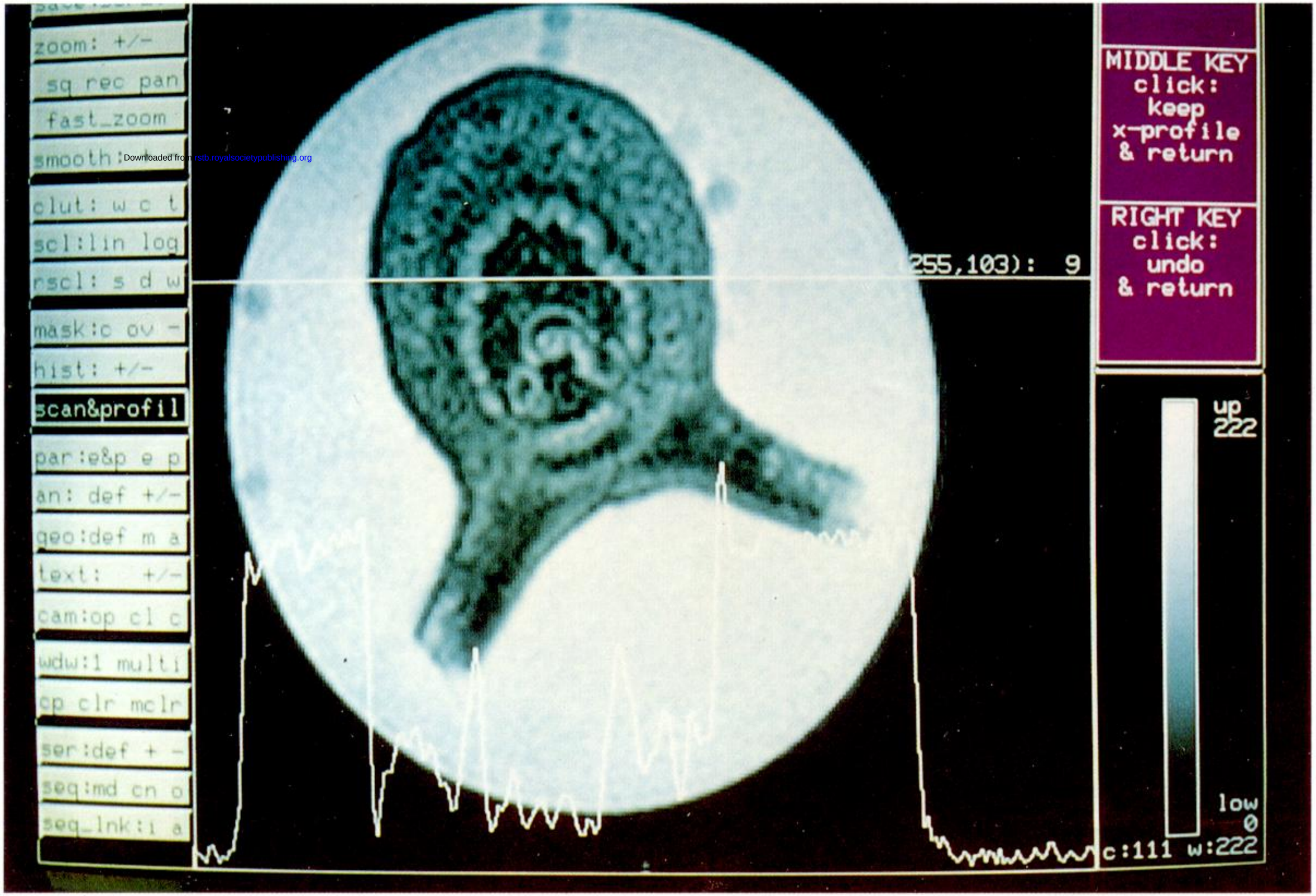




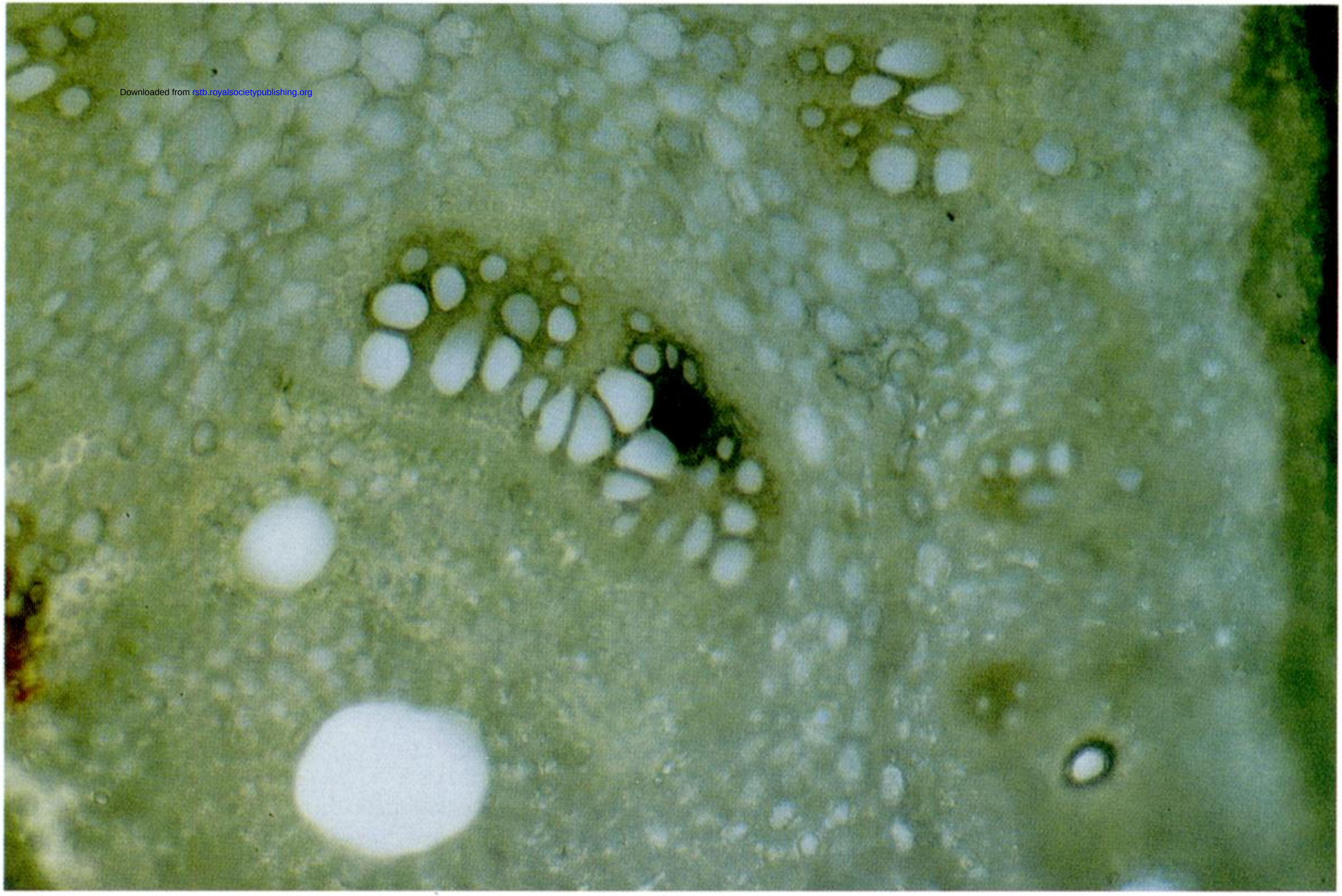
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES



gure 1. Typical cross-sectional $^{1}$H-spin-echo NMR image of the region close to the midrib of a leaf of an *Anacardium excel-m* tree growing near Panama City, Panama. The leaf was excised at 35 m height during the rainy season. Slice thickness projection was 400 μm, field of view 5 mm × 5 mm, magnetic field strength 11.7 T, the magnetic gradient up to 350 T m$^{-1}$ and the matrix 128 pixels × 128 pixels. The spin echo time was 3.4 ms. The column shows on a linear scale the rcentage of water concentration in the tissue (red = 0% and violet = 100%). Note that with the exception of the pith reg-n (which contains air) the tissue is well supplied with water. The average signal intensity is as high as in the surrounding ater (E. Kuchenbrod, A. Haase, R. Benkert, F. Meinzer & U. Zimmermann, unpublished data).




gure 2. Typical $^{1}$H-spin-echo NMR image and signal intensity profile of a cross-section of an excised leaf of *Argyrodendron ralatum* (Queensland, Australia, leaf cut at 32 m height). The tree had been subjected to prolonged drought. Experimen- conditions were as in figure 1 (E. Kuchenbrod, A. Haase, R. Benkert, H. Schneider, G. Zimmermann & U. Zimmer- ann, unpublished data). It is evident that the signal intensity throughout the tissue is very low indicating the presence a substantial amount of air and correspondingly a relatively low water content. Xylem pressure probe measurements nfirmed this result (see figure 8).



gure 9. Insertion of the microcapillary of the xylem pressure probe into a xylem vessel of a leaf of *Anacardium excelsum* lose to the midrib). The tip of the microcapillary was filled with Indian ink. Note that the dye was sucked into only one the vessels (R. Benkert, F. Meinzer & U. Zimmermann, unpublished data).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