



## *Tansley review*

# Water ascent in tall trees: does evolution of land plants rely on a highly metastable state?

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## Summary

The Cohesion Theory considers plant xylem as a 'vulnerable pipeline' isolated from the osmotically connected tissue cells, phloem and mycorrhizas living in symbiosis with plant roots. It is believed that water is pulled exclusively by transpiration-induced negative pressure gradients of several megapascals through continuous water columns from the roots to the foliage. Water under such negative pressures is extremely unstable, particularly given the hydrophobicity of the inner xylem walls and sap composition (lipids, proteins, mucopolysaccharides, etc.) that prevents the development of stable negative pressures larger than about  $-1$  MPa. However, many plant physiologists still view the Cohesion Theory as the absolute and universal truth because clever wording from the proponents of this theory has concealed the recent breakdown of the Scholander pressure bomb (and other indirect methods) as qualified tools for measuring negative pressures in transpiring plants. Here we show that the arguments of the proponents of the Cohesion Theory are completely misleading. We further present an enormous bulk of evidence supporting the view that – depending on the species and ecophysiological context – many other forces, additional to low tensions, can be involved in water ascent and that water can be lifted by a series of watergates (like ships in staircase locks).

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## I. Introduction

No topic in plant physiology has a longer history or a more voluminous literature than antigravitational water ascent in higher plants. This phenomenon has puzzled plant physiologists and physicists since the time of Hales (1727). But, in spite of this, there is no firm conclusion. The literature and botanical textbooks published in the last four decades give the superficial impression that the water-lifting problem is solved. The belief is that water is pulled through the xylem conduit by tension gradients produced by evaporational water loss. This hypothesis is called the 'Cohesion Theory' and was introduced by Dixon & Joly (1894, 1895) and Askenasy (1895). There is no doubt that this unifying theory is appealing because it is simple – water can only be pulled to dizzying heights because pumping can be excluded. Indeed, it is a principle of science to search for simplicity. William of Occam, a 14th century philosopher, made the dictum known as the principle of parsimony '*Essentia non sunt multiplicanda praeter necessitatem*' (entities should not be multiplied beyond necessity). Einstein is quoted as saying, 'in the whole history of science from Greek philosophy to modern physics, there have been constant attempts to reduce the apparent complexity of natural phenomena to some simple, fundamental ideas and relations' (Vilée, 1962).

However, the problem of the simple Cohesion Theory is that sizable negative pressures must exist in the xylem vessels of tall trees. This means that the xylem water is in a metastable state like superheated water. Scientists from other disciplines have great difficulties in imagining how stable water flow against gravity can occur under such conditions, particularly as the xylem sap is confined by leaky walls (e.g. Canny, 1995a). In the light of the hydraulic, osmotic and electrical coupling of the xylem with the heterogeneously composed (multiphase) surrounding tissue, it is extremely hard to envisage how the xylem elements can be treated as isolated, 'vulnerable pipelines' for water movement. It is therefore not surprising that the vigorous debate among the scientists of the 19th century about the mechanism of water lifting also continued after the introduction of the Cohesion Theory and the indirect demonstration of Renner (1925) that moderate negative pressures can exist in the xylem under some circumstances.

Retrospectively, a turning point in the pros and cons debate was the impressive experiment of Preston (1952) who demonstrated that tall trees survived overlapping double saw-cuts made through the cross-sectional area of the trunk to sever all xylem elements. This result, confirmed later by several authors (e.g. Mackey & Weatherley, 1973; Eisenhut, 1988; Benkert *et al.*, 1991), was obviously not in agreement with the Cohesion Theory. Preston and other authors at his time called for serious modifications of the theory. But the normal scientific advance of hypothesis followed by observation, then followed by a new or revised hypothesis and further observation did not

occur. In 1965 Scholander and colleagues used the modified pressure bomb technique of Dixon (1914) and claimed without proof that this archaic tool can measure negative xylem pressure values in the megapascal range and that the measured values were of the order of magnitude predicted by the Cohesion Theory. The 'triumphal procession' of the pressure bomb technique in plant physiology and ecology during the last 4 decades is hard to understand when the original data of Scholander and co-workers are considered. Scholander *et al.* (1962) found positive, sub-atmospheric to slightly negative pressure values (down to  $-0.1$  MPa) for mangroves using Renner's technique. Three years later, the same authors published pressure values of  $-3.3$  to  $-6.0$  MPa for the same species using the pressure bomb. It is surprising that Scholander and co-workers did not look into the discrepancy of the data as is usual in science. Today, results contradicting the Cohesion Theory are ignored and alternative theories are discarded. Many plant physiologists view the statement that water is pulled exclusively by transpiration-induced tension gradients as the absolute and universal truth.

In this review article we demonstrate that the present interpretation of the pressure bomb data is based on a misconception and that negative xylem pressure values of several megapascals exist only in the realm of science fiction since xylem sap composition, the features of the xylem wall and the hydraulic coupling of the xylem with the tissue prevent the development of stable tensions larger than about 1 MPa. We also present powerful evidence that a paradigm change away from the Cohesion Theory is cogently required in the light of the data obtained with the minimally invasive xylem pressure probe technique (Balling & Zimmermann, 1990). This technique allows the direct measurement of pressure in a single xylem vessel of an intact higher plant. Probing of the vessels of a broad variety of plant species has given clear-cut evidence that moderate negative pressures can exist in the xylem of higher plants. However, probe measurements in well-hydrated leaves high in tall trees or in salt-tolerant trees have also revealed that the xylem tension values are frequently too low to be consistent with the Cohesion Theory (Zimmermann *et al.*, 1994a,b). Moreover, advanced NMR imaging technology has shown (Zimmermann *et al.*, 2002c) that the basic assumption of continuous water-filled xylem elements is not necessarily fulfilled for branches of tall trees, even under predawn conditions. Today, there is a bulk of evidence that water supply to the uppermost foliage of tall trees is achieved by a finely tuned interplay of various forces acting in the xylem, in the multiple phases of the tissue and at liquid/gas interfaces. Support of lifted water volumes, e.g. by occasional axial barriers in the xylem or adherence to gel matrices, seems to be an additional strategy of tall trees to keep the various forces (including tension) at moderate (model-proved) values. The analogy to the lifting of ships by consecutive watergates (staircase locks) is obvious, even though for ships there is only one force involved.

The unifying Cohesion Theory was obviously an approximation to the truth in some circumstances. We are faced with the same situation as the physicists after 1905 when Einstein showed that the law of the Conservation of Energy had to be modified to allow for the interconversion of matter and energy. In the present state of the art it is not possible to formulate a new unifying theory for water ascent in tall trees. Therefore, we suggest the use of the terms 'Multi-Force' or 'Watergate' Theory to account for the experimental results obtained with the novel advanced technology.

When reading the extensive literature published in recent years on this subject, we were really surprised by the many desperate attempts made by proponents of the pressure bomb technique and the Cohesion Theory to call into question the results obtained by the pressure probe and NMR imaging techniques. Clever wording conceals that the pressure bomb is a conceptual artefact and that only rather limited negative pressures develop within the xylem conduit upon transpiration. Before writing this review article, we debated on whether to comment on the arguments by proponents of the Cohesion Theory against the probe and NMR imaging work because they are very polemic and pervert the facts to a large extent. However, we came to the conclusion that real progress in the field can only be expected in the future if the entire scientific community is involved in the long-debated question how the foliage of a tall tree is supplied with water. This is, of course, only possible if we show that the arguments of the proponents of the Cohesion Theory are inadequate and misleading.

## II. Can water sustain negative pressures?

### 1. Thermodynamic considerations

Physicists and physico-chemists always have difficulties accepting that negative pressures can exist in condensed phases (Laschimke, 1989, 1990; Hahn, 2000). Equilibrium thermodynamics shows that the absolute hydrostatic pressure,  $P$ , in a gas or condensed phase is given by:

$$P = T dP/dT - dU/dV \quad (1)$$

where  $T$  is the absolute temperature,  $U$  the internal energy, and  $V$  the volume.

For an ideal gas  $dU/dV$  is zero because there is no interaction between the molecules by definition. For liquids (and solids) the term  $dU/dV$  may easily exceed the term  $T dP/dT$  because of the attraction (cohesion) forces between the molecules. Under these conditions, negative absolute pressures, i.e. tensions larger than 0.1 MPa, can be expected (Bentrup & Zimmermann, 2000). However, water under negative pressure is in a metastable state because the equilibrium vapour pressure of water is +2 kPa at room temperature. Thus, the local input of energy or substantial lowering of the enormous activation energy barrier for bubble nucleation results in

cavitation, i.e. in the breakage of the tensile water followed by the transition of a large amount of water into the vapour phase.

Consideration of a two-compartment system consisting of a solvent and a solute phase separated by a solute-reflecting membrane (which mimic very closely the xylem/cell assembly) also demonstrates that negative pressures can develop in the solvent phase under some circumstances. At thermodynamic equilibrium, the chemical potentials of water,  $\mu_w$ , in the solvent (xylem) and solute (cell) compartments must be equal (Gibbs, 1961):

$$\mu_{wx} = \mu_{wc} \quad (2)$$

where the subscripts  $x$  and  $c$  denote the solvent (xylem) and solute (cell) compartment, respectively. The chemical potential of water is given by Eq. 3 if an isothermal system is considered:

$$\mu_w = \mu_{w0} + \bar{V}_w P + RT \ln a_w \quad (3)$$

where  $R$  = gas constant,  $\mu_{w0}$ ,  $\bar{V}_w$ ,  $a_w$  = standard chemical potential, partial molar volume and activity of water, respectively.

It follows from Eqs 2 and 3 that:

$$\bar{V}_w P_x = \bar{V}_w P_c + RT \ln a_{wc} \quad (4)$$

If the reduction of the water activity is envisaged exclusively by osmotically active (low-molecular weight) solutes, Eq. 3 can be transformed into Eq. 4 because of  $RT \ln a_w = -\bar{V}_w \pi$  (Chang, 1981), whereby it is assumed that the reflection coefficient of the solute is  $\sigma = 1$ , i.e. that the full osmotic pressure calculated from the van't Hoff equation is exerted (Dainty, 1963; Zimmermann & Steudle, 1978):

$$P_x = P_c - \pi_c \quad (5)$$

Inspection of Eq. 5 shows that thermodynamic equilibrium between the solvent and the solute phases (xylem and cell) can be brought about in two ways upon an increase in the osmotic pressure of the solute compartment,  $\pi_c$  (Renner, 1915; Strugger, 1943; Mauro, 1957; Balling & Zimmermann, 1990): either  $P_c$  increases and  $P_x$  remains constant (Pfeffer, 1881) or  $P_x$  decreases and  $P_c$  remains constant (Hepp, 1936). In the latter, relatively unfamiliar equilibrium state, negative pressures can be established in the solvent compartment once the osmotic pressure in the solute compartment exceeds 0.1 MPa (*c.* 40 mOsmol) at atmospheric pressure.

Stationary negative pressures were demonstrated by Mauro (1965) by using a modified Hepp-type osmometer (Hansen, 1961). The osmometer consisted of a closed water-containing brass chamber separated by a semipermeable membrane from an open, PEG 6000-containing compartment. The pressure

in the water phase was sensed by a displacement transducer sealed to one end of the brass chamber. After extremely careful removal of 'gas nuclei' negative pressures could be established in the water compartment once the osmotic pressure of the PEG 6000 solution exceeded 0.1 MPa. Stepwise increase of the solute concentration resulted in more negative pressures, but simultaneously the frequency of cavitation increased. At pressures of  $-1$  MPa cavitation occurred within a fraction of a second. Similar results were found by Balling *et al.* (1988) when using a glass chamber as water compartment and the water-filled xylem pressure probe for pressure sensing (see section III.1).

## 2. Vulnerability of tensile water

Cavitation apparently prevents the development of large stable negative pressures in static osmometer experiments. Even less stable negative pressure values are expected for continuous mass flow because of the input of kinetic energy (!) required to induce flow (Preston, 1952). Therefore, the make-or-break question for the Cohesion Theory is whether long-lived negative pressures of  $-1$  down to  $-10$  MPa (or lower) can be considered realistic for the xylem of transpiring tall trees, salt-tolerant trees and other higher plants subjected to environmental stress. The question can also be formulated in a different way: Are the cohesive and adhesive forces within the water-filled conducting xylem elements large enough to keep the xylem water continuously under extremely negative pressures during ascent?

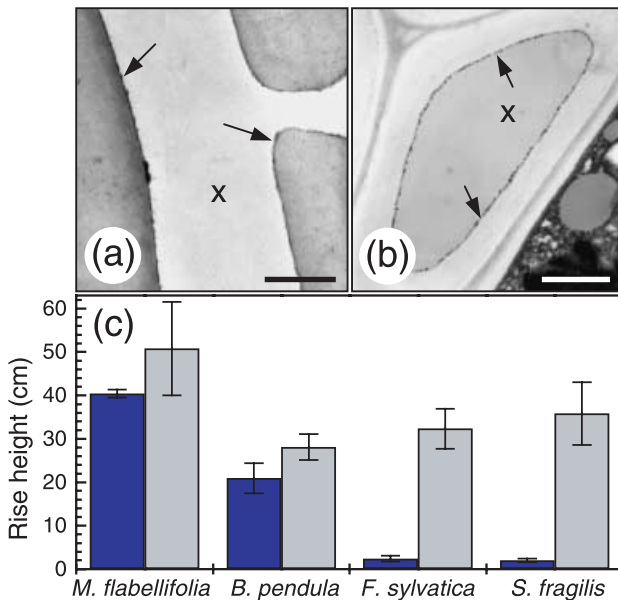
Attempts to estimate the cavitation threshold of tensile water were first made by Berthelot (1850) who dissolved a tiny gas bubble in a sealed water-containing glass tubing by heating. On cooling the water suddenly ruptured. From the temperature values, the expansion coefficients of water and glass, and the compressibility of water, the cavitation threshold was calculated. Variants of this method were used until quite recently (see Dixon, 1909; Ursprung, 1915, 1916; Kenrick *et al.*, 1924; Temperley, 1947; Trevena, 1967; Hayward, 1970; Henderson & Speedy, 1987). The cavitation thresholds determined by these methods ranged between  $-1.6$  and  $-20$  MPa. A much less negative cavitation threshold (around  $-0.5$  MPa) was found by Reynolds (1878). This author spun a water-containing glass capillary, which was closed at one end, on a centrifuge and calculated the tension from the speed at which the water column broke. Using Reynolds' method with tap water saturated with air, Temperley & Chambers (1946) found negative pressure values ranging from only 0 MPa to  $-0.6$  MPa. In the light of these and many other results Preston (1952) concluded that 'the Cohesion Theory stands on less sure ground than it at first sight appeared'.

The current proponents of the Cohesion Theory have ignored Preston's warnings as well as observations that the cibarial pumps of xylem-feeding insects could generate a maximum suction of only 0.3 MPa (Mittler, 1967; Raven,

1983; Andersen *et al.*, 1992). They use the experiments of the physicist Briggs (1949, 1950, 1955) as a 'smoking gun' for the existence of **stable** xylem pressures in the megapascal range (e.g. Steudle, 1995, 2001). Briggs repeated the experiments of Reynolds by using a carefully cleaned Z-shaped glass capillary filled with deionised, distilled, ultra-filtered and degassed water in a vacuum chamber. The Z-shape prevented water loss from either side of the capillary. Briggs (1955) found a great variation in the cavitation threshold when using different Z-shaped capillaries. The lowest pressure at which breaking of the water columns occurred was about  $-30$  MPa. This value is obviously sufficient for tension-driven water ascent even in the tallest trees. However, the key problem is that the experimental conditions selected by Briggs cannot simply be transferred to the xylem. Smith (1991, 1994) showed that the cavitation threshold is elevated dramatically when the Z-shaped capillary is not enclosed in a vacuum chamber and non-degassed water is used ( $-0.258 \pm 0.337$  MPa;  $n = 192$ ). Transient negative pressures of  $-1.0$  MPa were recorded in only very few cases. Smith also demonstrated that the cavitation threshold shifted to values around vacuum if the inner surface of the capillary was non-wettable or if organic contaminants were not carefully removed.

Some proponents of the Cohesion Theory (e.g. Tyree *et al.*, 1984; Salleo & Lo Gullo, 1986) argue that the cavitation threshold is more negative in small-sized capillaries (being more in the range of xylem vessels). However, there is no experimental evidence for this assumption. The experiments of Brereton *et al.* (1998) can also not be used as evidence for a capillary diameter-dependent cavitation threshold as suggested by Tomos & Leigh (1999). Brereton *et al.* (1998) measured cavitation of distilled water as it was released from pre-compression at 100 MPa (!) to atmospheric pressure in silica capillaries with diameters ranging from 10 to 100  $\mu\text{m}$ . Pre-compression stabilises resident vapour microbubbles or nuclei by forcing them to the bottoms of sharp-angled crevices, where an almost infinite force would be necessary to re-dissolve them, against the resistive forces of surface tension. Because of the large surface-to-volume ratio the number of 'free' microbubbles or nuclei will, therefore, be considerably less in small capillaries than in large, shifting the cavitation threshold to larger tensions. It is clear that a method of nuclei removal that requires pressures of 100 MPa has not yet been invented by nature.

Holbrook *et al.* (1995) recently asserted that 'glass tubes are an inappropriate model system for assessing the stability of water under tension in the xylem' thereby implying that glass tubes are expected to be more vulnerable to cavitation than xylem vessels. This is an astounding argument because the ramified anatomy of the vessels, the rough topography of the xylem walls, xylem sap ingredients as well as appreciable amounts of dissolved oxygen (Gansert *et al.*, 2001) should prevent the development of large tensions (see e.g. Trevena, 1987; Young, 1989; Adamson & Gast, 1997). An even more



**Fig. 1** Evidence for hydrophobic xylem walls. (a, b) Transmission electron micrographs of cross-sections of a rehydrated branch of *Myrothamnus flabellifolia* (a) and of water-conducting branch wood of *Betula pendula* (b). It is obvious that the inner xylem walls are covered with an osmiophilic (lipid) lining (arrows; x = xylem element; bars = 1  $\mu$ m). (c) Acropetal rise heights of water (blue columns) and benzene (grey columns) in cut air-dry branches of *M. flabellifolia* and twigs of *B. pendula*, *Fagus sylvatica* and *Salix fragilis* 24 h after solvent contact. The columns (= mean values  $\pm$  SD;  $n \geq 3$ ) evidence faster climbing of benzene than of water. For more details see Schneider *et al.* (2000b) and Zimmermann (2003). (b) and (c) reproduced from Zimmermann (2003), with kind permission of Physiko-Medica, Würzburg, Germany.

important factor for the vulnerability of tensile xylem sap is the reduced wettability of xylem walls. Scott (1966), using electron microscopy, found that the walls of empty xylem vessels of *Ricinus* species were covered with a suberin-like layer, which resembled in texture the isolate external cuticle and intercellular lipid linings. Recently, Schneider *et al.* (1999, 2003), Wagner *et al.* (2000) and Westhoff *et al.* (unpublished data) also evidenced by staining with lipophilic dyes and transmission electron microscopy that the inner walls of the xylem elements of desiccated and re-hydrated branches of the resurrection plant *Myrothamnus flabellifolia* as well as of water-filled vessels of birch and lime branches were lined with an osmiophilic lipid layer (Fig. 1a,b). One component of this layer seem to be phospholipids as revealed by  $^{13}\text{C}$  NMR-MAS spectroscopy (Schneider *et al.*, 2003) and Raman spectroscopy (Rösch *et al.*, 2004) on *M. flabellifolia* branch pieces. Further evidence for xylem lipid linings arrived from refilling experiments of air-dried branches of *M. flabellifolia* as well as of twigs of birch, beech and willow. Fig. 1c shows that benzene ascends as rapidly as or even faster than water in the xylem conduit.

Even if xylem elements are not covered by lipid linings, the chemistry of lignin tells us that the xylem walls are less

wettable than generally believed because lignin is hydrophobic (Siau, 1984; Laschimke, 1989, 1990; Smith, 1994). Even extremely wettable cellulose surfaces can become hydrophobic because organic material is rapidly accumulated due to the high free energy of the surface. This produces a firmly adherent coating on the hydrophilic wall regions (Baier & Meyer, 1986). Therefore, the finding of Copeland (1902), Haberlandt (1909, p. 292) and other contemporary authors that tiny (hydrophobic) gas bubbles can adhere to the inner xylem walls in a necklace-like manner (so-called Jamin chains) is not very surprising. Even Grace (1993), a proponent of the Cohesion Theory, believes that cavitation may not be spatially random but associated with bubble growth from the xylem walls. Investigating a large variety of trees, Laschimke (1989, 1990) and Laschimke & Laschimke (1998) showed additionally that gas bubbles on xylem walls are flattened. These observations can be taken as further clear-cut evidence for the hydrophobicity of the inner xylem walls of a variety of tree species.

The above brief considerations demonstrate that the effects of adhesion upon tensile water have greatly been underestimated, presumably because of the name 'Cohesion Theory' used for the description of (transpiration-induced) tension-driven water ascent. The hydrophobicity of the xylem walls and the composition of xylem sap make it extremely unlikely that **stable** negative xylem pressures in the megapascal range can exist. As we will see in section V, this conclusion is further substantiated when the hydraulic coupling between the xylem and the tissue cells (including the phloem) is incorporated into the analysis.

### III. Negative xylem pressures of several megapascals: fact or mystery?

The question arises why many plant physiologists believe in extremely negative xylem pressures, even though the above considerations demonstrate that such pressures should not exist in the xylem vessels. The problem is mainly due to the measuring device. The xylem pressure probe and the leafy twig/vacuum line technique yield stable tensions up to about 0.7 MPa, whereas the pressure bomb yields the extremely high tensions required by the Cohesion Theory. The question can, therefore, be formulated in a different way: what parameters are measured by the various devices and what are their limitations?

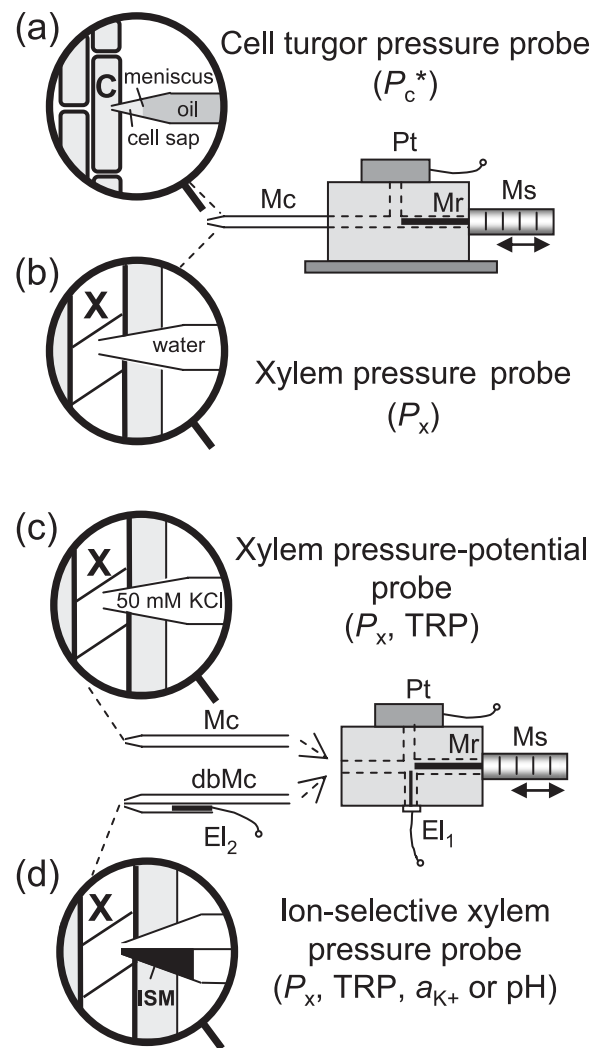
#### 1. The xylem pressure probe: direct measurement of xylem pressure in intact plants

The xylem pressure probe is a variant of the cell turgor pressure probe developed by Råde and Zimmermann and applied initially to the determination of turgor pressure and other water relations parameters of giant algal cells from turgor pressure relaxation and clamp experiments (Zimmermann

*et al.*, 1969; Steudle & Zimmermann, 1971; Zimmermann & Steudle, 1974; Zimmermann, 1978, 1989; Wendler & Zimmermann, 1982, 1985; Ortega *et al.*, 1992; Murphy & Smith, 1998; Tomos & Leigh, 1999). The electrical properties of the tonoplast and the plasmalemma can also be determined separately if the probe is combined with vacuolar microelectrodes and perfusion assemblies (Wang *et al.*, 1997; Ryser *et al.*, 1999; Heidecker *et al.*, 2003a,b; Mimietz *et al.*, 2003). Measurements of the water relations parameters of tissue cells of higher plants were first reported by Hüskén *et al.* (1978) using a sophisticated modification of the probe. However, this micro-pressure probe failed the practical tests and the present version (used by many laboratories for turgor pressure measurements as well as for sap sampling from single cells) is identical to the original version (see Tomos & Leigh 1999). The 'new' device of Steudle (see Fig. 2 in his review article of 2002a) is a re-invention of the pressure probe of Råde and Zimmermann and deserves, therefore, no further discussion. This also applies to the so-called root pressure probe (Steudle & Jeschke, 1983; Steudle, 1993) that was adopted from Zimmermann & Mehlan (1976) and is used for measuring bulk water relations parameters of excised roots. This approach does not yield information at the single-cell level and, due to the multi-phase root compartment, interpretation of data obtained by the root pressure probe is based on many unproved assumptions.

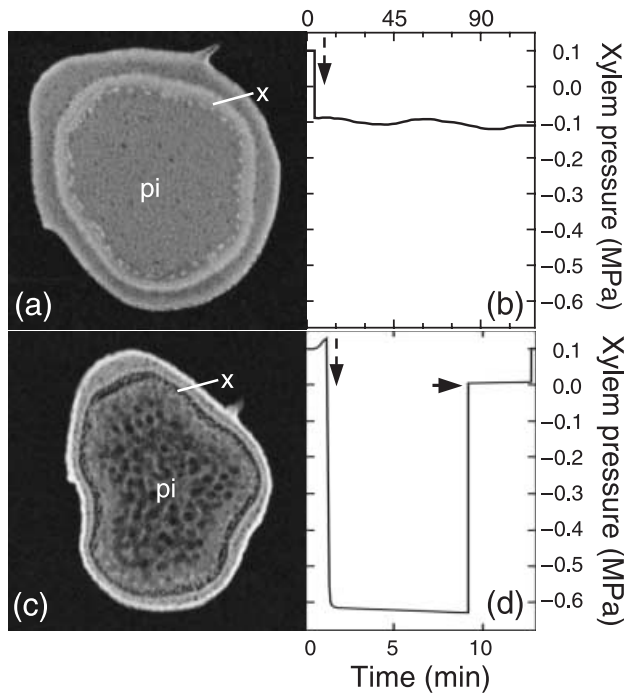
The cell turgor pressure probe senses the turgor pressure by a pressure transducer mounted in a 50- $\mu$ l perspex chamber and sealed to a glass microcapillary that is inserted into the cell (Fig. 2a). The entire probe system is filled with low-viscosity oil. Upon probing, the turgor pressure pushes cell sap into the very tip of the microcapillary. When the position of the oil/cell sap boundary is clamped close to the cell surface (by displacement of a metal rod introduced into the probe chamber) the pressure within the probe equals the turgor pressure of the cell. In pressure relaxation experiments turgor pressure changes are induced by osmotic challenges or transpirational changes while keeping the position of the oil/cell sap boundary constant. Changes in cell volume and turgor pressure, respectively, are brought about by deliberately changing the position of the oil/cell sap boundary (for details, see Zimmermann, 1989).

In the case of the xylem pressure probe (Fig. 2b), it must be emphasised that the components of the probe need to be carefully manufactured and thoroughly cleaned before use to promote complete wetting. Similarly, special care must be taken to avoid gas nucleation at the interfaces between the different components of the probe. For the same reason the oil must be replaced by de-ionised and degassed water. If these crucial factors are not considered, cavitation can easily be induced at the metal rod and at the perspex surface (see e.g. Wei *et al.*, 1999b). For measuring xylem pressure a water-wettable pressure transducer is required. Generation of negative pressures down to  $-1.4$  MPa showed that the water-wettable transducers operate linearly (Balling & Zimmermann, 1990; Zimmermann



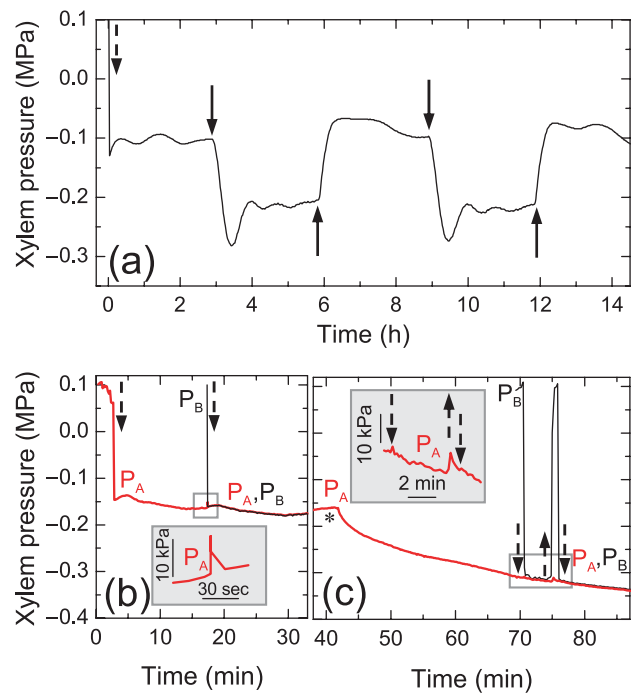
**Fig. 2** Schematic diagrams of the cell turgor pressure probe (a), xylem pressure probe (b), xylem pressure-potential probe (c) and ion-selective xylem pressure probe (d). Abbreviations: c = cell,  $P_c^*$  = cell turgor (=  $P_c - P_{am}$ ), Mc = microcapillary, Pt = pressure transducer, Mr = metal rod, Ms = micrometer screw, x = xylem vessel,  $P_x$  = xylem pressure, TRP = *trans*-root potential, dbMc = double-barrelled microcapillary, ISM = ion selective matrix,  $a_{K^+}$  =  $K^+$  activity in the xylem sap,  $El_1$  = Ag/AgCl electrode for TRP recording and  $El_2$  = electrode for  $a_{K^+}$  or pH recordings. For more details, see text.

*et al.*, 1994a). The ability of the xylem pressure probe to measure  $-1$  MPa has been demonstrated by using a Hepp-type osmometer (see section II.1) and other model systems (Thürmer *et al.*, 1999). Further effort to test whether the xylem pressure probe can measure even more negative pressures as demanded by Steudle (2003) is obviously wasting time because stable negative xylem pressures being significantly below  $-0.6$  MPa have not been found for higher plants. Wei *et al.* (2001) claimed to have measured stable xylem pressures down to  $-1$  MPa, but only an absolute pressure value of  $-0.62$  MPa was documented (Wei *et al.*, 1999a,b).



**Fig. 3** Typical  $T_1$ -weighted  $^1\text{H}$  NMR spin echo images of the water distribution and xylem pressure recordings (given in absolute values) in the shoot of well-watered (a, b) and drought-stressed (c, d) about 2-month-old *Nicotiana tabacum* plants. The  $^1\text{H}$  NMR images (repetition time 0.2 s, echo time 9.8 ms, slice thickness 1 mm, spatial resolution =  $20 \times 20 \mu\text{m}^2$ ) demonstrate that most xylem vessels ('x') of the well-watered plant were filled with water (a; high signal intensity), while more than 80% of the vessels were embolised after 1-wk drought (c; no signal intensity). Correspondingly, repeated xylem probing showed that most vessels of the well-watered plant were under negative absolute pressure (b; c.  $-0.1$  MPa), whereas in the drought-subjected plant only slightly sub-atmospheric pressure values were recorded indicating that most vessels were embolised. Only occasionally, stable xylem pressures down to  $-0.62$  MPa could be recorded for some time before cavitation ended the experiment (horizontal arrow in d). Note that drought resulted in shrinkage of the shoot cross-sectional area and in the formation of extended intercellular cavities in the pith ('pi') evidencing considerable water loss from the tissue cells (c). Dashed arrows in (b) and (d) indicate vessel impalement. (d) reproduced from Zimmermann *et al.* (1994a), with kind permission of Blackwell Science, Oxford, UK.

For probing a vessel in an intact plant the microcapillary is inserted slowly into the tissue, usually at an angle of about  $20^\circ$  to the transverse plane. If the vessels are filled with water (see e.g. the cross-sectional  $^1\text{H}$  NMR image of the water distribution in the shoot of tobacco in Fig. 3a) impalement is **always** detected by a rapid change of the pressure readings from above-atmospheric values to sub-atmospheric, positive or negative values (Fig. 3b). The advance of the microcapillary is then immediately stopped. Pressure equilibrium between the vessel and the probe is established within a few seconds. It is evident from Fig. 3b that the vessels of this tobacco plant were under a moderate negative pressure of  $-0.1$  MPa (light irradiance  $6 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). Subsequent pressure recordings are



**Fig. 4** Typical xylem pressure recordings on shoots of a 2-month-old *Lycopersicon esculentum* plant (a) and on roots of 2-wk-old hydroculture *Zea mays* seedlings (b, c). Vessel impalements are indicated by downward-directed dashed arrows. (a) Response of the xylem pressure when the plant was subjected to repeated short-term light regimes ( $6$  and  $150 \mu\text{mol m}^{-2} \text{s}^{-1}$  light irradiation; arrows). (b, c) Xylem pressure recordings by two pressure probes ( $P_A$  and  $P_B$ ) inserted consecutively into the same vessel (b) and in two adjacent vessels (c). Measurements were performed at a light irradiation of  $700 \mu\text{mol m}^{-2} \text{s}^{-1}$ . In (c) the xylem pressure was further lowered before insertion of probe  $P_B$  by addition of  $25 \text{ mM}$  NaCl to the nutrient medium (asterisk). As indicated in the insets, insertion of the second probe  $P_B$  resulted in a pressure spike in probe  $P_A$  when the same vessel was pierced (b). In the case of two adjacent punctured vessels (c) a pressure spike could only be recorded by  $P_A$  upon removal of probe  $P_B$  (upward-directed dashed arrow), but not upon insertion or reinsertion several micrometers away from the first impalement site. Note that both probes read always the same xylem pressure value independent of the various manipulations. For further details, see Schneider *et al.* (1997a). (b) and (c) redrawn after Schneider *et al.* (1997a), Oxford University Press, Oxford, UK.

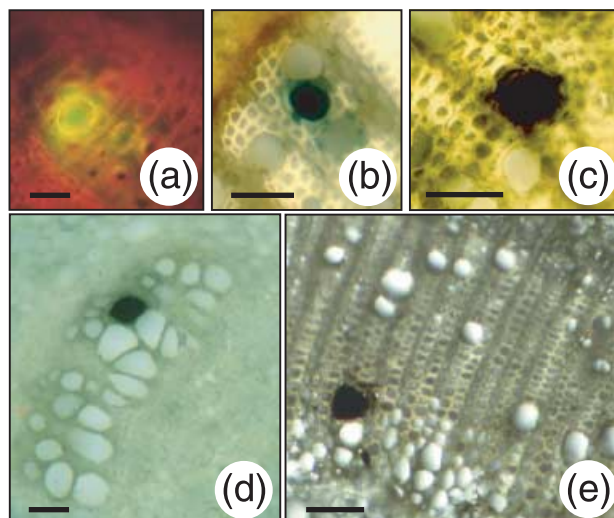
usually stable for several hours (part of a pressure trace is shown in Fig. 3b), even though weak oscillations can occur at low light irradiance as exemplified for tomato in Fig. 4a. Volume (pressure) pulses injected by displacement of the metal rod in the probe dissipate very quickly indicating that the punctured vessels are connected to other conducting vessels (Balling & Zimmermann, 1990; Benkert *et al.*, 1991). In herbaceous plants, such as tomato (Fig. 4a), an increase in the light irradiation to more than about  $50$ – $100 \mu\text{mol m}^{-2} \text{s}^{-1}$  commonly results in a marked decrease in xylem pressure to more negative values (around  $-0.3$  to  $-0.4$  MPa) which is frequently followed by a back-regulation to a slightly less negative value (see Fig. 4a). This typical 'overshoot' reaction is apparently linked

with changes in stomatal conductance (Farquhar & Cowan, 1974; Raschke, 1975; Schneider *et al.*, 2004). At very high light irradiations strong oscillations in transpiration can occur that are reflected somewhat delayed in xylem pressure (Wegner & Zimmermann, 1998; Schneider *et al.*, 2000a; Zimmermann *et al.*, 2002a; see also Fig. 14 further below). Xylem pressure changes can also be induced by changes in the ambient relative humidity. Decrease in relative humidity results in a drop of xylem pressure towards more negative values which can be reversed by increasing the relative humidity again (Zimmermann *et al.*, 2002a; Schneider *et al.*, 2004).

Manipulations of transpiration as well as osmotic challenges can also be used to identify the proper placement of the probe in a conducting vessel when the initial xylem pressure is in the positive, sub-atmospheric range (e.g. Benkert *et al.*, 1991, 1995; Zimmermann *et al.*, 1994a; Zhu *et al.*, 1995; Schneider *et al.*, 1997a,b; Thürmer *et al.*, 1999).

When a xylem vessel is targeted in an inappropriate way, cavitation can occur upon piercing. Frequently, there is a subsequent leak. Probe-related cavitation can also occur during xylem pressure measurements. Leaks can easily be detected and identified by a pressure increase to atmospheric while cavitation leads to an instantaneous shift of the pressure to +2 kPa (Fig. 3d and also Figs 12a,c further below). Cavitations within the xylem can clearly be distinguished from cavitations within the probe. In the latter case no pressure is built up in the probe when a volume pulse is applied and gas bubbles can be seen in the probe.

Doubts about probe function have also been allayed by numerous other experiments. When the probe was placed into a vessel of an excised shoot segment of tobacco that was sealed at both ends and bathed in PEG 6000 solutions of increasing concentrations, xylem pressures down to about -0.4 MPa could be established (Balling & Zimmermann, 1990). Insertion of the probe into the xylem of a cut leaf sealed to the lower end of the glass capillary of a Hepp-type osmometer (Balling *et al.*, 1988; Balling & Zimmermann, 1990) yielded negative pressure values that corresponded to the osmotic pressure in the PEG 6000 solution reservoir (up to 0.3 MPa; Balling & Zimmermann, 1990). Consecutive insertion of two probes into the root xylem of intact maize hydroculture plants also excluded artefacts arising from probing (Schneider *et al.*, 1997a). Fig. 4b shows an experiment performed in nutrient solution at a light irradiation of about  $700 \mu\text{mol m}^{-2} \text{s}^{-1}$ . Placement of two probes in the same vessel was proved by filling the capillary tip of one probe with fluorescein solution, the other one with alcian blue. The inset in Fig. 4b shows that the introduction of the second probe ( $P_B$ ) induced a small pressure spike in the vessel that was detected by the first probe ( $P_A$ ). After equilibration both probes read the same value. In Fig. 4c a negative pressure of about -0.3 MPa was established by NaCl addition to the nutrient solution before insertion of probe  $P_B$ . This time probe  $P_B$  punctured an adjacent vessel. In this case the reading of probe

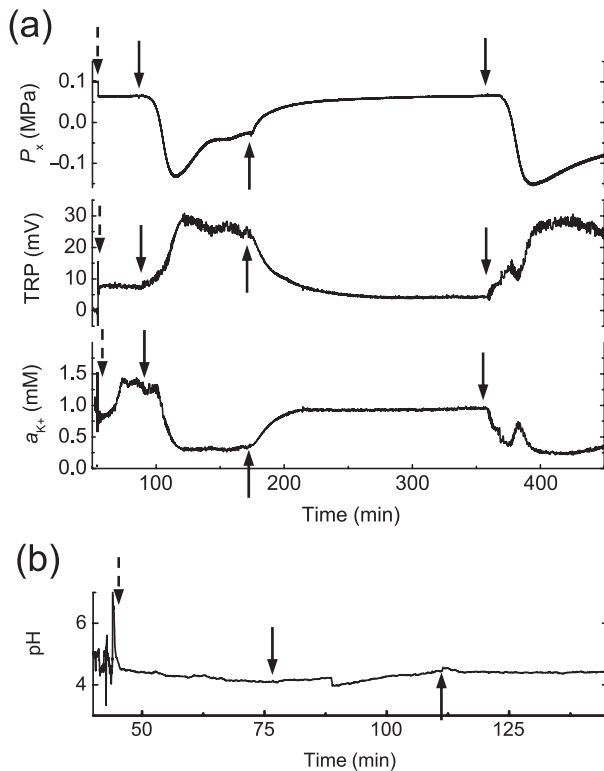


**Fig. 5** Light microscopy cross-sections of probed xylem vessels of a shoot of *Solanum tuberosum* (a), a seedling stem of *Rhizophora mangle* (b), a twig of *Fagus sylvatica* (c), a leaf petiole of *Anacardium excelsum* (d) and a shoot of *Nicotiana tabacum* (e). Before insertion the tip of the probe microcapillary was loaded with Na-fluorescein (a), alcian blue (b) or India ink (c–e) solution. Note that the dyes were sucked into only one of the vessels. Bars: 50  $\mu\text{m}$ . (c) and (d) were taken from Zimmermann *et al.* (1993a,b), with kind permission of BIOS Scientific Publishers, Oxford, UK, and The Royal Society, London, UK, respectively.

$P_A$  remained unaffected, even when  $P_B$  was gently removed after about 4 min and then reinserted several micrometers away from the first insertion site. However, intermediate removal of  $P_B$  led to a small pressure spike registered by  $P_A$  (see inset in Fig. 4c). Upon replacement of the saline solution by nutrient medium both probes responded identically (data not shown).

Probing of water-conducting vessels is further supported by insertion of probes filled partly with degassed (low- and high-molecular weight) dye solutions. Owing to the tension in the xylem, the dye solution is sucked from the microcapillary into the vessel upon impalement (Balling & Zimmermann, 1990; Benkert *et al.*, 1991; Zimmermann *et al.*, 1993a,b, 1994a,b, 2002a). Inspection of the cross-sections of roots, shoots and leaf petioles of various herbaceous and woody plants under the light microscope always revealed that the dye was confined to a single vessel at the insertion point (Fig. 5). In the case of fluorescein (Fig. 5a), the dye was transported with the transpiration stream as revealed by cross-sections made above the insertion point at regular intervals (Benkert *et al.*, 1991). The flow rates were in the same order of magnitude as determined by the heat pulse technique (Huber, 1932) and by NMR flow imaging (Kuchenbrod *et al.*, 1996, 1998).

Zimmermann and co-workers (1991) have also shown that pneumatic pressure applied to the roots of tobacco immersed in water was transmitted to the xylem. At low bomb pressures the xylem pressure probe (inserted into a vessel outside the bomb) responded immediately and almost linearly, whereas at higher overpressures the response curve was more sigmoid.



**Fig. 6** Typical on-line recordings of xylem pressure ( $P_x$ ), *trans*-root potential (TRP),  $K^+$  activity ( $a_{K^+}$ ) and pH, respectively, in the root xylem of *Zea mays* plants by using the ion-selective xylem pressure probe. Vessel impalements were performed at a light irradiation of  $10 \mu\text{mol m}^{-2} \text{s}^{-1}$  and are indicated by downward-directed dashed arrows. (a) Changes in  $P_x$ , TRP and  $a_{K^+}$  in the root xylem of a 21-d-old plant when subjected to a repeated short-term light regime (downward-directed arrows: increase of light irradiance from 10 to  $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ ; upward-directed arrows: return to low light irradiation). (b) Analogous pH measurements in the root xylem of a 9-d-old seedling. The responses of  $P_x$  and TRP upon an increase in light irradiation were similar to those in (a) and are therefore not given. Note that changes in  $P_x$  are reflected in corresponding changes of  $a_{K^+}$ , but not of pH. For more details, see Wegner & Zimmermann (2002) and Wegner *et al.* (2004).

Additionally, when the roots were cut, 1 : 1 responses of the xylem pressure probe over a large pressure range were recorded upon pressurisation (Balling & Zimmermann, 1990).

Xylem pressure changes induced by mechanical destruction of the tissue cells due to the impalement process were excluded by using a probe incorporating an Ag/AgCl microelectrode and filled with degassed electrolyte solution (Fig. 2c; Wegner & Zimmermann, 1998; Wegner *et al.*, 1999; Schneider *et al.*, 2000a; Zimmermann *et al.*, 2002a). Insertion of this xylem pressure-potential probe into the root xylem of hydroculture plants yielded resistance values that were on average even higher than those published in the literature (e.g. Anderson & Higinbotham, 1976). Location of the probe in a xylem vessel was indicated by changes in xylem pressure and in the *trans*-root potential upon illumination (Fig. 6a). Except for probe sites at the root base and along the root of very young wheat

plants the *trans*-root potential responded usually about 1–3 min faster than the xylem pressure. Very recently, Wegner & Zimmermann (2002) additionally integrated a  $K^+$ -selective electrode into the xylem pressure-potential probe (Fig. 2d). On-line measurements of the  $K^+$  activity in individual vessels of hydroculture plants probed at the root base showed (Fig. 6a) that an increase in light irradiation resulted in a concomitant drop of the xylem pressure and xylem sap  $K^+$  activity accompanied by a depolarisation of the *trans*-root potential. The effects of light on all three parameters were reversible. The  $K^+$  activity values at low irradiation agreed well with those deduced from xylem exudates of cut leaf veins and of xylem sap collected with the conventional xylem pressure probe (Lohaus *et al.*, 2000). Correct reading and placement of the probe in a vessel without injury was also demonstrated by immersing cut roots in media containing elevated  $K^+$  activities. Furthermore, use of xylem pressure-potential probes, in which a pH-sensitive electrode had been integrated (see Fig. 2d; Wegner *et al.*, 2004), also yielded pH-values (Fig. 6b) which were in the order of magnitude expected for the xylem (Davis & Higinbotham, 1969; Miller, 1985). In contrast to  $K^+$ , the pH remained constant upon short-term illumination. However, it increased when the root was exposed to bicarbonate (data not shown), providing evidence for the hypothesis of Mengel *et al.* (1994) that the reduced availability of Fe in plants growing on calcareous soil ('lime chlorosis') is due to an alkalinisation of the xylem sap when bicarbonate is taken up.

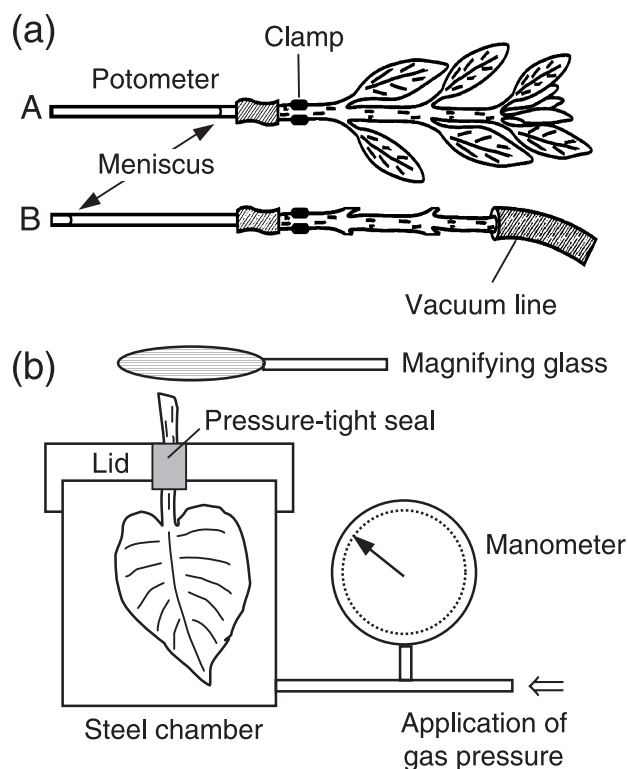
In the light of the bulk of evidence given above doubts about the proper location and function of the xylem pressure probe are obviously unfounded. Despite this, the leading proponents of the Cohesion Theory persistently argue that the probe is not placed in a conducting vessel (see Appendix 1) because of the xylem pressures around 0 and  $-0.1$  MPa measured in mangroves and at large heights of tall forest trees. So far, stable xylem pressures down to *c.*  $-0.6$  MPa were only found in laboratory-sized herbaceous plants when exposed to extremely strenuous conditions or severed from roots before probing (see Fig. 3d and Zimmermann *et al.*, 1994a, 1995a; Schneider *et al.*, 1997a, 2004). It is important to note that at these negative pressures the leaves were nearly turgorless, independent of the plant species investigated. Furthermore, these pressure values could only be recorded in very few vessels. Most of the vessels that were impaled appeared to be gas-filled since their pressure was close to atmospheric.  $T_1$ -weighted  $^1\text{H}$  NMR images of various heavily stressed plants confirmed the results of the xylem pressure probe experiments. As exemplified for an initially well-watered tobacco plant (Fig. 3a), more than 80% of the xylem vessels were filled with air after 1-week drought (Fig. 3c). Consistent with the assumption that the majority of the vessels was cavitated, flow-weighted  $^1\text{H}$  NMR imaging of severely drought-stressed, wilted plants revealed that the xylem volume flow had dropped dramatically. Interestingly, upon watering flow signals could be recorded within several hours accompanied with the reoccurrence of

moderate negative pressures (data not shown). If such flow-weighted NMR imaging measurements are not made (Holbrook *et al.*, 2001; Clearwater & Clarke, 2003), erroneous conclusions may be drawn about the functional state of the vessels.

As discussed in section V.3, it is extremely unlikely that stable negative xylem pressures can exist when the turgor pressure dropped to zero. Nature has obviously limited the magnitude of negative pressures that can develop in the xylem sap. This conclusion is also supported by the average xylem pressure values measured with the leafy twig/vacuum line technique (Renner, 1925; Scholander *et al.*, 1955, 1962).

## 2. The leafy twig/vacuum line technique

In Renner's technique an excised leafy twig is attached to a potometer (A in Fig. 7a). When water uptake has stabilised, the flow resistance is increased by squeezing or by making saw-cuts into the twig from opposite sides. This leads to a temporary drop in water flow (associated with a transient xylem tension increase as shown by the pressure probe; Benkert *et al.*, 1991). When the temporary drop has passed, the leaves are removed and a vacuum line is attached to the decapitated end of the twig (B in Fig. 7a). This is again accompanied by a change in water consumption. The unknown tension in the xylem is calculated from the ratio of water volume flow through the leafy twig to that through



**Fig. 7** Schematic diagrams of the leafy twig/vacuum line technique (a) and the pressure bomb (b). For details, see text.

the twig/vacuum line system, by assuming that the vacuum line creates an absolute sub-atmospheric pressure value of +0.01 MPa. The average xylem pressure data initially published by Renner (1911, 1912) ranged between  $-1$  and  $-2$  MPa. During the following years, however, he received severe criticism by his colleagues (e.g. Ursprung & Blum, 1916; Nordhausen, 1916, 1919a,b, 1921). As a consequence, Renner improved his technique and published in 1925 an overview of tension values measured on twigs of various higher plants and under various environmental conditions. The majority of the xylem pressure values ranged between  $-0.2$  and  $-0.4$  MPa. The most negative xylem pressure of  $-0.8$  MPa was measured on a cut twig of *Forsythia suspensa* (after introduction of a double clamp). Renner (1925) also measured positive, sub-atmospheric values under some circumstances. However, modern literature only cites the very negative pressure values of 1911 and 1912, presumably because these pressure values are more consistent with those measured by the pressure bomb technique.

An important artefact that can falsify the tension data is the entry of air into the xylem upon cutting (see Pfeffer, 1881, p. 133). This problem was eliminated by Renner by cutting the twig under water. A further problem involved in the interpretation of the leafy twig/vacuum line data is the assumption that the effective cross-sectional area for water flow remained unchanged during the entire experiment. This is not necessarily the case because the leaves were not replaced by a corresponding number of vacuum pumps (see set-up B in Fig. 7a). According to the Hagen-Poiseuille law, the volume flow, but not the flow velocity is directly proportional to the square of the water-conducting area. Therefore, despite an increase in the flow velocity the total flow in the twig/vacuum line system could be lower than that in the intact twig, if the number of conducting xylem vessels decreases due to the experimental manipulations (see Zimmermann *et al.*, 1993a).

Nevertheless, the method of Renner allows at least the magnitude of the average xylem tension to be estimated that can develop in a cut transpiring leafy twig that is continuously watered by an attached potometer.

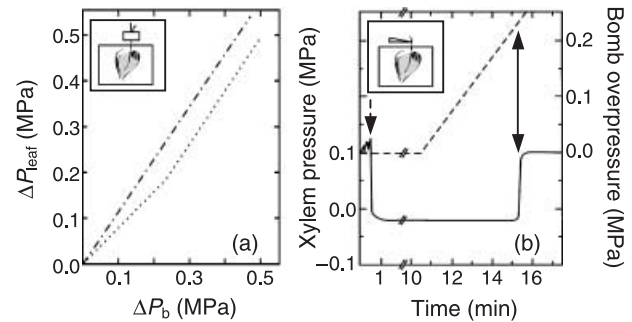
## 3. The pressure bomb technique: a conceptual artefact

The pressure bomb technique (Scholander *et al.*, 1965) was never validated in appropriate, well-defined air-water model systems in which negative pressures of known magnitude could be established. The method has only been compared with the root pressurisation (Passioura & Munns, 1984) and the psychrometric method (see e.g. Kaufmann, 1968a,b; Klepper & Ceccato, 1969; Barrs *et al.*, 1970; West & Gaff, 1971; Turner *et al.*, 1984), but these techniques have also never been calibrated and tested in model experiments. Agreement between the data obtained by the various approaches only suggests that 'sometimes these [...] methods are estimating the same quantity, but this is not proof that the quantity is the tension that

prevailed in the xylem water columns before cutting' (Canny, 1995b, p. 344). It is also surprising retrospectively that publication of many conflicting data (Tobiessen *et al.*, 1971; Hellkvist *et al.*, 1974; Connor *et al.*, 1977; Koch *et al.*, 1994) and some critical evaluations of the pressure bomb (Begg & Turner, 1970; Ritchie & Hinckley, 1971; Janes & Gee, 1973; Baughn & Tanner, 1976; Turner & Long, 1980) have not evoked a broadly based critical reassessment of the pressure bomb technique and the other methods.

With the pressure bomb, publication of stable negative xylem pressure values of  $-1$  MPa down to  $-17$  MPa became commonplace (e.g. Kappen *et al.*, 1972). The method is simple and thus very popular. A leafy twig is placed into a steel chamber with the cut end protruding through a pressure-tight seal to the ambient atmospheric pressure (Fig. 7b). Gas pressure is applied to the specimen with a rate of about  $0.3$  MPa/min. The overpressure at which water appears at the cut surface, the so-called balancing pressure,  $P_b$  (defined in relation to atmosphere), is postulated to be numerically equal to the tension ( $= -P_x + 0.1$  MPa) that existed in the xylem before cutting. This explanation implies (see e.g. Scholander *et al.*, 1965; Boyer, 1967; Passioura, 1982; Jones, 1992; Holbrook *et al.*, 1995; Tyree, 1997; Steudle, 2003) that the tension in the xylem is instantaneously relieved upon cutting of the leaf. Water is osmotically forced from the xylem into the adjacent tissue cells and the xylem sap is withdrawn from the cut end. The balancing overpressure is needed for forcing the water from cells back into the xylem elements and to re-establish the original tension.

However, from the beginning there have been many doubts about what the bomb in fact measures. The dilemma of interpreting pressure bomb data is that the contribution of the many processes to the  $P_b$ -value generated by cutting transpiring leaves and by subsequent application of gas pressure is not predictable and presumably highly species- and environment-dependent. Upon cutting capillary tension is created at the cut end which may considerably exceed the values that are predicted by the radii of the lumens of the conducting elements because of the varying diameters and/or the non-circular cross-sections of the vessels and tracheids (Finn, 1989; Zimmermann *et al.*, 1994a; Schneider *et al.*, 2000b). Tyree & Hammel (1972) assumed that  $P_b$  exclusively reflects the pressure at which the capillary force becomes zero, i.e. at which the cut surface becomes wetted (see their Eq. 6). Pressure probe work on air-cut leaves and roots has indeed shown (Zimmermann *et al.*, 1995a; Benkert *et al.*, 1995) that under well-hydrated conditions negative pressures in the xylem are not exclusively 'transpiration-born', but rather 'capillarity-born'. Probing of excised roots of maize and barley yielded similar results (Zhu *et al.*, 1995; Schneider *et al.*, 1997b). Today Tyree (1997) and Wei *et al.* (2000b) postulate that the pressure bomb is measuring only equilibrium negative pressure values in leaves because of the dissipation of hydrostatic and osmotic pressure gradients in the multi-phase plant organs



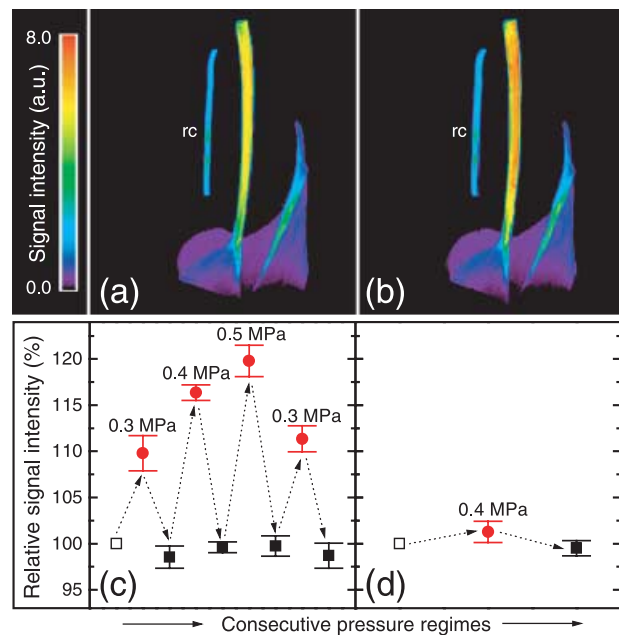
**Fig. 8** External pressure transmission in leaves of *Nicotiana tabacum* plants. (a) Excised leaves were mounted into a pressure bomb, and a pressure transducer was directly sealed to the cut end of the petiole protruding through the seal of the bomb (inset). Pressure changes in the leaves upon external gas pressure application ( $\Delta P_b$ ) are shown for a leaf preinfiltrated with water (dash-dotted line) and for an untreated leaf (dotted line). (b) Time course of changes in absolute xylem pressure (solid line) upon application of external gas pressure (dashed line; zero pressure = atmospheric pressure) measured by a xylem pressure probe inserted into the leaf petiole protruding through the seal of the bomb (inset). Vessel impalement is indicated by a dashed arrow. Double-headed arrow indicates pressurisation of the xylem sap associated with water release at the cut end. For discussion, see text and Zimmermann (2003). Taken from Balling & Zimmermann (1990), with kind permission of Springer-Verlag, Heidelberg, Germany.

upon cutting and stop of transpiration when the leaf is placed into the bomb (see e.g. Rygol *et al.*, 1993; Zimmermann *et al.*, 1991, 1992, 1993a). This assumption implies that the balancing pressure is not numerically equal to the tension previously existing in the xylem of the intact plant at the site where the leaf is cut. The discrepancy between the two values may be quite large, in particular when leafy twigs are taken from heights above 10 m as done for example by Scholander *et al.* (1965; see also Tobiessen *et al.*, 1971). In this case, the gravitational potential term vanishes upon cutting that should lead to large changes in the gradients of xylem pressure and in the turgor pressure of the hydraulically linked tissue cells provided that the fundamental assumption of the Cohesion Theory of the water columns being continuous over the total height of a tall tree is valid (see sections IV and V.1). Unfortunately, from the nomenclature used by Tyree and co-workers it is very often not clear whether the authors refer to the xylem tension in the intact plant or to the new artificial equilibrium value.

A further key problem is the transmission of external pressure to the xylem which is assumed by the users of the bomb technique to occur instantaneously. Scholander and his co-workers (1965) saw this quite clearly at the beginning of the 'pressure bomb era'. Balling & Zimmermann (1990) studied the pressure transmission by sealing a pressure transducer onto the cut end of leaf petioles of tobacco protruding through the seal of the pressure bomb (Fig. 8a). Only in leaf petioles pre-infiltrated under vacuum with water, the pressure response upon increasing the bomb pressure was 1 : 1; otherwise, it was significantly less up to a bomb overpressure of about 0.25 MPa.

In accordance with previous results of West & Gaff (1971, 1976), these findings evidence that air-filled spaces had to be compressed before pressure could be transmitted directly. Wei *et al.* (2000b) recently repeated the experiment of Balling & Zimmermann (1990) on excised, non-infiltrated leaf specimens. They traced the significant discrepancies between the applied bomb pressures and the pressure transducer readings back to the compression of air bubbles in embolised vessels. Surprisingly, they argued that this finding does not invalidate the pressure bomb as a measuring tool for negative xylem pressures when the cut surface is exposed to atmosphere. The finding of Balling & Zimmermann (1990) on detached leaves with their cut surface outside the bomb that the negative xylem pressure of non-infiltrated specimens – in contrast to infiltrated specimens – only responded to overpressures of 0.25 MPa (Fig. 8b) makes this statement obsolete.

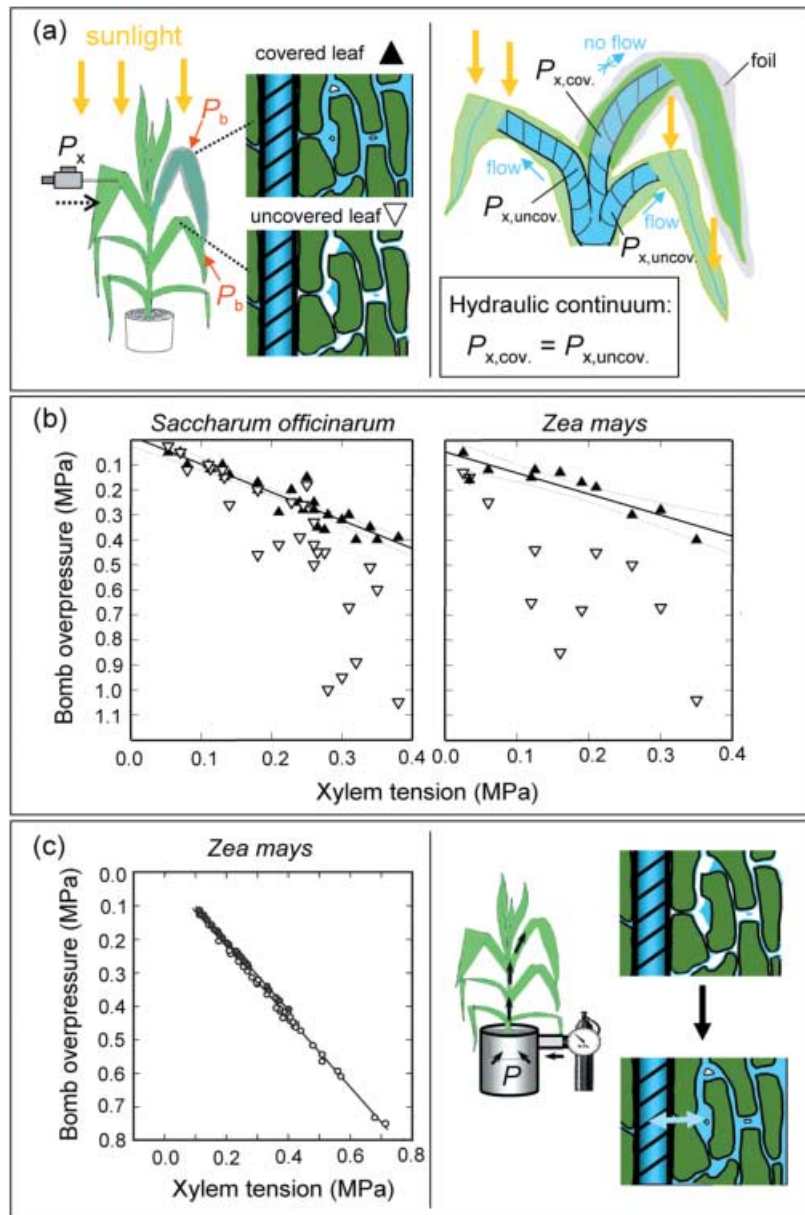
$^1\text{H}$  NMR imaging of petioles of cut hydrated *Epipremnum aureum* leaves subjected to pressurisation also gave evidence that the overpressure cannot be set equal to the original (or equilibrium) xylem tension (Zimmermann *et al.*, 2000; Schneider *et al.*, manuscript in preparation). Leaves of this plant were selected because of their stiffness and low compressibility that prevented movement of the leaf within the bomb (mounted into the bore of a NMR magnet) during the measurements. Only data for uniform pressure application ( $0.1\text{ MPa min}^{-1}$ ) are shown in Fig. 9 because the Scholander bomb arrangement yielded the same results. It is obvious from Figs 9a–c that  $T_1$ -weighted images showed a significant increase in signal intensity with increasing overpressure. By contrast, the spin density (i.e. the water content of the petiole) remained constant within the limits of accuracy (Fig. 9d). The effects of pressurisation on spin-lattice relaxation time,  $T_1$ , were completely reversible.  $T_1$  depends on the compartment size confining the water (Mansfield & Morris, 1982) and on the oxygen concentration (Chen *et al.*, 1998). Since pressure-induced changes in the oxygen concentration could be excluded by control experiments using  $\text{N}_2$ -exposed leaves, the results can **only** be explained by a pressure-induced shift of water within the leaf petiole from large water-filled to air-filled compartments or to small cells (because of the volume dependence of the volumetric elastic modulus of the cell wall, Zimmermann, 1978). The criticism of Wei *et al.* (2000a, p. 147) that ‘a movement of water from the symplast into the tissue apoplast can be neither detected nor quantified because of a lack of resolution’ is irrelevant. What is relevant is that **uniform pressure application leads to water movement** within the leaf resulting in the generation of pressure gradients due to the anisotropic compressibility features of the multi-phase leaf system. Similar conclusions have been drawn from xylem pressure probe measurements in intact, weakly transpiring tobacco plants placed in a hyperbaric chamber (Balling & Zimmermann, 1990; Zimmermann *et al.*, 1991). Changes in xylem pressure could be recorded over 2 h before the tissue equilibrated with ambient pressure (up to 0.5 MPa). The



**Fig. 9** Typical  $T_1$ -weighted and spin-density-weighted  $^1\text{H}$  NMR experiments on well-hydrated leaves of the liana *Epipremnum aureum* subjected to uniform pressure regimes by using a pressure bomb placed in the bore of the NMR magnet. Note that  $T_1$ -weighted images represent the compartment size confining the water whereas the spin density images reflect the water content of the tissue. In the case of  $T_1$ -weighted experiments, application of overpressure (given in relative values) led to an increase of the signal intensity within the leaf petiole as indicated by the colour change from yellow to red (a = control; b = 0.5 MPa; signal intensity increase c. 20%). The pressure effects on the signal intensity (●) were reversible as shown by intermediate pressure releases (■ in c; mean values  $\pm$  sd,  $n = 4$ ). By contrast, in corresponding spin-density-weighted experiments the signal intensity remained unaffected by the pressure regime (d). Note that the values in (c) and (d) were normalised to the reference images which were taken before the first pressure application (□). Note further that the signal intensities of the water-filled reference capillary (rc) remained unaffected by the pressure regime both in  $T_1$ -weighted and spin-density-weighted experiments. For discussion of the results, see text and Zimmermann (2003); for experimental details, see Zimmermann *et al.* (2000). (a,b) taken from Zimmermann *et al.* (2000), with kind permission of Elsevier, Amsterdam, The Netherlands.

equilibrium xylem pressure corresponded to the original value as expected in the light of Eq. 4 stating that the absolute hydrostatic pressures in the water and solvent phase are independent of the ambient pressure (a ‘bizarre’ phenomenon according to Passioura, 1991). The observations of our group were recently confirmed by Wei *et al.* (2000b) by exposing *Tsuga canadensis* branches to uniform pressure in a pressure bomb. Astoundingly, even this did not provoke a shift in the persistent view of these authors that the pressure bomb is an appropriate tool for measuring xylem pressures.

Strong evidence for the interference of air-filled spaces with pressure transmission also arrived from the experiments of Melcher *et al.* (1998). In this study (Fig. 10a, left-hand side),



**Fig. 10** Xylem pressure probe vs pressure bomb-based balancing overpressure measurements. The schematic diagrams in (a) show the experimental procedure used by Melcher *et al.* (1998). Before the experiment, when the leaves were well watered (upper drawing on the left-hand side), one leaf was covered with aluminium foil before onset of transpiration, while a pressure probe was inserted into the xylem of an adjacent, exposed leaf. Upon progressive increase in transpiration evaporational water loss leads to the development of large air-filled spaces in the uncovered leaves (illustrated by the large white areas in the lower drawing), but not in the covered leaf (upper drawing). Because of the hydraulic connection the xylem pressure in the covered leaf ( $P_{x, cov.}$ ) should be the same as in the adjacent uncovered leaves ( $P_{x, uncov.}$ ) independent of transpiration (right-hand side of (a); green leaf areas: full hydration; mottled leaf areas: incomplete hydration). (b) Plots of the balancing overpressure values,  $P_b$ , measured on the covered and an adjacent uncovered leaf vs the corresponding tension values yielded a nearly 1 : 1 relationship for covered leaves (▲; dotted lines indicate 95% confidence). Large discrepancies (that increased with transpiration) were found between the balancing overpressures and tension values of uncovered leaves (▼), since excessive overpressure is needed for compression of the air-filled spaces. (c) The approach of Wei *et al.* (1999b) differs essentially from that of Melcher *et al.* (1998) in that pneumatic pressure was applied to the roots before the  $P_b$ -values were determined. This leads to a refilling and compression of the air-filled spaces as shown in the drawings (right-hand side) and, because of the establishment of a hydraulic continuum in the leaves, also to a 1 : 1 relationship between the tension values and balancing overpressure values of uncovered leaves (left-hand side, redrawn from Wei *et al.*, 1999b; ● = covered leaves; ○ = uncovered leaves). Extrapolations of the straight lines to pressure values that were not measured were omitted. For a detailed discussion of the results, see text and Zimmermann (2003). (b) and (c) modified after Zimmermann *et al.* (2000) and Wei *et al.* (1999a), respectively, with kind permission of Elsevier, Amsterdam, The Netherlands.

one or two leaves of sugarcane or maize were covered with aluminium foil at predawn to prevent substantial transpiration during the day. In a nearby light-exposed, transpiring leaf of the same plant the xylem pressure probe was introduced into a vessel. As shown schematically in Fig. 10b (right-hand side), significant differences in xylem tensions between the neighbouring covered and light-exposed leaves can be excluded because of the hydraulic continuum of xylem sap (see also Passioura, 1982; Benkert *et al.*, 1991; McCutchan & Shackel, 1992). For determination of  $P_b$  covered and uncovered leaves were excised simultaneously from the same plant. In order to prevent dehydration after excision, the leaves were covered with plastic bags just before excision. The excised leaves, still in plastic bags except for the cut end, were sealed into a pressure bomb. A nearly 1 : 1 relationship (up to a tension of 0.4 MPa;  $P_x = -0.3$  MPa) was found between xylem tension values measured with the xylem pressure probe and the bomb (Fig. 10b). By contrast, at noon when the transpiring leaves were exposed to light irradiances of 1500–2000  $\mu\text{mol m}^{-2} \text{s}^{-1}$  and the proportion of the air-filled spaces within these leaves increased, the balancing overpressures of uncovered leaves were up to 0.6 MPa (200%!!) greater than those of the covered leaves and the tension values measured directly with the xylem pressure probe, respectively. This demonstrates that substantial overpressures were required to establish a hydraulic continuum within the periphery and the xylem of the uncovered leaves. Wei *et al.* (1999b, p. 1203) argued that ‘the presumption that adjacent leaves should have nearly identical pressures is clearly wrong’. These authors repeated the experiments of Melcher *et al.* (1998) under so-called ‘well-defined conditions’ by using maize plants being not irrigated for 1 d or more prior to the experiment. During probe measurements, the xylem pressure was artificially adjusted by root pressurisation in order to verify insertion of the probe tip into a xylem vessel. For determination of the balancing overpressure values leaf blade tissue was subsequently taken from the leaf in which the probe was inserted. In contrast to Melcher *et al.* (1998), Wei *et al.* (1999a,b) reported a 1 : 1 correlation between relative  $P_b$ -values and xylem tensions up to 0.7 MPa ( $P_x = -0.6$  MPa) also for the weakly transpiring leaf blade tissue (data for the non-transpiring leaves are not given for larger pressures, see Fig. 10c). Even though accurate details of the entire experimental procedure are not presented, it is clear that preceding root pressurisation led to a refilling of air-filled spaces and cells as illustrated in Fig. 10c on the right-hand side (see also the NMR results in Fig. 9). Under these highly artificial conditions excessive overpressure is, of course, not required. The question remains why Tyree, Steudle and Wei try to create the impression that the pressure bomb has been calibrated down to  $-1.0$  MPa by using the pressure probe and can thus be employed for measuring xylem pressure even in highly transpiring and/or dehydrated plants.

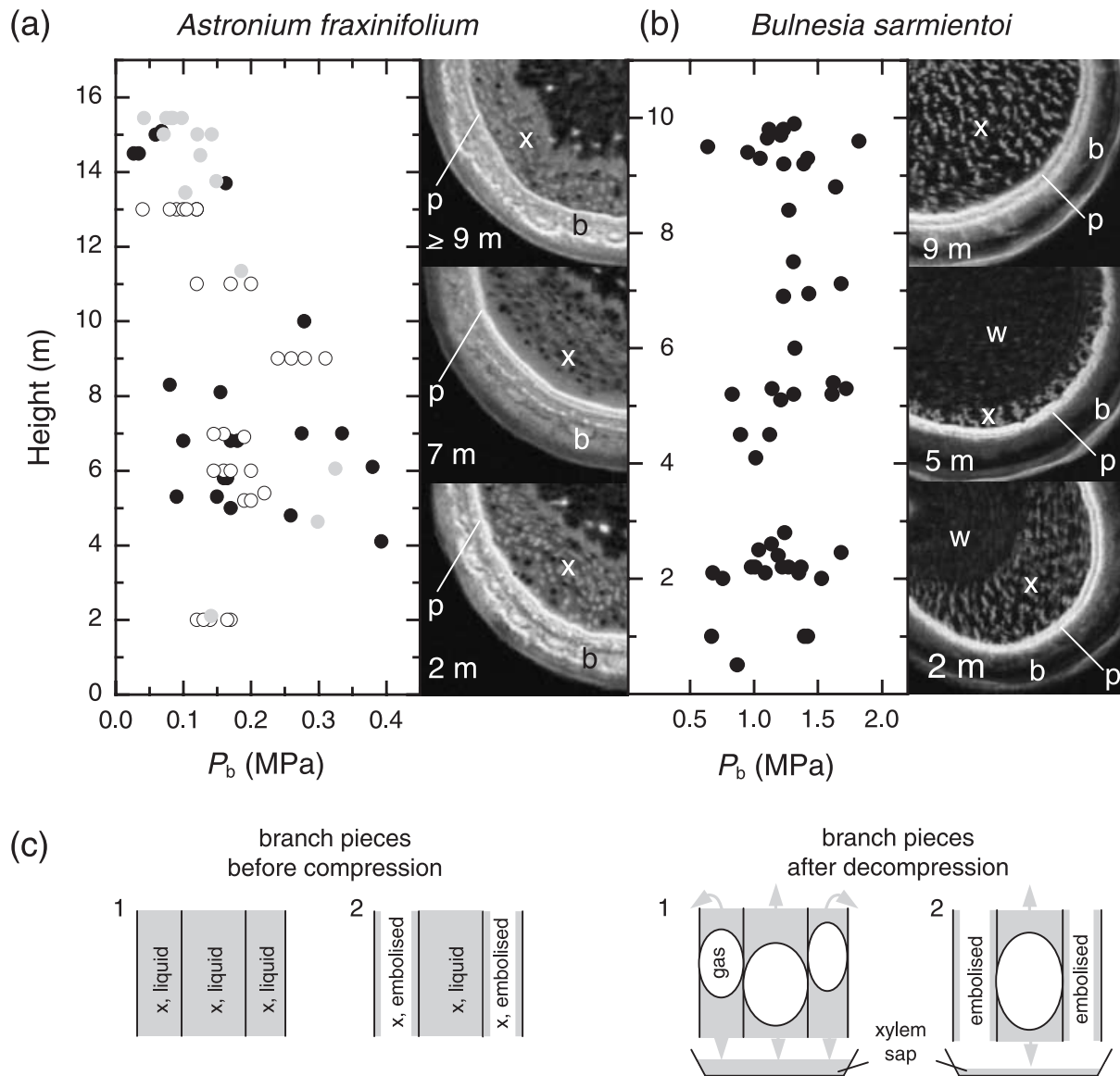
Meinzer *et al.* (2001, p. 245) suggested recently that ‘pressure bomb measurements should routinely be made on both

covered and exposed leaves, but even the covered leaf balance pressures can be ambiguous if substantial cavitation has occurred and if the anatomy of the stem to which the leaf is attached allows water to be forced into non-conducting tissue during pressurisation’. However, such bomb data are also ambiguous when the xylem sap does not consist of pure water. In this case, corrections of the  $P_b$ -values are required (Smith & Lüttge, 1985; Murphy & Smith, 1994) which can be quite dramatic if mucilage or proteins are present, as found in mangroves and other salt-tolerant trees (see sections IV and V.6). In *Rhizophora mangle*, excessive overpressures of about 3 MPa were required to compensate for the xylem mucilage, irrespective of the difference in overpressure between covered and uncovered leaves (0.6–0.8 MPa; Melcher *et al.*, 2001). This clearly reflects the dilemma that applicants of the pressure bomb technique are faced with.

In the last years many desperate efforts were made by the proponents of the Cohesion Theory to ‘rescue’ the bomb as a measuring tool for xylem pressure. The inconsistencies of the various approaches are discussed in detail in Appendix 2. Tyree (1997), one of the outstanding proponents of the pressure bomb technique and Cohesion Theory, hit the mark by saying recently that the interpretation of pressure bomb data in terms of xylem pressure is based on hypothesis. This implies that stable negative pressures of the order of megapascals measured by this technique are not facts, but belong in the realm of science fiction.

#### IV. The continuity of the xylem water columns: fact or hypothesis?

The second assumption of the Cohesion Theory, i.e. the continuity of the water columns, was also challenged recently (Zimmermann *et al.*, 2002c). Evidence of the filling state of the vessels can be obtained by rapid xylem sap sampling. Pure xylem sap can be expelled from the vessels when gas bubbles are formed in the xylem by subjecting branch pieces to a compression/decompression cycle (see also Schill *et al.*, 1996). Most importantly, this method only functions if the water columns are **continuous** over some length (see schematic diagram in Fig. 11c). If the vessels are (partly) embolised, no or less water can be extracted in contrast to centrifugation. For example, this was found for branches of *Astronium fraxinifolium*, a tree growing in the Chaco of Paraguay at moderately saline sites, under predawn conditions where refilling should have been completed. While several hundred microlitres of xylem sap could be extracted by a compression/decompression cycle from branch pieces collected at 13–15 m height, no or only very tiny amounts of liquid could be collected from branch pieces further down (Fig. 11c). Centrifugation yielded similar results, although the volume of the extracted xylem sap was, on average, much larger, even in branch pieces where the compression/decompression method failed. Clear-cut evidence that the water columns in branches



**Fig. 11** Xylem filling status of tall salt-tolerant Chaco trees in dependence on height. Evidence for the ‘water availability state’ in the xylem vessels at predawn was obtained by balancing overpressure measurements on leafy twigs,  $T_1$ -weighted  $^1\text{H}$  NMR imaging on leafless twigs (protected against water loss) and extraction of xylem sap from branch pieces using the compression/decompression method (described in section IV). (a) *Astronium fraxinifolium*: Balancing overpressure values of leafy twigs taken from three trees (black, white and grey circles) were much lower close to the ground level and towards the apex than at intermediate heights of 4–8 m. Cross-sectional  $^1\text{H}$  NMR images revealed that at intermediate heights the lumens of the majority of the xylem vessels were filled with air, while most vessels at 2 m and above 9 m height were filled with water (x = xylem, p = phloem, b = bark). Xylem sap extraction yielded substantial amounts when branches were taken at 2 m and at 9 m height and above, but considerably less when taken from intermediate heights. (b) *Bulnesia sarmientoi*: Balancing overpressure values were height-independent within the limits of accuracy. Cross-sectional  $^1\text{H}$  NMR images taken at 2 m showed a circular xylem area of extremely weak signal intensity (w) that increased towards intermediate heights dramatically and disappeared then at higher heights. Xylem sap extraction yielded comparable amounts over the entire height. (c) The scheme illustrates that the amount of xylem sap (shaded areas) expelled by the compression/decompression method can be taken as a quick relative indicator for the filling status of the vessels of the branches of a given tree because sap can only be expelled from a vessel upon pressure release by the spontaneously formed gas bubbles when it was not embolised before treatment (1: filled vessels; 2: partly gas-filled vessels). For more details, see text and Zimmermann (2003). Graph in (a) modified after Zimmermann (2003), with kind permission of Physiko-Medica, Würzburg, Germany.

between heights of 2 m and about 8 m were partly interrupted was obtained by  $^1\text{H}$  NMR imaging on defoliated twigs protected against evaporational water loss before measurements. It is evident from Fig. 11a that most xylem vessels of a twig taken at 2 m height showed high signal intensity, i.e. they were obviously filled with water. Towards 7 m height the number of vessels exhibiting high signal intensity decreased dramatically, whereas at 9 m height the majority of the vessels showed again high signal intensity. Staining experiments demonstrated that the lumens of the vessels were not completely filled with mucilage, proteins or other compounds that could suppress  $^1\text{H}$ -signal intensity. This indicated that the vessels exhibiting zero signal intensity were filled with air and, in turn, that at intermediate heights the water columns in the twigs of this tree were interrupted. The height-dependent filling state of the vessels with water is obviously reflected in the balancing overpressure values of leafy twigs. As shown in Fig. 11a,  $P_b$  increased up to a height of 8 m from about 0.1 MPa to 0.4 MPa and decreased again towards larger heights. This suggests that the Scholander pressure bomb arrangement can be used for estimating the 'water availability state' in the vessels under field conditions. The conclusion is corroborated by measurements on the Chaco tree *Bulnesia sarmientoi* rooting in extremely saline water. In this case, the predawn balancing overpressure values of the leafy twigs were almost constant over the entire height (Fig. 11b). Consistently, xylem sap extraction from branch pieces by a compression/decompression cycle yielded comparable xylem sap volumes over the entire heights. Staining of microscopic sections and  $^1\text{H}$  NMR imaging (Fig. 11b) revealed that the water-conducting cross-sectional areas of twigs taken at intermediate heights were reduced by extremely high concentrations of mucopolysaccharides (together with amylose and proteins; Zimmermann *et al.*, 2002c). The asymmetric clogging of vessels by mucopolysaccharides apparently resulted in a more or less homogenous water content of the vessel lumens of the twigs over the entire height.

The finding on *A. fraxinifolium* evidences that the assumption of the water columns being continuous at predawn is not necessarily true. The current data base is too poor to generalise this statement. However, in the light of the simple experiments of Sachs (1887, p. 269) it is quite likely that the water columns in many other trees may also be discontinuous, particularly during the day. Thus, we have to postulate the involvement of other forces together with watergates for water lifting.

## V. The 'Multi-Force' or 'Watergate' Theory

Canny (1995b) has recently introduced a new unifying theory to explain water lifting in tall trees. This so-called 'Compensating-Pressure Theory' has been challenged in a series of strenuous, partly impertinent critiques (Comstock, 1999; Tyree, 1999; Stiller & Sperry, 1999). We have also criticised Canny's theory for some thermodynamic reasons

(Zimmermann *et al.*, 1995b). However, our main objection is that it is premature to introduce a new unifying theory that explains all-inclusively the mechanism of water lifting in higher plants including trees. In our opinion, plants are too complicated and the data basis is too poor to formulate a coherent concept about the mechanism of water ascent at the present state of the art. Therefore, in the light of the above considerations we propose to identify experimentally the various interacting forces and flows that can operate in plant species under various environmental conditions while simultaneously elucidating the structural features of the xylem elements involved.

### 1. The driving forces: some general remarks

The chemical potential (partial molar Gibbs energy) does not contain terms for gravitational and electrical fields or surface interactions and capillary effects. As shown by Gibbs (1961) and other authors (Andrews, 1972, 1976; Roderick, 2001; Zimmermann *et al.*, 2002a), in a static water column within a xylem element at thermodynamic equilibrium the chemical potential of water,  $\mu_w$ , (and of any other component in the liquid) declines as the gravitational potential energy increases with height,  $h$ . However, their sum is a constant throughout the column:

$$\mu_w + M_w g h = \text{const.} \quad (6)$$

or, with the use of Eq. 3:

$$-M_w g h = \bar{V}_w (P_{x,h} - P_{x,h=0}) + RT \ln(a_{x,h}/a_{x,h=0}) \quad (7)$$

where  $M_w$  is the molecular mass of water.

The existence of gradients in the chemical potential of water at thermodynamic equilibrium without the occurrence of flow demonstrates that it is not correct when stating that flow spontaneously occurs along chemical potential gradients. In mechanics or electricity the direction of spontaneous changes is always such that the system goes from a higher potential-energy state to a lower one. In thermodynamics the system is not quite so simple because we have to take into account that the chemical potential comprises both energy and entropy factors. For this and other reasons treatment of long-distance water transport by Ohm's law analogy (Tyree, 1997; Wei *et al.*, 1999a,b; Steudle, 2002b; see also further below) is utterly wrong (Cowan, 1965). As highlighted recently by Roderick (2001) the central issue is that the theory of Gibbs predicts that water (or other components) would spontaneously move across the boundary surface between different phases if the chemical potential is not equal on both sides (see section II.1). Thus, the incorporation of cellular compartments, xylem segmentation into osmotically active compartments, interruption of water columns, water attraction by hydrogel phases and other phase boundaries (e.g. between root cells and mycorrhiza-forming fungal hyphae)

into the analysis of water ascent in a transpiring plant is a cogent necessity (Zimmermann *et al.*, 1993a,b, 1994a, 1995b). For the same reasons, the introduction of a so-called water potential instead of the chemical potential of water as well as the traditional approach in plant physiology to assume that the total water potential of a system can be expressed as the sum of matric potential, gravitational potential *etc.* is erroneous or at least very questionable (Dainty, 1976; Zimmermann & Steudle, 1978; Roderick, 2001; Zimmermann *et al.*, 2002a). Equally, the practice of reporting a single estimate of the water potential for an entire tissue and for soil has no meaning because the chemical potential of water will be different in each phase of such multi-phase systems.

In summary, there is no doubt that Eqs 6 and 7 are an inadequate basis for a complete description of multiphase systems such as higher plants. Under non-equilibrium conditions other forces and flows can come into play that are not included. Coupling between non-conjugate flows and forces *via* semipermeable membranes and barriers makes it even more difficult to arrive at a quantitative treatment of water ascent. However, the equations are of great heuristic value because they show that gradients in the activity of water can be just as important as (transpiration-induced) tension gradients for the compensation of the gravitational potential term. They also demonstrate that the magnitude of the tension is much less when a water volume that was lifted to a certain height is supported by appropriate axial barriers, by adherence to hydrogel films on the inner xylem walls or by other stabilising means. In this case, the height, *h*, in the equations reduces to the height between the supporting sites. Thus, less tension is needed to shift the water further up, i.e. water is lifted analogously to a ship lifted by serial watergates.

## 2. Transpiration-bound water ascent

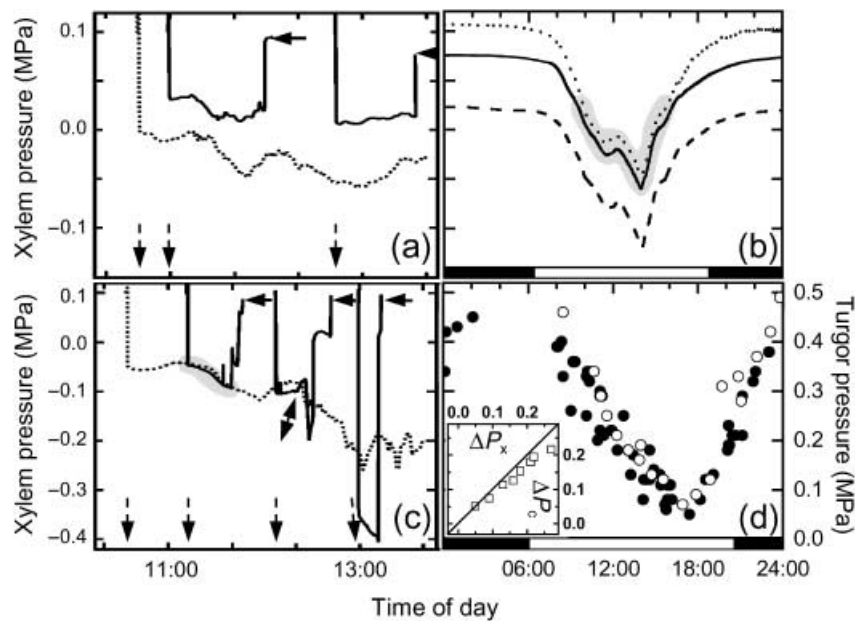
Vessel probing has demonstrated that transpiration-induced tension obviously plays a crucial role in water lifting as long as water continuity in the vessels is maintained. For herbaceous, laboratory-sized plants it appears that transpiration-induced tension is apparently the dominant driving force, admittedly in co-operation with the water relations parameters of adjacent tissue cells (Haberlandt, 1909, p. 291; Renner, 1915; Strugger, 1943; Balling & Zimmermann, 1990; see next section).

Tension is also an important driving force in lianas where water has to be lifted to larger heights. This is obvious from xylem pressure probe measurements at 1 m and 5 m height on a *c.* 10-m-tall liana (*Tetrastigma voinierianum*) grown in a tropical greenhouse and watered regularly (Benkert *et al.*, 1995; Thürmer *et al.*, 1999). Due to branch bending at 9 m height the measuring points were about 17 and 13 m, respectively, away from the roots. The xylem pressure probe measurements revealed that the magnitude of the pressure values depended strongly on weather conditions. On cloudy and rainy days the xylem pressure remained in the positive, sub-atmospheric

or slightly negative range over the entire day (Figs 12a,b). Occasionally, under these conditions pressure values at ground level were equal to or more negative than those at 5 m (Fig. 12a). By contrast, exposure of the leaves to sunshine after a cloudy morning resulted in a drop of the xylem pressure at 5 m down to  $-0.4$  MPa, but not at 1 m height (Fig. 12c). Lower negative pressures could not be recorded, even in concomitant measurements at 9.5 m. On very sunny days, negative pressures could only occasionally be read at 5 m and in particular at 9.5 m around noon. Repeated insertion of probes revealed that cavitation had occurred in the majority of the vessels. This is in accordance with cryo-scanning electron microscopy results on herbaceous plants under field conditions (Canny, 1997b, 2001). Negative pressures at 5 m and 9.5 m height could only be recorded again during the afternoon.

Whether a tension gradient was formed or disappeared in the morning hours on sunny days depended on the exposure duration of the leaves to sunshine. In the light of the innumerable leafy branches of the liana it can be expected that local transpiration varied considerably over the entire height (Hinckley & Ritchie, 1970). A base-to-apex-directed positive tension gradient developed only when tension increased first in the upper parts of the plant. However, between 1 m and 5 m the gradients were very often significantly smaller than between 5 m and 9.5 m. Local changes in tension were apparently not immediately propagated, and this led to temporary decreases or increases, respectively, of the pressure gradients between the measuring points (Figs 12b and 12c, shaded areas). This finding is in agreement with results of Huber & Schmidt (1936), Morikawa (1974) and Goldstein *et al.* (1998) (see also Swanson, 1967; Lassoie *et al.*, 1977) who demonstrated that changes in sap flow in the lower part of the trunk lagged behind those in the upper part.

From our standpoint, the probe data clearly revealed that the spatial development of negative pressures over long distances does not follow necessarily the simplistic notion that water is lifted by transpiration-induced tension gradients through 'pipelines' with impervious walls. However, Wei *et al.* (1999b) interpreted the xylem pressure probe data in favour of the Cohesion Theory. They criticised Thürmer *et al.* (1999) for not taking into account flow resistances caused by the hydraulic architecture and the upside-down arrangement of part of the liana branches. Theoretically, downward-directed flow may lead to tension gradients smaller than  $10 \text{ kPa m}^{-1}$ . However, as mentioned above, the key point is that no tension gradients or even tension gradients of opposite direction were observed on rainy and cloudy days (see e.g. Figs 12a and 12b), i.e. when transpiration and, in turn, flow was very low at ground level (relative humidity 84%, light irradiation  $12\text{--}27 \mu\text{mol m}^{-2} \text{ s}^{-1}$ ; Benkert *et al.*, 1995). Therefore, we would expect a tension gradient within the water column comparable in magnitude and direction to that observed during the night (Fig. 12b) if the assumption of Wei *et al.* (1999b) is applied. However, this contradicts the experimental findings. The



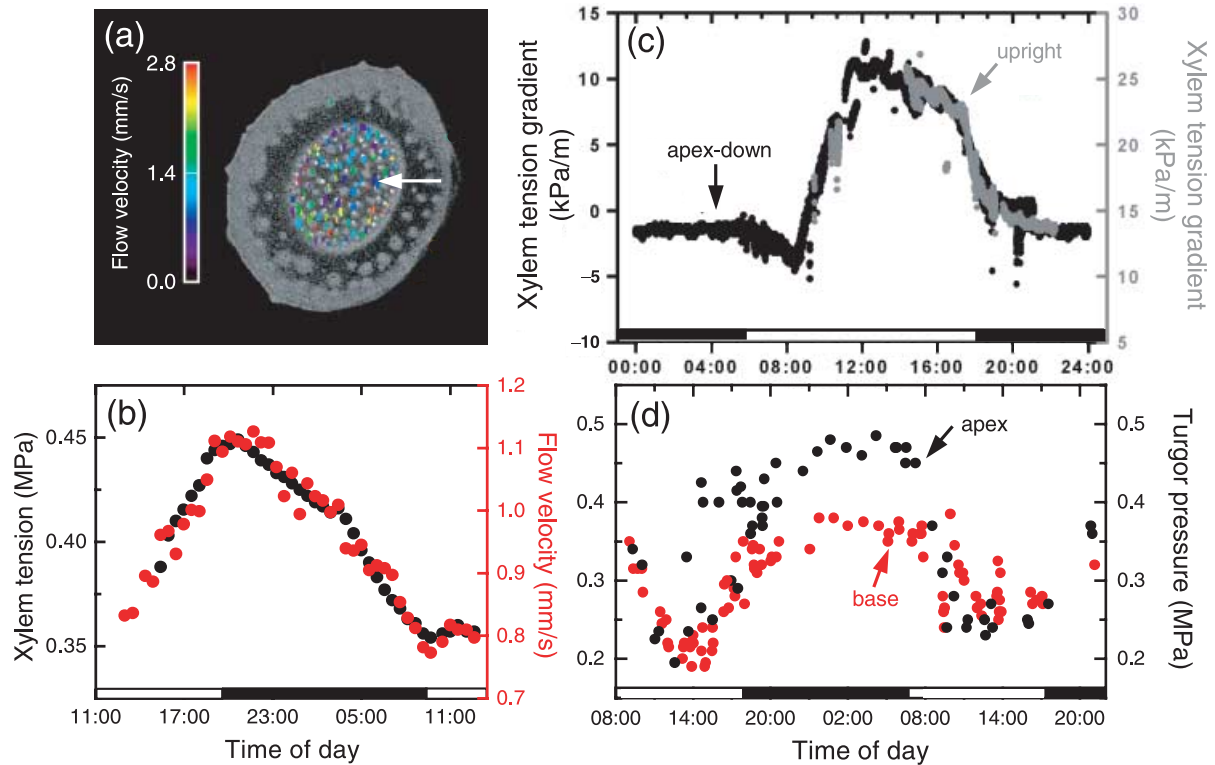
**Fig. 12** Diurnal changes in xylem pressure (a–c) and turgor pressure (d) in the liana *Tetrastigma vainierianum* under greenhouse conditions. Xylem pressure: Concomitant xylem pressure recordings in (a) and (c) were performed at 1 m (dashed line) and 5 m (solid line) height as well as in (b) at 9.5 m height (dotted line). Vessel piercing in (a) and (c) is indicated by a downward-directed dashed arrow, cavitations or leaks are marked with a horizontal arrow. Weather conditions were in (a) cloudy and rainy, in (b) cloudy and in (c) up to noon cloudy and then very sunny (marked with a double-headed arrow). Note that the tension gradient in (a) was opposite to that expected from the Cohesion Theory and that in (b) and (c) the tension gradient partly disappeared (shaded areas). Turgor pressure: Measurements were performed at 1 m height (○) simultaneously with xylem pressure recordings (not shown) and at 5 m height (●) on very sunny days. As shown in the inset, a nearly 1 : 1 relationship existed between turgor and xylem pressure changes at 1 m height. For discussion, see text and Zimmermann (2003); for more experimental details, see Benkert *et al.* (1995) and Thürmer *et al.* (1999). (a,c) from Benkert *et al.* (1995) and (b,d) from Thürmer *et al.* (1999), with kind permission of Springer-Verlag, Heidelberg, Germany.

further assertion of Wei and colleagues that the gravitational potential term must be provided with a negative sign because of the apex-down orientation of the branches is not correct: Water has to be lifted from the roots at ground level up to 1 m, 5 m or 9.5 m and the gravitational potential is independent of the path.

Wistuba *et al.* (2000) recently added further evidence for the correct interpretation of the probe data on *Tetrastigma vainierianum*. These authors measured the xylem tension gradients in up to 14-m-tall, well-hydrated specimens of *Epipremnum aureum*. Flow-weighted  $^1\text{H}$  NMR imaging together with probe measurements revealed that changes in transpiration induced parallel changes in xylem tension and flow (Figs 13a,b), thus contrasting the findings on plants subjected to drought (section III.1). This liana did not exhibit branches such as *T. vainierianum*, thus xylem tension gradients could be determined under controlled upright, horizontal and inverted (i.e. apex-down) orientations. As expected, the absolute values of the xylem tension gradients were different for all three orientations, but the diurnal changes in the magnitude of the gradients were nearly identical (note the different scaling of the ordinates in Fig. 13c; data for horizontal orientation are not shown). During the night the xylem tension gradients attained generally much lower values

than during the day. For the upright orientation they were still slightly larger than the value of  $10 \text{ kPa m}^{-1}$  as required for tension-driven water lifting. Similar values were also found for horizontally oriented plants. Apex-down plants showed negative gradients, i.e. the xylem tensions were higher towards the elevated shoot base than near the apex at ground level. Interestingly, around midnight the magnitude of the negative tension gradient was much less than expected theoretically from a standing water column (as predicted by the Cohesion Theory). However, at predawn the xylem pressure at the apex became gradually more positive, leading to a shift of the gradient to slightly more negative values. Upon exposure of the shoot base to sunlight at 6 m height, the xylem pressure dropped into the negative pressure range with the formation of a tension gradient of down to  $-5 \text{ kPa m}^{-1}$  (Fig. 13c). This gradient reversed when the leaves at the apex at ground level also became exposed to the sun. This supports the above finding on *T. vainierianum* that local changes in pressure can occur that are not propagated immediately through the entire xylem conduit.

The findings on the two lianas as well as the tensions of up to 0.2 MPa measured in the xylem of well-hydrated leaves of the tropical rainforest tree *Anacardium excelsum* at 35 m height (Zimmermann *et al.*, 1994a) demonstrate that forces other than transpiration-induced tension must be involved in



**Fig. 13** Diurnal changes in xylem flow velocity and in the longitudinal gradients of xylem tension and turgor pressure measured on the shoots of well-hydrated *Epipremnum aureum* plants. Laboratory conditions: (a) Coloured flow velocity  $^1\text{H}$  NMR cross-sectional image measured in the xylem of a horizontally oriented liana. For correlation of flow with anatomy the flow velocity map was superimposed on a black-and-white  $T_1$ -weighted spin echo image (taken at 16:30 h at a flux density of about  $20 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). (b) Diurnal flow velocity changes extracted from the region in (a) marked by an arrow and the corresponding changes in xylem tension measured concomitantly. Greenhouse conditions: (c) Diurnal changes in the base-to-apex-directed xylem tension gradients of upright (grey) and apex-down (black) oriented plants recorded with two xylem pressure probes that were at least 4.1 m away from each other. Data were pooled from 2 d. Note that negative values of the gradients during the night mean that the tension was higher at the base than at the apex. Note further that the absolute values of the upright and apex-down gradients differed (see scaling of the ordinates), even though the diurnal changes in the gradients were comparable. (d) Diurnal changes in the turgor pressure at the apex and the base measured concomitantly with two cell turgor pressure probes on an apex-down plant. Note the development of large turgor pressure gradients during the night. Redrawn from Wistuba *et al.* (2000), with kind permission of Thieme-Verlag, Stuttgart, Germany.

water movement that come to the fore under certain conditions (see also Meinzer *et al.*, 2001). Concomitant turgor pressure measurements on both lianas as well as on herbaceous plants showed that the chemical potential of water of the adjacent tissue cells plays an important role in locally 'buffering' the xylem pressure against rapid drops towards more negative pressures upon transpirational water loss.

### 3. Cell osmotic pressure-coupled water ascent

The great advantage of the frame given by thermodynamics of irreversible processes is that it describes and quantifies couplings between flows and forces. The very popular description of water flow in terms of electronic circuits (e.g. Tyree, 1997; Wei *et al.*, 1999a,b) contradicts this because coupling of water movement in the xylem with other forces and flows in the tissue is neglected. The application of Ohm's law to water flow in the xylem assumes that living cells are not involved in water

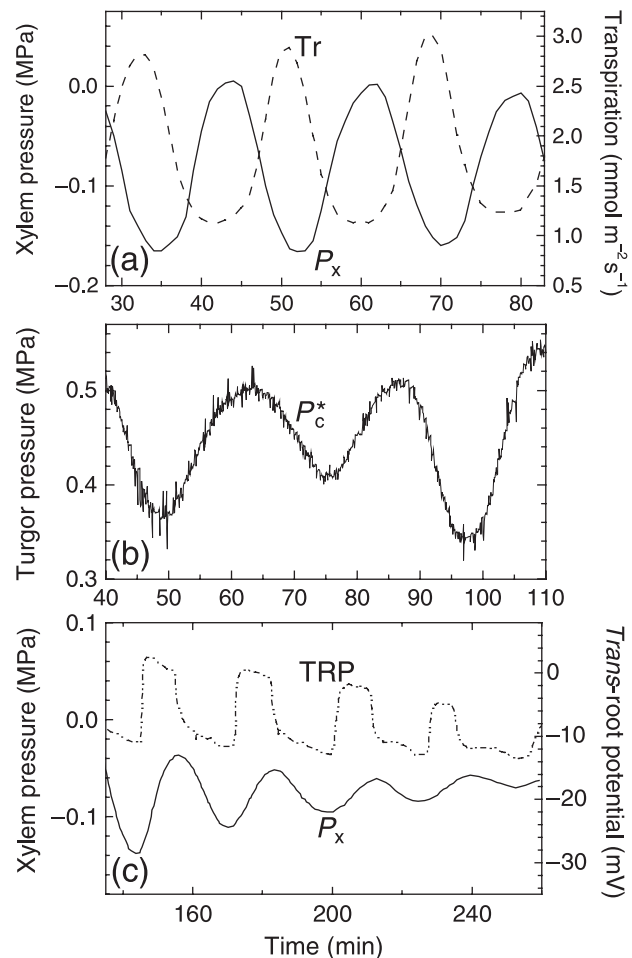
lifting. For justification, Sperry *et al.* (1996) and other authors cite the experiments of Strasburger (1891) who reported that cut lianas 'remained alive' for considerable time periods after addition of water-soluble poisons or after boiling of the shoot. Strasburger's lianas were, at maximum, 15 m tall. As shown above, sufficiently negative pressures to hold the water columns against gravity were measured by the xylem pressure probe on intact lianas. Interestingly, Strasburger reported several times that the xylem of the lianas contained mucilaginous substances that can additionally contribute to water lifting (section V.6). Furthermore, Strasburger's interpretation is based only on the observation how long it took the leaves to wilt (Canny, 1995b; Zimmermann *et al.*, 2002b). Ewart (1908) demonstrated that poisons such as formalin killed only part of the xylem parenchyma. For similar reasons Haberlandt and other authors stated that the experiments of Strasburger are not conclusive (see Haberlandt, 1909, p. 295; Ursprung & Blum, 1916). Therefore, it is quite obvious to us that the experiments of

Strasburger have been completely misinterpreted by the current proponents of the Cohesion Theory (e.g. Steudle, 2002b). Obviously, they have not read the work of Renner (1915), Münch (1930, p. 49), Strugger (1943) and other authors up to the 1950s. These authors have clearly seen that the development of tension in the xylem cannot be considered separated from the tissue cells: 'Turgor pressure must decrease with the development of negative pressure in the xylem. If the cells are turgorless and the plant is completely wilted, the value of the negative pressure equals the osmotic pressure of the parenchyma cells' (translated from Strugger, 1943, p. 184). As a consequence of their considerations Renner and Strugger have described this relationship between the xylem pressure and the 'water potential' ( $P_c - \pi_c$ ) of the cells by an equation which is identical to Eq. 5. Application of this equation to the xylem/tissue cell two-compartment system requires that the osmotic pressure in the xylem liquid,  $\pi_x$ , is taken into account (Balling & Zimmermann, 1990):

$$P_x - \pi_x = P_c - \pi_c \quad (8)$$

It is important to point out again that  $P_c$  in Eq. 8 is the absolute hydrostatic pressure within the cells and not the turgor pressure. It is equally crucial to mention that Eq. 8 assumes that the xylem and the adjacent tissue cells are always at thermodynamic equilibrium. Water exchange between the xylem and the adjacent cells usually occurs in a few seconds (Zimmermann, 1989; Steudle, 1989, 1992; Moore & Cosgrove, 1991; Malone, 1993), presumably due to the presence of aquaporins (Chrispeels & Maurel, 1994; Kaldenhoff *et al.*, 1998; Kaldenhoff & Eckert, 1999; Johansson *et al.*, 2000). Therefore, local thermodynamic equilibria between the two compartments at any height of the plant can be assumed, even though the entire xylem or tissue compartments may not necessarily be at thermodynamic equilibrium.

According to Eq. 8 the xylem pressure in higher plants should be determined by the water relations parameters of the adjacent cells and *vice versa*, but not *a priori* by the transpiration rate. This is supported by the finding that at irradiation rates below *c.*  $50 \mu\text{mol m}^{-2} \text{s}^{-1}$  changes in transpiration are not reflected in changes of xylem pressure (Benkert *et al.*, 1991; Schneider *et al.*, 2004; see also Appendix 1). Recent measurements on hydroculture maize and well-watered soil culture tomato plants also demonstrated that transpirational changes induced by changes in light irradiation from 10 to  $150 \mu\text{mol m}^{-2} \text{s}^{-1}$  did not lead to exactly linearly correlated changes in xylem pressure (Schneider *et al.*, 2004). Furthermore, Schneider *et al.* (2004) measured xylem pressures in small inverted tomato plants and then submerged the entire foliage in water. Although an increase in xylem pressure was registered immediately upon leaf submersion, the xylem pressure remained in the negative range as long as the leaves were kept in the submerged state. Upon removal of the water, the xylem pressure decreased very rapidly back to the original value.



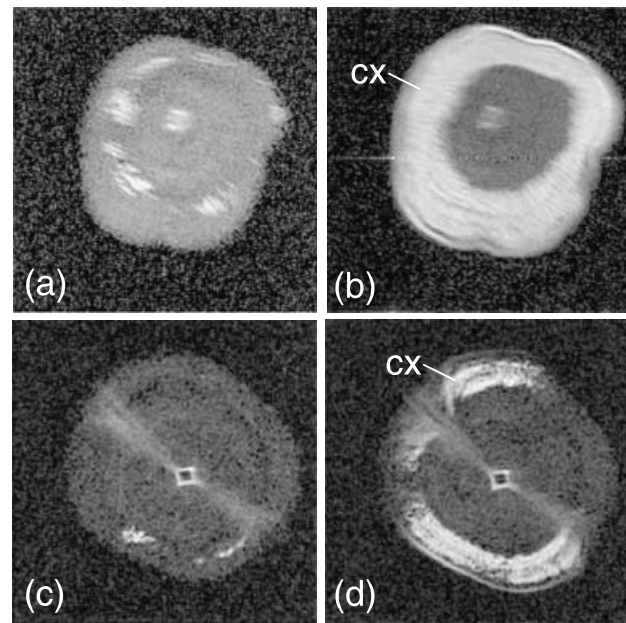
**Fig. 14** Oscillations in the transpiration rates of *Triticum aestivum* hydroculture plants (Tr; dashed trace in a) occurring at a light irradiation  $> 300 \mu\text{mol m}^{-2} \text{s}^{-1}$  resulted in corresponding, somewhat delayed oscillations of root xylem pressure ( $P_x$ ; solid traces in a and c), of the turgor pressure of root cortical cells ( $P_c^*$ ; b) and of the trans-root potential (TRP; dash-dotted trace in c). Measurements were performed by using a conventional steady-state porometer, the xylem pressure probe, cell turgor pressure probe and the xylem pressure-potential probe, respectively. For more details, see text and Schneider *et al.* (1997b), Wegner & Zimmermann (1998), Zimmermann *et al.* (2002a) and Zimmermann (2003). Taken from Zimmermann *et al.* (2002a), with kind permission of Kluwer Academic Publishers, Dordrecht, The Netherlands.

Measurements on laboratory-sized herbaceous plants and lianas have given further evidence that xylem pressure and turgor pressure are linked with each other. When water uptake through the roots becomes rate-limiting in response to a significant increase in transpiration, a parallel drop in both xylem and turgor pressure is observed resulting in the establishment of a new water equilibrium between the xylem and the surrounding cells. For instance, measurements in roots of hydroculture wheat plants at a light irradiation of about  $300 \mu\text{mol m}^{-2} \text{s}^{-1}$  (Zimmermann *et al.*, 2002a) showed that oscillatory changes in transpiration and xylem pressure (Fig. 14a) were reflected

in analogous changes of turgor pressure of the cortical cells (Fig. 14b). The ratio of xylem to turgor pressure changes was, however, only about 1 : 0.3, i.e. less than expected from Eq. 8. Similar results were found for light-induced pressure changes in maize roots (Zimmermann *et al.*, 2002b). The most likely reasons for these low ratios of xylem to turgor pressure changes are hydraulic resistances in the endodermis, large unstirred layer effects (Schneider *et al.*, 1997a,b) and/or dramatic changes of the radial, flow-dependent turgor pressure and osmotic pressure gradients within the root cortex that may be accompanied by cell shrinkage (Zimmermann *et al.*, 1992; Rygol *et al.*, 1993). Consistently, higher ratios of xylem to turgor pressure changes were found for the shoots of well-hydrated potato plants (1 : 0.5; Schneider *et al.*, 2004). Corresponding measurements of the shoot xylem and leaf turgor pressure of the re-hydrated resurrection plant *Myrothamnus flabellifolia* yielded even higher values (1 : 0.7; Schneider *et al.*, 1999). A nearly 1 : 1 relationship between changes in shoot xylem pressure and leaf turgor pressure existed in the case of the lianas *Epipremnum aureum* and *Tetrastigma voinierianum* (Fig. 12d, inset).

More insight into the hydraulic coupling between xylem pressure and turgor pressure was obtained by measurements on *E. aureum* lianas under different plant orientations (Wistuba *et al.*, 2000). In upright, about 6-m-tall plants no longitudinal turgor pressure gradients could be measured between ground level and 5 m height, neither during the day nor during the night. In horizontally placed plants, by contrast, a base-to-apex-directed turgor pressure gradient of 18 kPa m<sup>-1</sup> developed during the night, with maximum turgor pressure values at the apex of 0.33 MPa. For apex-down plants, an even higher turgor pressure value towards the apex was measured (Fig. 13d). Under these gravity-supported conditions a value of up to about 0.5 MPa was reached during the night resulting in a large longitudinal cellular turgor pressure gradient of 22 kPa m<sup>-1</sup> between the base and the apex. This finding can **only** be explained by the assumptions that (i) the osmotic pressure of the apical tissue cells is higher than that of the basal cells and that (ii) under upright (against gravity) and less under horizontal conditions (no gravity) water uptake through the roots is not sufficient for complete compensation of the water loss of the apical cells before day-break. This has the important consequence that the longitudinal cellular osmotic pressure gradient from the roots to the apex represents an additional tension component for anti-gravitational water lifting **through the vessels**. This force is obviously responsible for the establishment of xylem pressure gradients that were lower than predicted for a standing water column.

Unbalanced radial cell osmotic pressure gradients may also contribute to xylem water lifting in lianas because it is very likely that radial turgor (and osmotic) pressure gradients exist along the shoot of these lianas as found in willow and beech twigs (Zimmermann *et al.*, 1993a). Recent <sup>1</sup>H NMR imaging experiments performed during xylem refilling of *M. flabellifolia*



**Fig. 15** Axial and radial refilling patterns of cut air-dry branches of the resurrection plant *Myrothamnus flabellifolia* upon exposure of the basal cut end to tap water (a,b) and to 50 mM raffinose solution (c,d). The T<sub>1</sub>-weighted <sup>1</sup>H NMR images measured 6 cm away from the cut end were taken 30 min (a), 70 min (b), 63 min (c) and 130 min (d) after exposure to the refilling medium. Note that axial water rise occurred initially only in a few clusters of xylem elements and that raffinose delayed considerably radial water spreading within the conducting xylem area ('cx'). Images were reproduced from Wagner *et al.* (2000), with kind permission of Blackwell Science, Oxford, UK.

also strongly supported the view that radial osmotic forces are very important in water lifting (Figs 15a,b; Wagner *et al.*, 2000). In the light of a mass of evidence Hinckley *et al.* (1978, p. 37) came to the same conclusion that 'water flow in tree stems occurs radially as well as vertically'. The radial flow of water between living tissues within and near the cambium and the sapwood has been assessed based (i) on changes in tissue water status (literature quoted in Hinckley *et al.*, 1978), (ii) on the accumulation of xylem-added radioisotopes in the phloem (e.g. Fraser, 1958; Owston *et al.*, 1970), (iii) on fluctuations in tree stem diameters (e.g. Kramer, 1983; Zweifel *et al.*, 2001; Sevanto *et al.*, 2003), and (iv) on sap flow measurements (Goldstein *et al.*, 1998; Meinzer *et al.*, 1999). Hinckley *et al.* (1978) estimated that internally stored water in tall trees can supply as much as 15% of the total amount of water transpired during the summer (see also Pfeffer, 1881, p. 119; Ewart, 1908; Jarvis, 1975). Loss of tissue water is manifested in a decrease in trunk diameter during the day. The proponents of the Cohesion Theory assume that this is caused by the collective shrinking of many vessels under high tension. However, this is very unlikely because of the extremely high value of the volumetric elastic modulus of the xylem walls. <sup>1</sup>H NMR imaging studies on non-irrigated tobacco plants

have given direct evidence (Fig. 3c) that a significant amount of water stemmed from shoot cells.

The tight coupling between xylem pressure and cell turgor pressure explains straightforwardly why changes in xylem and turgor pressure (and, in turn, flow) in the lower parts of lianas and trees lag behind those in the upper parts – a conclusion already drawn in the 1970s by many authors (e.g. Morikawa, 1974; Hinckley *et al.*, 1978). It also explains why the xylem pressure at 1 m and 5 m height of the liana *T. voiniarianum* very often assumed nearly identical values: Fig. 12d shows that the turgor pressure values measured at these two measuring points were very similar.

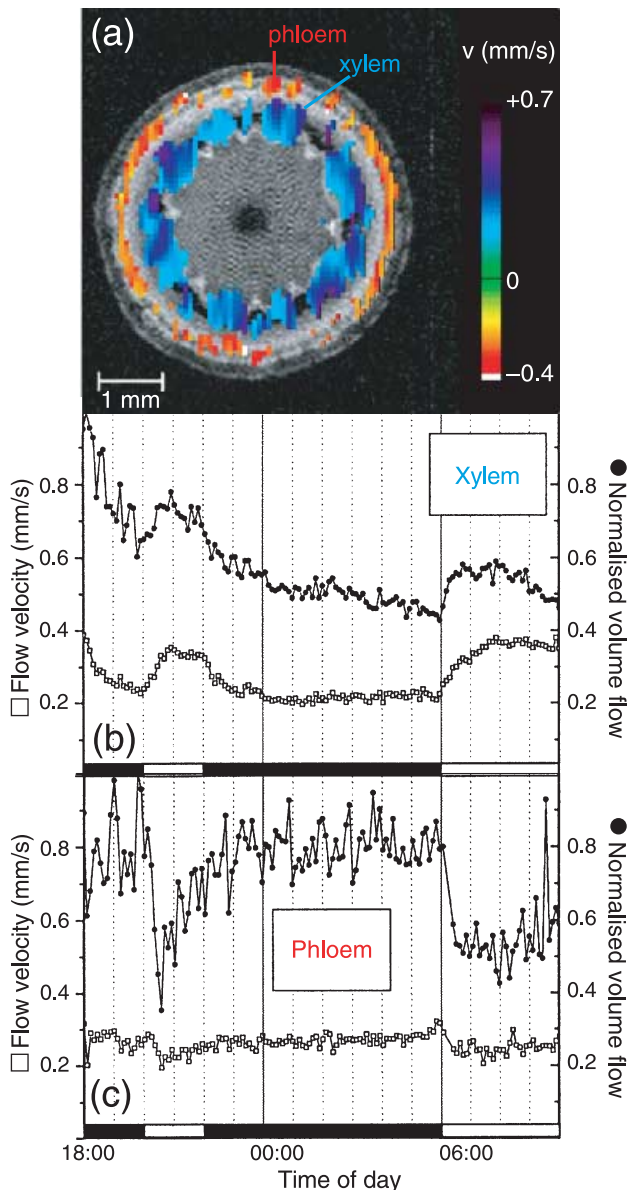
If the osmotic pressure of the xylem and of the cells remains unaltered during transpiration, then the lowest **stable** negative xylem pressure which can theoretically exist in a vessel is reached when the turgor pressure becomes zero, i.e.  $P_c = 0.1$  MPa (Balling & Zimmermann, 1990; Schneider *et al.*, 1999; Wistuba *et al.*, 2000). Consistent with this, the minimum stable xylem pressure measured in *T. voiniarianum* was around  $-0.4$  MPa when turgor pressure had dropped from about 0.45 MPa before day-break to about 0.05 MPa (see Figs 12c,d). The turgorless state is obviously the ‘point of no return’ for **stable** negative xylem pressures. When transpiration is going on, but water supply through the roots is limited, tension within the xylem will increase extremely rapidly until cavitation occurs. This is exactly what we found by vessel probings of various plants upon water shortage (section III.1). Turgor pressure values higher than about 1 MPa are not reported in the literature. The turgor pressures of *Anacardium excelsum* leaves at 30 m height measured by the cell turgor pressure probe were between 0.4 and 1.0 MPa. For *Rhizophora mangle* rooting in sea water the stem and leaf turgor pressure was between 0.24 and 0.49 MPa. Equally, it is very unlikely that the osmotic pressure of turgorless cells can balance extremely negative xylem pressure values (e.g. Steudle, 2003) because this implies that the cellular osmotic pressure must be of the order of the osmotic pressure of sea water (!). Dramatic increase of cellular osmotic pressure by plasmolysis is conceivable, but has never been observed and would also represent an unfavourable survival strategy of a (tall) higher plant. Thus, consistent with our conclusions in section II it is very unlikely that **stable** xylem pressures being significantly below about  $-1$  MPa can exist even if osmoregulation (i.e. *de novo* synthesis of osmolytes by the cells; Steudle, 2003) or an increase in the intracellular osmotic pressure due to plasmolysis are taken into account.

Wei *et al.* (1999b, pp. 1203–1204) have recently seen this substantial argument against the Cohesion Theory. They claimed that in woody living cells, the turgor pressure ‘could swing to negative values as water loss progresses past the turgor loss point, because the lignified wall prevents cell collapse. Even if these rigid living cells cavitate, equilibrium will continue to exist between the cavitated living cells and the xylem vessels’. This stands in stark contrast to Tyree’s statement in 1976 saying that reports on negative turgor pressure are fallacious.

We agree with this early statement because structure and composition of the cell interior provides innumerable nucleation sites that prevent any generation of a pressure below vacuum.

#### 4. Xylem-phloem re-circulation

The xylem is in intimate contact not only with the adjacent parenchyma cells, but also with the phloem *via* the rays. Thus, some authors have considered the hydraulic interconnection between xylem and phloem as being very important in radial cell osmotic water flow and, in turn, also in diurnal and seasonal fluctuations in stem size (Zimmermann & Brown, 1980; Hinckley *et al.*, 1978). Evidence for an internal solute/solvent recycling system between xylem and phloem operating like ‘cable cars’ was first postulated by Haberlandt (1892) in the light of transpiration measurements on tropical plants. Muenschler (1922) and Münch (1930, p. 74) as well as Tanner & Beevers (1990) have added evidence to this hypothesis. Hydroculture maize and barley plants grown at both 50–60% and >95% relative humidity exhibited similar growth rates, even though water loss by transpiration (and guttation) was reduced by a factor of 2–4 at elevated relative humidity. Similar results were recently obtained for sunflowers (Tanner & Beevers, 2001) indicating that transpiration is not absolutely crucial for antigravitational water transport (see also Lampinen & Nojonen, 2003). Direct proof for water lifting by xylem-phloem re-circulation was obtained by flow-weighted  $^1\text{H}$  NMR imaging. First attempts to measure phloem flow by NMR imaging were made by Köckenberger *et al.* (1997). However, a single flow data set required 4.5 h and the measurements could only be performed on non-transpiring seedlings of *Ricinus communis*. Technical improvements by Rokitta *et al.* (1999a,b; Peuke *et al.*, 2001) reduced the acquisition time to 3.5–7 min. Furthermore, the use of larger magnet bores allowed simultaneous measurement of the volume flows in the xylem and phloem of shoots of well-watered leafy plants of *R. communis* (Figs 16a–c). As expected in the light of the pressure probe and flow data measured on well-hydrated *Epipremnum aureum* plants (Fig. 13b), xylem flow velocity and volume flow increased and decreased in parallel during a light-dark regime (Fig. 16b). By contrast, the phloem flow velocity remained unaltered during a light/dark regime while the volume flow increased – in many plants quite dramatically – during the dark period (Fig. 16c). This finding suggests that sieve pores which are plugged by protein threads during the light period become temporarily open during darkness in order to re-circulate xylem volume flow.  $^1\text{H}$  NMR spin echo images acquired for twigs taken from different heights of various tall trees also suggest that the phloem plays a crucial role in uptake and re-circulation of water because this compartment exhibits signal intensities as high as pure water, even under limited water supply (e.g. Fig. 11; Zimmermann *et al.*, 2002c and unpublished data). Furthermore, its location close to the bark suggests that the phloem may also be involved



**Fig. 16** Quantitative determination of volume flow and flow velocity in the xylem and phloem of the shoot of an about 5-wk-old *Ricinus communis* plant by using flow-weighted  $^1\text{H}$  NMR imaging. (a) Typical flow velocity map (blue colour: upward-directed xylem flow, red colour: downward-directed phloem flow) superimposed on a virtual cross-section of the shoot obtained with the FLASH imaging technique (Rokitta *et al.*, 1999b). (b) and (c) Volume flow (●) and flow velocity (□) changes in the xylem and phloem, respectively, upon a light/dark regime. Volume flow data were normalised to the maximum flow value (for experimental procedure and data evaluation, see Rokitta *et al.*, 1999a). Note that xylem volume flow and flow velocity changed in parallel upon changes in illumination, while the flow velocity in the phloem remained constant throughout the experiment even though the volume flow increased dramatically during darkness. Taken from Rokitta *et al.* (1999a), with kind permission of Springer-Verlag, Heidelberg, Germany.

in reverse transpiration, i.e. water uptake from the atmosphere through the bark that is then fed into the re-circulation process (see section V.7). We agree with van Bel (1990) that the re-circulation between xylem and phloem is an undervalued route for water and solute transport.

## 5. Xylem osmotic pressure-bound water flow

Inspection of Eq. 7 indicates that the gravitational potential term of the water columns in the xylem conduit can also be compensated by an appropriate decrease of the chemical activity of water with height without development of a tension gradient by transpiration. Water activity decreases with increasing concentration of osmotically active solutes. Thus, gradients in osmotic pressure within the vessels could be a driving force for water ascent. Xylem osmotic pressure-driven water flow seems to be an all-year-round mechanism of water lifting in tropical trees (Molisch, 1898; Faber, 1915; Braun, 1983, 1984; Barker & Becker, 1995 and references cited therein). The xylem saps of many trees of moderate climate also contain carbohydrates, amino acids and other osmotically active solutes. These are secreted by accessory cells along the vessels into the xylem sap. The osmotic pressure exerted by these solutes (the so-called 'stem pressure') has been considered by many authors as a driving force to explain bleeding of maple, birch, alder and oak in spring (Pfeffer, 1881, p. 157; Lepeschkin, 1927; Löhr, 1953; Sauter *et al.*, 1973; Ferguson *et al.*, 1981; Essiamah & Eschrich, 1985; Ahtonen & Kallio, 1989; Biles & Abeles, 1991; Harms & Sauter, 1992).

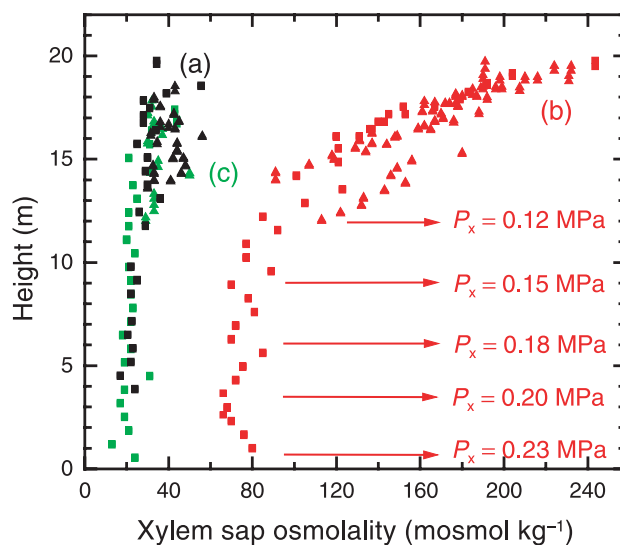
The idea of xylem osmotic pressure-driven water ascent in tall trees is obviously not new, but it has not been recognised by many plant physiologists that the solutes in the xylem sap can only develop an osmotic pressure if the xylem is segmented in axial compartments of certain length bounded by radial and axial solute-reflecting barriers (i.e. the so-called reflection coefficients,  $\sigma$ , of these barriers or boundaries must be  $0 < \sigma \leq 1$ ; Zimmermann *et al.*, 1993a,b, 1994a; Canny, 1995a). Segmentation into osmotically active compartments also appears to be a prerequisite for the establishment of relatively large longitudinal osmotic pressure (and, in turn, of hydrostatic pressure) gradients in the xylem conduit (Plumb & Bridgman, 1972; Andrews, 1972, 1976; Zimmermann *et al.*, 1994a). These would be subject to large fluctuations because of the concentration and dilution induced by the transpirational water loss during the day and the water uptake through the roots at night.

In radial direction, solute reflection is obviously achieved by the hydrophobicity of the lignified xylem walls and reinforced by lipid linings in some species (section II.2 and Fig. 1). Radial solute-reflecting sheaths have also been found around the vascular bundles of maize and sugarcane stems (Jacobson *et al.*, 1992; Welbaum *et al.*, 1992; Canny, 1993). Root pressure, i.e. hydrostatic overpressure within the root

xylem, also indicates that radial solute-reflecting barriers must ensheath the xylem. There is no debate among plant physiologists (e.g. Kramer, 1983; Schwenke & Wagner, 1992) that root pressure is important for xylem and tissue refilling as well as for generation of above-atmospheric xylem pressures during the night (e.g. Fig. 12). However, the magnitude of root pressure that can develop in the leaky xylem conduit is too small to explain hydrostatic pressure-driven water lifting to substantial heights. Field measurements on rooted plants of *Myrothamnus flabellifolia* revealed that root pressure drives xylem refilling up to a height of about 1 m suggesting absolute root pressure values of about +0.11 MPa (Schneider *et al.*, 2000b). The maximum root pressure recorded at the base of *Tetragium voimierianum* (see Fig. 12) and sugarcane with the xylem pressure probe was about +0.13 MPa (Benkert *et al.*, 1995). For 35-m-tall trees of *Anacardium excelsum*, no root pressure could even be recorded (Tyree and Meinzer, unpublished data). Higher pressures of up to about +0.4 MPa are reported in the literature for herbaceous plants (White, 1938; Miller, 1987; Steudle, 1993), but these were measured on excised roots sealed to a pressure transducer, i.e. the systems were practically closed in axial direction.

The structural features of axial, solute-reflecting barriers in the xylem of intact higher plants and trees have not yet been identified but they may be associated with blockages of the pits and perforation plates by resins, gels or lipids as well as by tyloses (Sachs, 1887; Morse, 1990; Canny, 1997a), with anatomy-related diffusion peculiarities and with immature vessel segments and/or other living cells. Even an air/water interface (e.g. formed in a cavitated vessel) can serve as an axial solute-reflecting barrier. It is obvious that most of the axial barriers meet the demands required by a watergate-based water lifting mechanism. Thus, much less osmotic force per pass is required than for a one-step water lift from the ground to the foliage. Such occasional transverse, solute-reflecting barriers should not impose large resistances to axial water lifting because, when necessary, these sites can be bypassed by osmotic pressure-driven water flow through the adjacent cells. This has been demonstrated quite nicely by  $^1\text{H}$  NMR imaging studies documenting that radial refilling of dry branches of *Myrothamnus flabellifolia* in the presence of high concentrations of electrolytes and sugars in the xylem liquid was considerably reduced compared to the controls (compare Figs 15c,d with Figs 15a,b).

First evidence for xylem segmentation into osmotically active compartments was very recently obtained by measuring the height dependence of the osmolality profile in up to 24-m-tall birches. For xylem sap collection the very accurate compression/decompression method was used. Typical examples of seasonal height-dependent xylem sap osmolality profiles measured in the trunk and the branches over three years are depicted in Fig. 17 for the time periods of leaf fall and dormancy (a), bud break and leaf emergence (b) and completion of leaf expansion (c). It is evident that during leaf emergence in late March and April the osmolality increased



**Fig. 17** Typical longitudinal xylem sap osmolality profiles of trunks (squares) and branches (triangles) of *Betula pendula* trees during leaf fall and dormancy (November; a), bud break and leaf emergence (early April; b) and completion of leaf expansion (late May; c). Xylem sap was extracted from freshly cut wood samples by using the compression/decompression method (see section IV). Additionally, the above-atmospheric, absolute hydrostatic pressure values,  $P_x$ , measured along the trunk during the period of bud break and leaf emergence with a digital manometer, are given. For further details, see text and Zimmermann (2003).

dramatically over the entire height with the formation of pronounced longitudinal gradients. The osmolality increased almost linearly up to the first branching at 11 m height (slope 1–2 mosmol kg<sup>-1</sup> m<sup>-1</sup>). Above this site, the concentration of glucose and fructose increased dramatically and the gradient became considerably steeper (about 26 mosmol kg<sup>-1</sup> m<sup>-1</sup>). Surprisingly, no differences in the slope values of the osmolality gradients between the branches and the upper part of the trunk could be recorded. Consistently, the xylem sap osmolality at the very top of the trunk and in the uppermost branches was practically identical (about 250 mosmol kg<sup>-1</sup>). The development of large osmolality gradients coincided with the occurrence of bleeding that could be observed up to a height of 21 m. Measurements of the absolute hydrostatic pressure in the xylem by use of tiny manometers revealed values of +0.2 to +0.3 MPa at the trunk base (Fig. 17) which dropped to slightly above-atmospheric pressure values at the uppermost bleeding site.

These findings evidenced that the axial osmolality gradient is transformed into an osmotic pressure gradient and then into an apex-directed hydrostatic pressure gradient. The radial solute-reflecting barriers required for the development of xylem sap osmotic pressures are obviously achieved by the lipid lining of the xylem walls of birches (Fig. 1). An axial, solute-reflecting barrier must be postulated at least at the site

of first branching because only this can straightforwardly explain the dramatic change in the slope of the osmolality gradient. When considering the size and direction of the hydrostatic pressure gradient it is obvious that the size of the gradient is sufficient to balance the gravitational potential term in Eq. 7, but its apex-oriented direction seems to be at variance with the profile of the osmotic pressure. However, due to the hydraulic coupling of the xylem liquid with the adjacent tissue cells water is pulled continuously into the osmotically unbalanced cells of the emerging leaves, thus keeping the hydrostatic pressure in the xylem around atmospheric. The mechanisms underlying the high water input into emerging leaves (the so-called 'growth water') apparently equal those being responsible for refilling of cells in the resurrection plant *Myrothamnus flabellifolia* (Schneider *et al.*, 2000a,b; Wagner *et al.*, 2000).

As indicated in Fig. 17 the osmolality (and hydrostatic pressure) gradients collapsed after leaf expansion in early May. During summer and autumn osmolalities of 10–30 mosmol kg<sup>-1</sup> were measured up to the height of first branching while further up to the apex the osmolality increased to about 60 mosmol kg<sup>-1</sup>. The reflection coefficients of the solutes are unknown, but it is likely that they are smaller than unity. Thus, one might hastily conclude in the light of the osmolality values that osmotic pressure-driven water lifting does not play an important role in leafy birches and, in turn, that transpiration-induced tension is the dominant force. However, upon cavitation at high transpiration rates and ongoing evaporational water loss strong concentrating effects of the solutes must occur. The high osmotic pressure in the remaining tiny liquid volumes together with the unbalanced osmotic pressure of the hydraulically coupled tissue cells could then provide a very efficient local driving force to bypass the cavitating vessels by water flow through the adjacent tissue. Unfortunately, the liquid in cavitating vessels is not accessible to the compression/decompression method (see section IV and Fig. 11c). However, strong support for the above view arrived from the finding on *Tetrastigma voinerianum*, sugarcane and other plants that the re-occurrence of negative pressures in previously cavitating vessels (that are under positive, sub-atmospheric pressure) in the afternoon always coincided with turgor pressure build-up (Zimmermann *et al.*, 1995b; Thürmer *et al.*, 1999; Wistuba *et al.*, 2000).

Thus, it seems very likely that osmotic forces within the xylem of birches still play an essential role even if the osmolality of the bulk xylem sap is considerably less than during leaf emergence. Since there is an increasing evidence that the xylem sap of other higher plants often contains substantial concentrations of osmotically active solutes (Braun, 1984; Smith & Lüttge, 1985; Biles & Abeles, 1991; Canny, 1993) it is quite likely that the solute concentrating effect of cavitation and the associated generation of high osmotic pressures is a fundamental principle for water lifting to the foliage of tall trees, particularly in cases of limited water supply. This

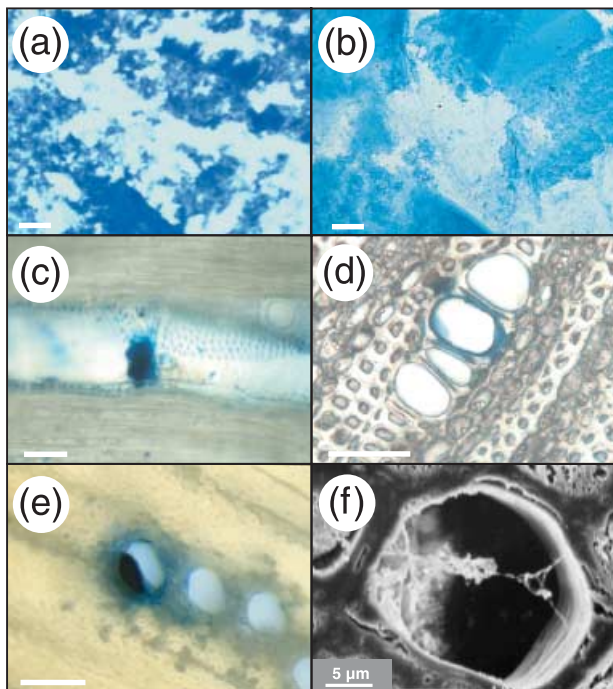
assumption is further supported by the findings discussed in sections V.6 and V.8 which also demonstrate that cavitation does not necessarily lead to a dysfunction of the respective vessels as believed by Tyree & Sperry (1989).

## 6. Mucilage-bound water ascent

Polymeric substances like proteins and mucilage can also reduce the activity of water. Therefore, transpiration-formed gradients of these substances can also cancel the gravitational potential term in Eqs 6 and 7 at constant hydrostatic pressure throughout the xylem conduit. In the presence of bulk mucilage, stabilisation of gradients can be envisaged by xylem segmentation (see above). Attachment of filamentary polymeric chains of mucilage and/or proteins to the xylem walls is another way to create stable gradients. Support of the lifted water volumes by appropriate gel matrix arrangements can further induce a watergate-based mechanism that significantly lowers the force per pass.

About 3 decades ago Plumb & Bridgman (1972) postulated that the xylem conduit of mangroves contains gel-like structures having a gradient in water activity to support the water column without pressure gradients. They hypothesised that the balancing pressure values of 3 to 6 MPa measured by Scholander *et al.* (1965) reflect the pressure required to squeeze water out of the gel structure of the xylem, but not tension. The hypothesis of Plumb & Bridgman was rejected soon after publication by proponents of the Cohesion Theory (e.g. Hammel, 1973; Richter, 1973; Scholander, 1973; Pickard, 1981), but has been recently revived. Today we know that not only cells of plants subjected to freezing, salt and drought stress (Goldstein *et al.*, 1991; Loik & Nobel, 1991; Watt *et al.*, 1994; McCully, 1999) contain large amounts of mucopolysaccharides, but also the xylem of mangroves and other salt-tolerant tall trees (Zimmermann *et al.*, 1994b, 2002b,c). Xylem mucopolysaccharides (which have a high binding capacity to electrolytes) were identified by staining with alcian blue in intact plants by using the xylem pressure probe (Fig. 5b) and/or in transverse and longitudinal microscopic sections (Fig. 18). Cryo-scanning electron microscopy of stem cross-sections of *R. mangle* revealed filamentary networks of mucopolysaccharides attached to the xylem wall as postulated by Plumb & Bridgman (Fig. 18f). Consistent with theory, the tensions in mangroves were only in the order of 0.1 MPa as revealed by the leafy twig/vacuum line technique (Scholander *et al.*, 1962) and the xylem pressure probe (Zimmermann *et al.*, 1994b).

The argument of Milburn (1996) that xylem mucilage is an artefact arising from wounding of immature xylem elements and cells can be rejected because xylem sap extracted by the compression/decompression method and by centrifugation contained (apart from proteins) large amounts of alcian blue-mucilage precipitates (Figs 18a,b). Furthermore, refilling of emptied vessels of pressurised branch or twig pieces with alcian



**Fig. 18** Presence of mucilaginous substances in fully functioning xylem vessels of salt-tolerant trees as evidenced by alcian blue staining (a–e) and cryo-scanning electron microscopy (f). Qualitatively similar mucilage-alcian blue precipitates are found in xylem sap extracted by compression/decompression of branch pieces of *Astronium fraxinifolium* (a) and by centrifugation of twigs of *Rhizophora mangle* (b). (c–e) Mucilage-alcian blue precipitates in the xylem of *A. fraxinifolium* (longitudinal section), *R. mangle* and *Bulnesia sarmientoi* (cross-sections), respectively, after subjecting the branch pieces or twigs to a compression/decompression cycle and subsequent refilling of the emptied vessels with 0.5% alcian blue solutions. (f) Filamentous mucilage network in the lumen of a xylem vessel of *R. mangle* preserved by cryo-scanning electron microscopy. Bars in (a–e) = 50  $\mu\text{m}$ . For further experimental details, see Zimmermann *et al.* (2002c). (c,e,f) reproduced from Zimmermann *et al.* (2002c), with kind permission of Springer-Verlag, Heidelberg, Germany.

blue solutions gave clear-cut evidence that the mucopolysaccharides in the extract stemmed from the vessels: Dye-mucilage precipitates were found attached to the inner xylem walls, but adjacent cells exhibited no staining (Figs 18c–e).

How is sap flow rate affected by mucilage, and are gel-based gradients in the water activity and small tension gradients sufficient to extract water from saline solutions? Model experiments with upright glass tubes filled with bulk pectin solutions have shown (Thürmer *et al.*, 1999) that relevant concentrations as required for gel-based water ascent (Plumb & Bridgman, 1972) do not significantly affect the hydraulic resistance of convective flow. Only at pectin concentrations >0.6% was a reduction in water flow observed. Furthermore, the effect of saline solutions on xylem pressure of transpiring plants is much less than generally believed. Xylem pressure in mangrove seedlings did not change significantly when the

roots were transferred from tap water to saline solutions with osmolalities of up to 1700 mosmol  $\text{kg}^{-1}$  (Zimmermann *et al.*, 1994b). This finding can be explained in two different ways, either that the activity of water in the xylem is changed in response to the external osmotic pressure changes or that the radial tissue reflection coefficients of NaCl,  $\sigma_{\text{NaCl}}$ , for roots of mangroves are close to zero. The latter assumption would mean that the effective osmotic pressure,  $\sigma\pi$ , against which water has to be extracted by the plant is much smaller than expected from the concentration of the osmolytes. Low radial root  $\sigma_{\text{NaCl}}$ -values can originate from large unstirred layers caused by structural characteristics such as membrane folding (Heidecker *et al.*, 2003a) or by abundant mucilage in the apoplast and symplast as is the case in salt-tolerant trees. Extremely low sap flow rates as measured e.g. in *R. mangle* (Zimmermann *et al.*, 1994b) may also contribute to the development of large unstirred layers as shown by xylem pressure probe measurements on roots of glycophytes subjected to saline challenges (Schneider *et al.*, 1997a,b). For the roots of non-transpiring or weakly transpiring maize plants,  $\sigma_{\text{NaCl}}$  was close to zero, whereas  $\sigma_{\text{NaCl}}$  increased up to unity with increasing transpiration and sap flow. Wheat and barley roots exhibited similar effects when subjected to osmotic challenges, except that the full external osmotic pressure ( $\sigma_{\text{NaCl}} = 1$ ) was seen at much lower light irradiation than in the case of maize. The reason for this is presumably that the root cortex of these glycophytes is significantly thinner than that of maize, thus concentration polarisation effects are reduced. The problem of water supply to salt-tolerant trees may be even less severe if we take into account the other forces discussed above and further below. Moreover, these trees have been quite ‘creative’ in developing mucilage-based mechanisms to save water on its way to the uppermost foliage and to reduce simultaneously evaporational water loss. The current data base is rather limited, but two mechanisms have been identified (section IV).  $^1\text{H}$  NMR imaging, in combination with the compression/decompression technique, has clearly shown (see Fig. 11) that the Chaco tree *Astronium fraxinifolium* reduces the water supply to branches and twigs at intermediate heights by interruption of the continuous water columns by gas-filled spaces. Filamentary gel networks and slime-like films on the xylem walls keep a mobile water continuum (postulated by Quincke more than a century ago; quoted in Sachs, 1887, p. 293).

The extremely salt-tolerant Chaco tree *Bulnesia sarmientoi* apparently uses another water saving strategy. Water supply to twigs at intermediate heights and transpirational water loss are minimised by clogging of a large part of the vessels by mucilage (and proteins) as shown by  $^1\text{H}$  NMR imaging and staining (Fig. 11b). This strategy is also used by the resurrection plant *Myrothamnus flabellifolia* even though this plant uses lipids for clogging of the water-conducting area in the leaf traces (Schneider *et al.*, 2003).

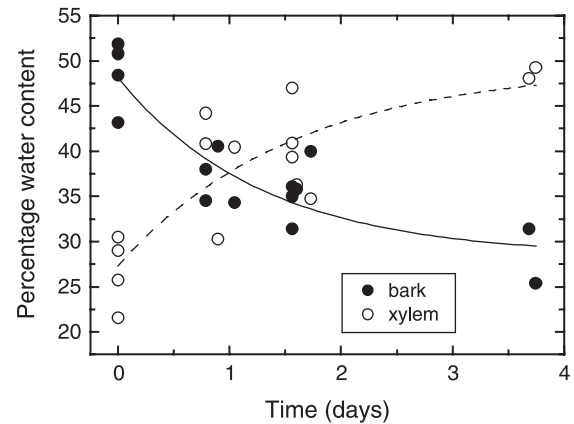
Mucilage, lipids and maybe also proteins in the xylem sap are obviously important for ‘xylem conditioning’. Their

functional potential seems to be multi-faceted, particularly that of mucilage, as we will see in the following section.

## 7. Reverse transpiration

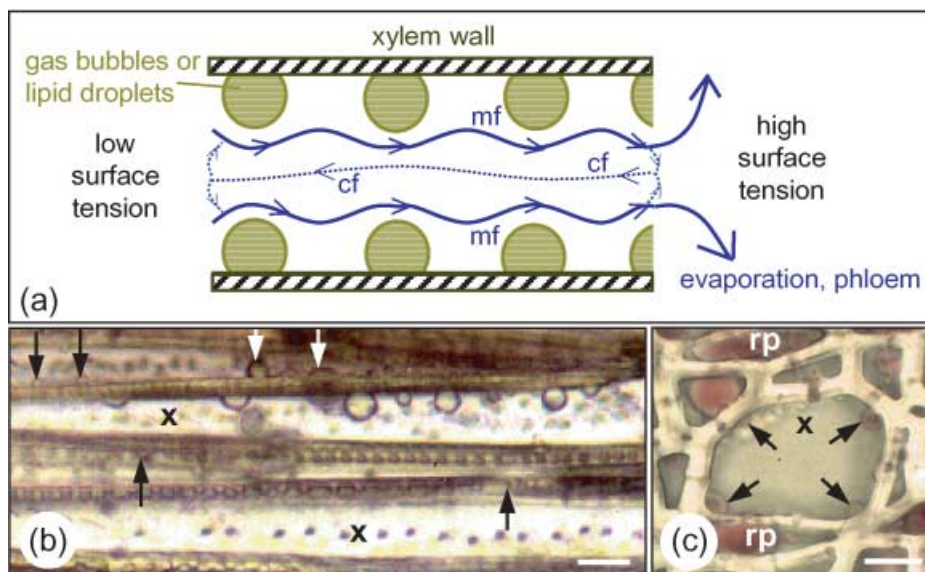
Extraction of xylem sap by the compression/decompression method and/or centrifugation has recently shown that not only salt-tolerant trees have mucopolysaccharides in their xylem. Even though the average concentration is significantly smaller, mucopolysaccharides have also been found in the xylem of willow and poplar (Zimmermann *et al.*, 2002b,c; unpublished data), of lianas (Strasburger, 1891, p. 688; Benkert *et al.*, 1995; Thürmer *et al.*, 1999) and of *Metasequoia glyptostroboides*, *Sequoia sempervirens* and *Sequoiadendron giganteum* (with the latter growing on fog-laden coasts). The mammoth trees additionally contained extremely high concentrations of mucilage in the bark and needles. Taking into account the large amount of dew in the canopies of tall trees at day-break (even in dry regions), it is not understandable from an economic and energetic standpoint why water should be lifted over large distances from the root when it is already available at the apex. Dawson (1998) believes that 30–40% of a redwood's water supply originates from fog. There are many reports in the literature indicating that moisture and rain can be taken up by the aerial parts of higher plants by a process termed reverse transpiration. For instance, Yates & Hutley (1995) exposed cut leafy twigs of an Australian subtropical rainforest tree to humidified air on one side only, but measured an increase in the 'water potential' of leaves on the other, dry side. This shows that water had been taken up by the leaves from the atmosphere and was redistributed within the twig. Possible moisture uptake sites at the leaves are considered to be guard cells, specialised epidermal cells and imperfections of the cuticle like cracks or thinnings (Meidner, 1954; Yates & Hutley, 1995; see also Boucher *et al.*, 1995 and references cited therein). Some authors have reported water uptake *via* the bark (e.g. Katz *et al.*, 1989). Lenticels (Esau, 1977), usually regarded as pathways for O<sub>2</sub> uptake by tree trunks, may also mediate water uptake.

We hypothesise that intracellular and xylem mucilages as well as mucilage-containing bark-adhering lichens and/or mosses, when present, may be involved in water uptake from the atmosphere by reverse transpiration. As outlined in section IV, the pressure bomb can be used for a qualitative estimation of the 'water availability state' of a leaf. Experiments with leaves of mangroves have shown (Scholander *et al.*, 1965; Zimmermann *et al.*, 1994b) that on cloudy days with intermittent rain the balancing overpressure,  $P_b$ , assumed extremely low values (around 0.1 MPa). Equally, upon onset of rain a significant drop in the  $P_b$ -values of peripheral leaves of the salt-tolerant tree *Astronium fraxinifolium* could be recorded within a few minutes whereas the  $P_b$ -values of the inner, non-exposed leaves of the tree remained unaltered for more than 1 hour. Additionally, when excised, weakly hydrated leaves of the salt-tolerant Chaco tree *Ruprechtia triflora* were exposed to a



**Fig. 19** Changes in the percentage water content of the bark and the xylem of twigs of *Bulnesia sarmientoi* after a rainy day ( $t = 0$ ) calculated from spin-density-weighted <sup>1</sup>H NMR images. During the following four sunny days, twigs were freshly taken from sites being in the shadow most of the time. The exponential fits show that the reduction of water content in the bark (●;  $R^2$  of the fit = 0.80) coincided quite well with the corresponding increase of water content in the xylem (○;  $R^2 = 0.69$ ). The percentage water content of the pith and the phloem as well as the total water content of the twig remained nearly constant (data not shown) suggesting that the increase in xylem water stemmed from the bark (Westhoff *et al.* unpublished).

humid atmosphere, water uptake was observed (Zimmermann *et al.*, 2002c). In *Bulnesia sarmientoi*, the morphology of the innumerable, relatively small leaves is ideal for water collection. Upon rainfall, the divided leaves cave in and assume a funnel-like configuration. Single leaf funnels of *B. sarmientoi* can trap several microlitres of water by capillary forces. The functional similarities between the leaves of *B. sarmientoi* and the water-impounding foliage of many arboreal bromeliads (termed 'tank'; Zotz & Thomas, 1999; Zotz & Hietz, 2001) is obvious. Furthermore, spin density-weighted <sup>1</sup>H NMR imaging on twigs of *B. sarmientoi* yielded direct evidence for the involvement of the bark in reverse transpiration and for internal redistribution of water (Fig. 19). After a rainy day about 50% of the total twig water was located in the bark. During the following four sunny days water was obviously shifted from the bark into the xylem, whereas the percentage of water in the pith and the phloem remained nearly constant (data not shown). The reduction in the water content of the bark with time coincided quite well with the corresponding increase in the water content of the xylem, particularly for twigs in shadow for most of the day (Fig. 19). The amount of water shifted from the bark to the xylem in sun-exposed twigs (about 10%) was less than in non-exposed twigs (about 30%; Fig. 19). This may be related to the higher total mucopolysaccharide and, in turn, water content of sun-exposed twigs in comparison to that of non-exposed twigs. Throughout the four sunny days, the total signal intensity per mm<sup>3</sup> tissue (in relation to pure water) was  $21 \pm 3\%$  in sun-exposed twigs and significantly higher than  $17 \pm 3\%$  in non-exposed twigs (t-test: confidence > 99.9%).



**Fig. 20** Interfacial streaming (Marangoni flow) along a liquid/gas or liquid/liquid interface induced by a gradient in surface tension. (a) Schematic diagram of Marangoni flow (mf) along a necklace of gas bubbles or lipid bodies adhering to the inner wall of the xylem. The interfacial flow is directed to the side of high surface tension (e.g. to the electrolyte-rich side or to the region of low temperature). Consequently, it causes flows to the transpiration sites and the phloem which may be partly reduced by a counterflow (cf) of water in the bulk. (b) Necklace arrangements of lipid bodies at the inner walls of the xylem (x) were found in longitudinal sections of rehydrated branches of *Myrothamnus flabellifolia* (arrows mark bodies being slightly out of focus; bar = 10  $\mu\text{m}$ ). (c) The corresponding cross-section (stained with Sudan III, rp = ray parenchyma cell) shows that the lipid bodies were flattened (arrows) as expected for hydrophobic surfaces (section II.2). For further explanations, see text and Zimmermann (2003). (b) and (c) reproduced from Zimmermann (2003) and Schneider *et al.* (1999), with kind permission of Physiko-Medica, Würzburg, Germany and Blackwell Science, Oxford, UK.

In the light of these data and the apparently ubiquitous occurrence of mucopolysaccharides in tall trees we think that reverse transpiration may represent an important strategy in water supply to the foliage of tall trees. Further studies in this direction will certainly be facilitated by the recent advent of high-resolution Computed Tomography (Fromm *et al.*, 2001) and of a portable NMR imager for field studies (Rokitta *et al.*, 2000).

## 8. Interfacial force-bound water ascent

Mucosubstances, but also other surface active compounds (e.g. phospholipids) stabilise gas bubbles, even under slightly negative pressures (Yount *et al.*, 1984). More than a century ago botanists realised that gas bubbles can induce water flow (see e.g. Sachs, 1887; Copeland, 1902; Haberlandt, 1909). Numerous experiments led Copeland (1902, p. 274) to the conclusion that the water ascent in trees will be understood only if there is a physical explanation of the repeated botanical observation of the passage of water between gas bubble and wall. The driving force for this water movement remained unknown at that time, but it is clear that Copeland and his colleagues had obviously discovered a very important flow mechanism that may operate permanently in salt-tolerant trees, but also temporarily in higher plants when cavitation has occurred. Thus, flow induced by non-stabilised gas bubbles

may be as important as local osmotic forces to meet the instantaneous demand for replacement of transpirational water loss during noon (when cavitation is frequent) as well as for subsequent refilling of cavitated vessels and adjacent cells during the afternoon (Canny, 1997b, 2001).

The discovery of Copeland and his colleagues shows that Eq. 7 is incomplete. Additional forces that arise at phase boundaries must be taken into account when the equilibrium is disturbed. Today it is known that at liquid/air or liquid/liquid boundaries interfacial flows are created due to surface tension gradients. Flow occurs from a region of low surface tension (e.g. low concentration of electrolytes, mucosubstances and proteins or high temperature) to a region of high surface tension (e.g. high solute concentration or low temperature). This so-called Marangoni streaming (Marangoni, 1871; Young *et al.*, 1959; Langbein, 1986; Langbein & Heide, 1986; Zimmermann *et al.*, 1993b; Adamson & Gast, 1997) causes a hydrodynamic pressure gradient and, in turn, a flow in the bulk solution. In Fig. 20a the corresponding flow profile is schematically shown for a necklace of gas bubbles adhering to the inner wall of a xylem vessel. If the capillary is not closed, i.e. if water is withdrawn at the site of high surface tension by evaporation and/or by the phloem, a net mass flow is created against gravity through the xylem vessel up to the solute-rich sites at the apex. The flow velocity of Marangoni streaming increases with increasing capillary diameter.

Calculations have shown (Zimmermann *et al.*, 1993b) that the velocities are in the range measured by the heat pulse and flow-weighted NMR imaging techniques (around  $0.2 \text{ mm s}^{-1}$  for 20- $\mu\text{m}$ -diameter vessels (Fig. 16b).

Marangoni streaming induced by cavitation-born or stabilised gas bubbles has not been demonstrated *in planta* yet. However, it apparently plays a crucial role in water lifting in the resurrection plant *Myrothamnus flabellifolia*. Upon wetting the lipid lining of the inner walls (Fig. 1a) and the lipids filling partly or completely the inter-vessel and parenchymal pits disintegrate to a large extent under formation of lipid bodies. Temperature-driven Marangoni streaming at the water/lipid interface seems to be involved in this process (for details, see Schneider *et al.*, 2000b; Wagner *et al.*, 2000). Interestingly, in microscopic branch sections of re-hydrated plants, necklaces of lipid bodies adhering to the xylem walls are seen (Figs 20b,c), thus suggesting that interfacial streaming also plays an important role in water lifting in the transpiring plant.

Taking the information given in this section and in section V.5 together it should be clear that the gaseous phase is likely to be very important in water ascent and is not merely a near-empty irrelevant space.

## 9. Electrical force-coupled water ascent

Xylem walls generally have a net negative bound surface charge neutralised by an excess of mobile cations in the sap close to the charged surface. The presence of negatively charged, gel-like compounds attached to the wall will also enforce the formation of an electrical double layer at the inner walls of the conducting xylem elements. Physicists have shown (e.g. Amin, 1982) that the energy stored in an electrical double layer at a charged interface is sufficient to compensate for large gravitational gradients. Furthermore, because there are more mobile cations than anions a positive current will be induced in the presence of a longitudinal electrical gradient that drags water along with it by frictional forces. This phenomenon is called electro-osmosis. *Vice versa*, if a pressure gradient exists, the water flow will result in the formation of a potential difference termed streaming potential (Helmholtz, 1879; Saxèn, 1892; Gouy, 1910; Mazur & Overbeek, 1951; Katchalsky & Curran, 1965). The general belief is that electro-osmosis is always less efficient than water shifting *via* pressure-driven flow (Dainty *et al.*, 1963). However, we agree with Tyree (1980) that this does not mean that electro-kinetic phenomena are not involved in xylem and/or phloem sap flow.

Attempts were made by several authors in the past to demonstrate that electro-osmosis can occur in higher plants or can be generated by the application of external currents or voltages (Fensom, 1962; Fensom *et al.*, 1965; Ginsburg, 1972; Tyree, 1980; De Boer & Prins, 1985). With the advent of the xylem pressure-potential probe it was possible for the first time to verify that in the xylem of root-severed, 25-cm-tall

tobacco plants stable axial potential gradients really exist (Schneider *et al.*, 2000a). Usually, negative potential values (down to  $-30 \text{ mV}$ ) could be recorded between the cut end and the insertion point of the probe (about 10 to 30 cm). An increase in light irradiation evoked a biphasic response that could be separated into a streaming potential and a component that was independent of flow.

At first glance, the electrical potential values measured in the xylem vessels of tobacco shoots in relation to the nutrition medium are too small to be of relevance for water lifting, particularly in taller plants. However, if we assume that the potential difference drops exclusively across the perforation plates and/or bordered pits they would be very important because of the coupling between water and ion transport. Evidence that long-distance water and ion flow may be considerably affected by electro-kinetic phenomena (or surface potential phenomena; Malone & Stanković, 1991) came also from xylem pressure-potential probe measurements in the root xylem of intact maize and wheat plants (Wegner & Zimmermann, 1998; Wegner *et al.*, 1999). As shown in Fig. 14, the oscillations in transpiration, xylem pressure and turgor pressure were accompanied by synchronous, periodic changes in the *trans*-root potential. These findings could reflect a loop system for negative or positive feedback regulation of xylem and turgor pressure after disturbance of the equilibrium state (Eq. 8). Such systems are well-known in algae and higher plants (Cram, 1976; Findlay, 2001; Dietrich *et al.*, 2001; Roelfsema & Hedrich, 2002; Heidecker *et al.*, 2003b).

Research on the coupling between hydraulic, osmotic and electrical forces is still in its infancy, but the few examples given here and in Fig. 6 demonstrate that electrical effects on water lifting cannot be ignored *a priori*, even if they only trigger other processes that ultimately lead, *via* coupling, to bulk water flow. Support for such a scenario was recently obtained by experiments in which different N-forms were fed to the roots of *Phaseolus vulgaris* in a split-root arrangement (Schulze-Till *et al.*, unpublished data). Root water uptake and flow was higher by a factor of up to 5 in the nitrate-fed compartment than in the ammonium-fed compartment as revealed by flow-weighted  $^1\text{H}$  NMR imaging. This was related to an up-regulation of aquaporin expression on the nitrate side and was accompanied by a hyperpolarisation of the *trans*-root potential compared to a depolarisation induced by ammonium (Wegner *et al.*, 1999).

## 10. Mycorrhiza-bound water lifting

Water uptake by plants against the capillary forces in dry soils has also puzzled plant physiologists for a long time and has enforced the belief that high tensions must exist in the xylem. Mucilaginous substances on root surfaces forming soil sheaths (McCully, 1999) could be one way to 'attract' water and ions from the soil, thus delaying dehydration of the roots and the

development of larger xylem tensions. Another and probably more important strategy is the dramatic enlargement of the absorption area. Experiments on *Arabidopsis thaliana* transformed with an anti-sense construct targeted to the plasma-membrane intrinsic aquaporin, PIP1b, revealed (Kaldenhoff *et al.*, 1998) that the decrease in the symplastic hydraulic conductivity of the root per unit area was counteracted by a five-fold increase of the root surface. Xylem tension and, in turn, water and nutrient uptake as well as morphology and development were practically identical in the anti-sense lines and the control plants. Similar results were found by Martre *et al.* (2002), demonstrating that the down-regulation of aquaporins is compensated by an increase in root mass. Nature achieves dramatic enlargement of the absorptive root surface by mycorrhization of the roots, i.e. by symbiosis with mycorrhiza-forming fungi. The surface of the roots of most land plants is mycorrhized (Hampp *et al.*, 1999). Beneficial effects on the host's water relations by mycorrhization are frequently reported (Boyd *et al.*, 1986; Hernández-Sebastià *et al.*, 1999). For example, comparative measurements on mycorrhizal plants with plants being free of fungus have evidenced that mycorrhization considerably facilitates water uptake into the roots, particularly under drought conditions (see e.g. Haselwandter & Bowen, 1996). The mechanism underlying hyphae-mediated water uptake is very poorly understood, but it is likely that it is of osmotic nature consisting of several steps (Augé *et al.*, 1986; Elmeskaoui *et al.*, 1995). Delivery of metabolites from the plant results in the generation of substantial intracellular osmotic pressures of the fungus. Thus, as in a watergate, water is 'lifted' from the surroundings into the cell and the subsequent increase in turgor pressure can then drive water into the neighbouring, osmotically unbalanced root cells.

Like xylem-phloem re-circulation, water uptake mediated by symbiosis with fungi as well as with other microorganisms (e.g. by mucopolysaccharide-producing bacteria; Dong *et al.*, 1997) seems to us to be a completely underestimated mechanism for water uptake by the proponents of the Cohesion Theory (e.g. Steudle & Peterson, 1998; Steudle, 2000). It allows water lifting at moderate transpiration-induced tension, even under conditions of water deficiency.

### 11. Capillarity-bound water ascent

Capillary pressure is often dismissed as being insufficient to account for water lifting in vessels because of the dependence of the volume flow on the fourth power of vessel diameter. However, water entering small vessels by capillary forces can leave predominantly radially (Canny, 1991) because their resistance to axial flow is large and their favourable surface-to-volume ratio leads to a relatively low resistance to radial flow. Radial water release from large vessels is also possible if the resistance to axial flow is increased by lipid linings (Schneider *et al.*, 2000b; Wagner *et al.*, 2000). Capillary

forces are also presumably involved in holding water columns against gravity because the maximum height at which water can be held depends on the diameter of the smallest portion of a capillary system (e.g. by perforation plates, bordered pits, terminal portions of the vascular system in leaf veins). Thus, theoretically a 100-m-tall water column can be maintained, even in large vessels in the absence of a transpirational pull or gradients in water activity. However, water columns held by capillary forces in large and long vessels are most likely unstable. Segmentation of the xylem or interruption of water columns by gas-filled spaces also seems to be a structural prerequisite for capillary effects as in the case of xylem osmotic forces.

## VI. Conclusions

When reading the voluminous old literature, we were amazed at the profound insights of the scientists at that time into the diversity of mechanisms that might be involved in water ascent against gravity. The question is why this and more recent work challenging the view that tension is the only driving force for water lifting was ignored in the last 4 decades by many plant physiologists. The reasons for this are presumably multifaceted. Canny (1993) is certainly right that spreading of the work by the old German scientists in the English-speaking world has been hampered, partly by the interruption of the supply of journals during the war and partly because it is written in German. A further reason is also that literature published in European Journals is often not cited by the scientific community in North-America. The third, and presumably most important reason is that the introduction of the pressure bomb technique has nipped any further controversial finding in the bud. The balancing pressure values on mangroves agreed with the belief at that time that the water uptake of these plants can only occur by ultrafiltration against high osmotic pressures. This was taken as sufficient proof. The extremely low balancing pressure values measured on mangroves on rainy days as well as the low tension values measured by the leafy twig/vacuum line technique were ignored because they did not fit into the traditional view. We are convinced that there is agreement in the scientific community that new methods must be subject to rigorous test procedures including the elucidation of their limits. It is obvious that deviation from this fundamental physical principle in favour of an empirical approach must lead to the introduction of several faulty concepts.

We understand that it is difficult to abandon the hypothesis of xylem sap being moved exclusively by tension and accept the evidence that it may in fact be subject to many forces. However, the pressure probe and NMR imaging data have demonstrated that the various theories developed by the old plant physiologists are not contradictory and may well complement each other in different higher plants and ecophysiological contexts, particularly if the water is not lifted in one

step from the ground to the uppermost foliage, but rather is lifted like ships that can overcome large height differences by serial locks. Occam's principle of parsimony mentioned at the beginning of this review article requires that a theory be pared to the bare essentials necessary to account for the phenomena observed. The problem is that we do not know at present the common biophysical, biochemical and/or structural factor(s) linking the many forces that are obviously involved in water ascent. The way out of this dilemma is to use the term 'Multi-Force' or 'Watergate' Theory as long as we have not achieved a more integrated view about the long-distance transport of water in higher plants. Future work in this field should assimilate Canny's statement (1995a, p. 233): 'Anything that is alive is too complicated to be fully comprehended by the human mind. Even the dead part of a plant contains sufficient complications to exercise all our ingenuity. [ ... ] We should humbly and thoughtfully plan our attempts to hold a dialog with the living plant'. A dialog with the plant can, however, only be held if we do not rely exclusively on massively invasive and, in some cases, 100-year-old techniques. Such techniques destroy the unique 'beauty of the herbal hydraulic machinery'. Pioneering progresses can only be expected from technology that is non- or minimally invasive. Sophisticated technology that meets these demands as e.g. ion-selective xylem pressure probes (Wegner & Zimmermann, 2002; Wegner *et al.*, 2004), advanced NMR imaging including a portable NMR imager for field studies (Rokitta *et al.*, 2000) and high-resolution Computed Tomography (Fromm *et al.*, 2001) is now available and awaits applications. If biophysical, physiological and biochemical parameters are recorded simultaneously and continuously at multiple sites of higher plants under various environmental conditions by using this new technology we feel confident (see also Meinzer *et al.*, 2001) that we will arrive at a deeper insight into the finely tuned concert of forces that supply the foliage of tall trees with water. Thereby, minimally invasive measurements in the xylem of trunks remain the greatest challenge. Knowledge of the forces and flows in this compartment are crucial to unearthing the truth about water lifting because branches, twigs and petioles may be segmented and separated from the main stream in the trunk. However, independent of the outcome of such experiments, in the light of the evidence reviewed here it is obvious that nature has developed a broad spectrum of complementary strategies for water transport against gravity to cope with various water deficiency situations without the necessity of developing incredibly negative pressures.

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## Appendix 1: Animadversion of the xylem pressure probe work

Doubts about the placement of the probe in individual xylem vessels are obviously unfounded. However, the leading proponents of the Cohesion Theory and users of the pressure bomb persistently argue that the probe is not placed in a conducting vessel.

Sperry *et al.* (1996, p. 434) insisted that (i) the pressure probe ‘may often be measuring gas rather than liquid pressure’ and that (ii) ‘pressure pulses resulting from brief injections of water into the punctured vessel [ ... ] would also be found in gas-filled conduits if the probe mouth was obstructed by the vessel wall’. Since the volume dependence of the internal energy is zero (Eq. 1) the gas pressure cannot drop below zero. However, the probe is reading negative pressure values, even in vessels being initially under positive, sub-atmospheric pressure (section III.1), thus indicating a water-filled element. Obstruction of the capillary tip can occur by taking up tissue material during penetration. But tip clogging is easily detectable because of very slow kinetics (minutes to hours) of pressure relaxation upon vessel impalement and upon the injection of a volume (pressure) pulse. Very slow pressure relaxations are also observed when the probe is positioned in partly dehydrated cell walls or other microporous structures within the plant that are drying out. In this case, the probe serves as an ‘external water source’ and water is sucked into the surrounding tissue by capillary forces, resulting in the development of moderate negative pressures (Zimmermann *et al.*, 1993b).

Tomos and Leigh (1999) claimed that, in the absence of transport, a 50 µm-diameter vessel will be filled with dye (fluorescein) solution up to a length of 0.5 m because of the injected volume (about 1 µl; Balling & Zimmermann, 1990). However, the dye solution is **sucked** immediately into the water-filled vessel upon puncture. Even if more dye solution is **injected** after the establishment of a constant pressure level, only the usual transient pressure peak is observed (Benkert *et al.*, 1991) as expected in the light of the incompressibility of water. Tomos and Leigh (1999) falsely assumed that the probed vessel is disconnected from its surrounding. These authors (p. 463) also criticised the double-pressure probe experiments shown in Fig. 4b: ‘it is possible that the major damage is done by first insertion and then both probes are measuring the same artefact’. However, upon disturbance a metastable system will relax very rapidly into a stable state, but not into another metastable state. Changes in the actual xylem tension will only occur when the probe is filled with compressible oil instead of incompressible water (see below).

Sperry *et al.* (1996, p. 434) argue that ‘the uptake of dyes injected into vessels does not prove a probed vessel is water-filled [ ... ] because, even if the injected vessel was embolised, the dye would be wicked through the vessel wall to surrounding functional vessels and be swept up the transpiration stream’. However, if the tip of the microcapillary is exposed to air, capillary forces will prevent withdrawal of the dye solution from the microcapillary and the pressure recorded by the pressure transducer decreases extremely slowly (see Zimmermann *et al.*, 1993b).

Milburn (1996) suggests that the xylem probe is inserted into living, immature vessels. Depending on their state of maturity, pressures should shift from high positive (immature cells undergoing vacuolation) to negative values (at final stages of maturation). However,  $K^+$  and  $H^+$  activities measured by the ion-selective xylem pressure probe (Fig. 6) as well as other evidence (see e.g. Meuser & Frensch, 1998) are not consistent with Milburn's argument.

Steudle and colleagues (e.g. Wei *et al.*, 2001, p. 554) claim that they have brought the direct measurements of negative xylem pressure values to maturity. They used a silicon oil-filled cell turgor pressure probe with water in the tip of the microcapillary. Wei *et al.* (1999b, p. 1201) argued that 'oil-filled probes seem to be superior to water-filled probes, which cavitate at  $-0.6$  MPa *versus*  $-1.3$  MPa'. The authors, however, created this negative pressure value by cooling sealed probes that were exclusively filled with silicon oil, thus eliminating nucleation at the water/oil boundary. Negative pressures of this order of magnitude can also be generated in sealed (water-filled) xylem pressure probes (see section III.1). Despite this, the main disadvantage of oil is its compressibility which is about one magnitude larger than that of water. This poses enormous problems when probing a vessel, as also realised by Wei *et al.* (2001, p. 550): 'a large amount of water (approximately  $4 \mu\text{l}$ ) must be introduced into the capillary tip to ensure that no silicon oil will be sucked into the xylem vessel'. Long-term recordings of negative xylem pressures are, therefore, not possible as evidenced by Wei *et al.* (1999a,b) and Steudle (2001, 2002a,b, 2003). The xylem pressure traces they published for maize plants at slightly negative xylem pressures cover a time scale of seconds to a few minutes only. Long-term recordings were only obtained when the pressure was increased meanwhile by root pressurisation to above-atmospheric, resulting in movement of water back into the probe.

Steudle also questioned very polemically many times the proper function and localisation of the xylem pressure probe, for instance (Steudle, 2001, p. 858): 'the results of Zimmermann and co-workers performed with the xylem pressure probe are most likely based on experimental artefacts that have not been checked for properly; in particular, the authors have not carefully proved the localisation of the probe'. Surprisingly, Steudle and colleagues selected the same criteria for proper probing as we: drop in pressure below atmospheric, response of the pressure to transpiration, root pressurisation and translocation of dye-labelled water from the probe tip into the vessel. Differences between results of Wei *et al.* (1999a,b) and those published previously by us often turn out to be merely a matter of display (e.g. plot of relative xylem pressure values instead of absolute ones, plot of pressure values instead of pressure changes, etc.). The statement of Wei *et al.* (1999b, p. 1199) that 'contrary to other findings (Benkert *et al.*, 1991), xylem pressure responses were substantial even at relatively low light intensities ( $150\text{--}260 \mu\text{mol m}^{-2} \text{s}^{-1}$ )' has also caused

great confusion among scientists. However, in the experiments of Benkert *et al.* (1991) the light irradiation was only changed between *c.* 40 and  $10 \mu\text{mol m}^{-2} \text{s}^{-1}$ , corresponding to very small changes in the transpiration rates of about  $0.38 \text{ ml h}^{-1}$  per plant.<sup>1</sup> At these transpiration rates the xylem pressure is 'buffered' exclusively by the chemical potential of water of the adjacent cells (see section V.3).

## Appendix 2: Xylem vulnerability, imaginary xylem pressures and other hypotheses

The web of inconsistencies surrounding the pressure bomb is also shown in the creative analysis of the so-called 'vulnerability' curves, i.e. the determination of balancing pressures at which the xylem becomes vulnerable to cavitation and embolism. In these experiments, the balancing pressure is measured by the pressure bomb whereas different methods are used for detection of the 'cavitation threshold'. Some authors (e.g. Milburn, 1966; Tyree & Dixon, 1983; Tyree & Sperry, 1989; Kikuta *et al.*, 1997) used acoustic emissions at audio or ultrasonic frequencies as an indicator for cavitation. Even though it is undisputed that cavitation can induce acoustic emissions, there are many possibilities for other origins of the clicks in higher plants, particularly during day-time and water shortage (e.g. aspirating pits, occurrence of flow turbulence, fusion of gas bubbles adhering to the walls, cracks in the cell walls being under strain, drying out of the bark, shrinking of stem diameter, xylem bending etc.). The acoustic signals are so numerous (rates of 10 per second in intact coniferous wood, and 100 per second in pieces of peeled wood left to dry on the bench; Grace, 1993) that it is extremely unlikely that they originate exclusively from cavitation events. Due to the inherent problems of the acoustic emission technique, it became more popular in the last decade to use the hydraulic conductivity as an indicator for 'cavitation' (Sperry & Tyree, 1990; Cochard *et al.*, 1992; Sperry *et al.*, 1996; Alder *et al.*, 1996). Unlike acoustic emissions, these measurements determine 'cavitation' in leafy branch segments of 4–80 cm length under non-flow conditions. The hydraulic conductivity is calculated by measuring the mass flow rate of water through the segments (usually flushed for re-hydration before use) and dividing it by the applied pressure gradient (commonly 30 to  $100 \text{ kPa m}^{-1}$ ). While dehydration is proceeding, a dramatic reduction in hydraulic conductivity is observed at a certain bomb pressure. It is assumed that this decrease in hydraulic conductivity is induced by 'cavitation', always followed by a complete embolism of the vessel (Pockman *et al.*, 1995; Sperry *et al.*,

<sup>1</sup>Note that due to a printing mistake a light irradiation of  $400 \mu\text{mol m}^{-2} \text{s}^{-1}$  instead of  $40 \mu\text{mol m}^{-2} \text{s}^{-1}$  is given in 'Materials and Methods' of the paper of Benkert *et al.* (1991). However, the total transpiration rates of the plants measured by weighing were so small that this printing mistake is obvious to plant physiologists, especially because the transpiration data were mentioned several times in the text.

1996). The corresponding balancing pressure is measured separately on detached leaves of the branch specimens.

Alternatively, 'vulnerability' curves are determined by placing branches in the pressure bomb with their cut ends protruding through two openings (Sperry *et al.*, 1996). While measuring the mass flow rate through the branch, the bomb pressure is increased. It is hypothesised that with an increase in bomb pressure gas is injected into the branch and that embolism is induced by 'air seeding', i.e. that xylem cavitation and embolism is nucleated by air pulled through the pit membrane pores rather than by cavitation of tensile water. Experimentally it is found that the pressures required for reaching the 'cavitation threshold' of fresh branches are lower compared to those that were pre-flushed by perfusion through the two protruding ends (in order to shift the xylem pressure to atmospheric). The difference between the two values is taken as a quantitative measure for the xylem tension in the fresh branch.

There are some other variants of these methods, but all of them are based on a poor physical background. Control experiments have not been performed to test some of the implicit assumptions. For instance, no evidence is given that the pressure drops linearly along the segment even though the main resistance and thus the largest pressure gradients should be at the pits of the vessel end walls. This can lead to a removal of cavitation nuclei around the pits during perfusion, but also during the 'flushing' pre-treatment. Physics also requires that the mass balance equation be fulfilled for water flow through the segment, i.e. that the outflow volume is equal to the inflow volume. Equally, it must be documented that the flow rate is constant over the entire segment. However, these may be minor points compared to the fundamental errors underlying the analysis of the 'vulnerability' curves. It is a great error to assume that cavitation will necessarily lead to dramatic changes in the hydraulic conductivity because most gas bubbles (particularly when stabilised by surface active substances) will adhere to the hydrophobic inner xylem wall and will thus not necessarily increase flow resistance (section V.8). Furthermore, it can easily be shown that breakage of the liquid column by cavitation leaves the vessel 99.98% full of liquid. When 1 gram of water is converted to vapour at the saturated vapour pressure it occupies about 45 litres, so it only needs the conversion of 2% of the liquid to vapour to increase the system volume by a factor of 1000. Also, water is practically incompressible, a pressure change from  $-0.4$  to  $0$  MPa will only change the volume of the liquid by around 0.02%. Any emptying of the vessel has to occur as the result of the subsequent evaporational water loss. The obvious inconsistency between the existence of very high tensions with the suction force of xylem-feeding insects (section II.2) is explained by Sperry *et al.* (1996, p. 434) in the same way: 'cavitation induced during insertion of the mouthpart of the insects into the vessel, occurs with minimal change in conduit water volume because of the incompressibility of water'. It is really

amazing to see how quickly the proponents of the Cohesion Theory assume the opposite standpoint when they are faced with inconsistent results. Even more puzzling is the determination of the balancing pressure at the so-called 'cavitation threshold'. According to the pressure bomb hypothesis, the balancing pressure value should be zero at this point because the xylem does not contain water. However, the bomb values at the 'cavitation threshold' are clustering around 1–5 MPa (Jones, 1992; Sperry *et al.*, 1996; Melcher *et al.*, 2001) implying that the determination of cavitation thresholds by measuring 'vulnerability' curves is obsolete. This desperate situation was also seen by Sperry *et al.* (1996, p. 432): 'Once a shoot is *completely* cavitated, there is no water in any of the conduits and xylem pressures do not exist. Under these circumstances, the pressure bomb method can give apparent xylem pressures that indicate overly negative [...] water potentials because the balance pressure is not achieved until at least part of the xylem path from mesophyll to cut surface is refilled'. The bedevilment was apparently solved by creating a new parameter that is measured by the pressure bomb. But what is an 'apparent xylem pressure' which is obviously an imaginary parameter? Further questions are what is then the meaning of 'vulnerability' curves, and how does the user of the bomb know that the leafy twig under investigation begins to enter the range of 'imaginary xylem pressures'? Tyree, Sperry and co-workers are forced to answer these questions.

The centrifugation experiments recently designed for supporting the cavitation thresholds deduced from vulnerability curves as well as for validation of the pressure bomb share the same shortcomings as the techniques described above. We would like to discuss these experiments in more detail because many current proponents of the Cohesion Theory believe (see e.g. Tyree, 1997; Tomos & Leigh, 1999; Steudle, 2003) that the centrifugation results have given clear-cut evidence for the validity of the pressure bomb hypothesis. The centrifugation experiments are based on a suggestion of Smith (1994) to place isolated whole xylem elements into the Z-tube of the set-up of Briggs (see section II.2) to determine the actual cavitation threshold of the xylem of various plants and trees. The idea is brilliant, but difficult to perform. Pockman *et al.* (1995) and Alder *et al.* (1997) used a less sophisticated approach by centring a pre-hydrated stem segment on the rotor of a centrifuge and spinning the segment at different speeds for at least 3 min. Then the stem segments were left in damp paper towels for 20–60 min to allow air to diffuse into vapour-filled conduits before 2–5 cm from each end was removed under water and the hydraulic conductivity was determined. Plots of the hydraulic conductivity *versus* the tension calculated from the centrifugal force yielded similar 'vulnerability' curves to those obtained with the methods mentioned above. Pockman *et al.* (1995) assumed that the bent ends that centre the water column in the Z-tubes as used by others are not necessary because of capillary forces acting at the inter-conduit pits. However, air/water interfaces at the

pits will only form when severe dehydration of the xylem occurs during and after centrifugation, i.e. when the stem segment enters the range of imaginary xylem pressures. Two years later, Alder *et al.* (1997) and Tyree (1997) recognised that water had been evaporated and thrown out by the rotational motion. However, the authors completely ignored this finding in their tension calculations. Furthermore, the integrated centrifugation equation used by these authors is based on the unproved assumptions that water columns are not interrupted and/or the distance of the air/water meniscus on either side from the rotation axis remains equal during centrifugation (Briggs, 1950). Apart from this, the argument of the authors that water was **only** released from the cut xylem elements is also extremely unlikely because of the high cohesive strength of water (!!). The long period between centrifugation and the determination of the hydraulic conductivity certainly imposes further complications in the interpretation of the data because of the instantaneous tension release followed by water moving between the various compartments after centrifugation has stopped. However, Tyree (1997) surprisingly excluded redistribution of water upon tension release after centrifugation. He assumed that the xylem pressure is preserved by the chemical potential of water of the dehydrated cells which obviously contradicts his previous and current sayings against the pressure probe data (Tyree, 2003 and section III.3).

The experiment of Holbrook *et al.* (1995) is faced with the same shortcomings as the various 'vulnerability' methods. They used an excised stem segment with a single leaf at its midpoint, and mounted the midpoint of the stem on the

rotating axis of a centrifuge-like set-up placed in a closed chamber. After centrifugation the authors removed the leaf and determined the balancing pressure value. In the light of the discussion in section III.3 tension in the xylem should be released instantaneously upon cutting (provided that no breakage of the water columns had occurred). Nevertheless, the authors found a 1 : 1 relationship between the (relative) pressure calculated from the centrifugation force and the balancing pressure. A possible explanation for the 1 : 1 correlation is that water was shifted into the periphery of the leaf (including the intercellular spaces) during centrifugation. Then, Newton's law that action has to equal reaction requires that the same force is needed to push water back from the tissue into the xylem. The problem for interpreting the data is the control experiment of Holbrook *et al.* (1995). It is obvious from the sketch in Fig. 1 of their paper that the control leaf, being not attached to the branch, was spun simultaneously, but not fixed close to the rotor axis of the centrifuge. In this case, the leaf is pressed against the wall of the chamber during rotation and, in turn, the centrifugal forces may act in a different way on the water in the control leaf compared to the fixed leaf. This could explain why the  $P_b$ -values of the control leaves were significantly lower than those of the attached leaves by 0.2 to 0.4 MPa. A correlation with the calculated rotational tension can also not be expected under these conditions. However, the finding that the  $P_b$ -values of the control leaves were significantly higher than the values of untreated leaves (0.05 MPa relative to atmosphere) evidences clearly that the control experiments were not properly designed.



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