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New evidence for large negative xylem pressures and their measurement by the pressure chamber method

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ABSTRACT

Pressure probe measurements have been interpreted as showing that xylem pressures below *c.* −0·4 MPa do not exist and that pressure chamber measurements of lower negative pressures are invalid. We present new evidence supporting the pressure chamber technique and the existence of xylem pressures well below −0·4 MPa. We deduced xylem pressures in water-stressed stem xylem from the following experiment: (1) loss of hydraulic conductivity in hydrated stem xylem (xylem pressure = atmospheric pressure) was induced by forcing compressed air into intact xylem conduits; (2) loss of hydraulic conductivity from cavitation and embolism in dehydrating stems was measured, and (3) the xylem pressure in dehydrated stems was deduced as being equal and opposite to the air pressure causing the same loss of hydraulic conductivity in hydrated stems. Pressures determined in this way are only valid if cavitation was caused by air entering the xylem conduits (air-seeding). Deduced xylem pressure showed a one-to-one correspondence with pressure chamber measurements for 12 species (woody angiosperms and gymnosperms); data extended to *c.* −10 MPa. The same correspondence was obtained under field conditions in *Betula occidentalis* Hook., where pressure differences between air- and water-filled conduits were induced by a combination of *in situ* xylem water pressure and applied positive air pressure. It is difficult to explain these results if xylem pressures were above −0·4 MPa, if the pressure chamber was inaccurate, and if cavitation occurred by some mechanism other than air-seeding. A probable reason why the pressure probe does not register large negative pressures is that, just as cavitation within the probe limits its calibration to pressures above *c.* −0·5 MPa, cavitation limits its measurement range *in situ*.

Key-words: cohesion-tension theory; water transport; xylem cavitation; xylem pressure probe.

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INTRODUCTION

Pressure probe measurements of xylem pressure range from atmospheric to *c.* −0·4 MPa (rarely to −1·0 MPa; pressures relative to atmospheric), while pressure chamber estimates on the same material may be considerably lower. Some have found the pressure probe results convincing enough to conclude that psychrometric and pressure chamber methods for estimating xylem pressures are artifactual, and that Dixon's cohesion-tension theory of water transport (Dixon 1914) must be discarded or considerably modified. The argument is made that, because the pressure probe directly measures xylem pressure, it is more reliable than the indirect pressure chamber or psychrometer techniques (Balling & Zimmermann 1990; Benkert, Balling & Zimmermann 1991; Zimmermann *et al.* 1993a,b, 1994; Canny 1995).

In this paper, we present new evidence for the existence of negative xylem pressures well below −0·4 MPa and for the ability of the pressure chamber technique to measure them. We offer alternative explanations for pressure probe results that are consistent with pressure chamber measurements of xylem pressure and with the cohesion-tension theory. The evidence we present was obtained in an experiment performed on a dozen woody species, both angiosperms and gymnosperms. Many of these results have been published or are soon to be published in other, broader contexts. Some results are unique to this paper. We present the collective data here to draw attention to their relevance for the current debate on xylem transport in plants.

The evidence is based on studies of xylem cavitation. Cavitation is the abrupt change from liquid water under negative pressure to water vapour (at vapour pressure). As water is withdrawn from the cavitated conduit, the vapour void expands to fill the entire lumen. Gas is prevented from passing to adjacent conduits by capillary forces (and torus aspiration in some gymnosperms) at the pit membrane of interconduit pits (Fig. 1; Tyree, Davis & Cochard 1994). Within hours of cavitation, the conduit becomes 'embolized' (air-blocked) as air

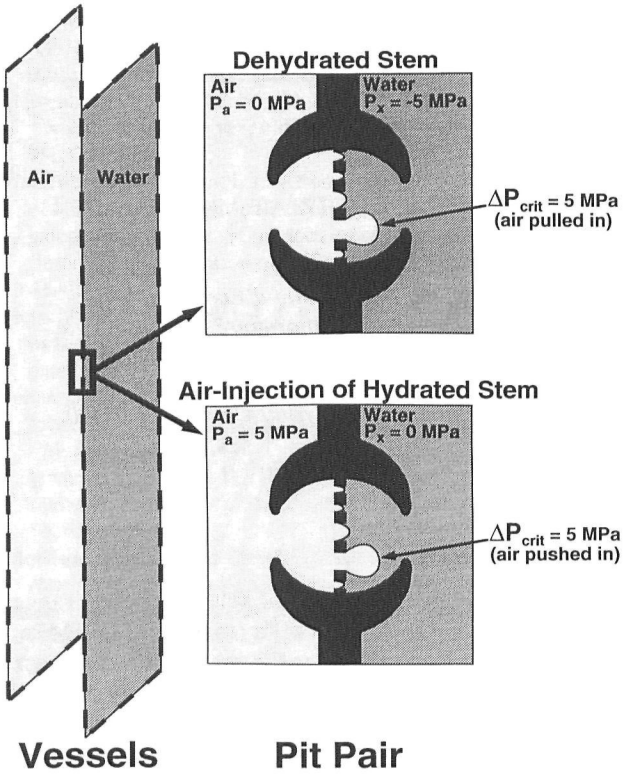

Figure 1. The air-seeding hypothesis and its corollary illustrated for the vessel-type of xylem conduit. Two adjacent xylem vessels are shown (left). Pits between vessels allow water flow but also prevent passage of an air–water meniscus in the event that one vessel becomes air-filled, as illustrated. The air-seeding hypothesis states that xylem cavitation in a 'dehydrated stem' (above right) is nucleated by air pulled through the pit membrane pores. This occurs when the air pressure (P_a, usually near 0) minus the xylem pressure (P_x, usually negative) across the air–water meniscus at the pores creates a pressure difference (ΔP_{crit}) sufficient to displace the meniscus. In the example shown, the ΔP_{crit} of 5 MPa is reached when $P_x = -5$ MPa. A corollary of the air-seeding hypothesis is that, during 'air injection of a hydrated stem' (lower right), where xylem pressure is atmospheric (0 MPa), ΔP_{crit} can be achieved by raising the air pressure (to +5 MPa in this example). This causes the water-filled conduit to become embolized by air injected through the pit membrane. The range of ΔP_{crit} can be determined in hydrated stems by measuring the loss of hydraulic conductivity in the xylem from embolism caused by air injection. This can be used to deduce the negative xylem pressure required to cause loss of hydraulic conductivity in dehydrated stems assuming that cavitation occurs by air-seeding.

diffuses in and pressures rise to atmospheric (Tyree & Sperry 1989; Lewis, Harden & Tyree 1994).

While there are many potential causes of xylem cavitation (Pickard 1981), the evidence strongly favours the 'air-seeding' hypothesis (Zimmermann 1983; Tyree *et al.* 1994). This states that cavitation occurs when air outside a water-filled conduit is aspirated into the conduit through pores in the wall. These pores will retain an air–water meniscus until the difference between the gas pressure (P_a) and xylem pressure (P_x) across the meniscus exceeds the capillary forces holding it in place. These forces are a function of pore diameter (d), the surface tension of water (T; 0·072 N m^{-1} at 20°C), and the contact angle between water and the pore wall material (Θ). The critical pressure difference (ΔP_{crit}) required to force air through a circular wetted pore is predicted from the capillary equation (Sperry & Tyree 1990).

$$\Delta P_{crit} = (4\,T \cos \Theta)/d, \quad (1)$$

where

$$\Delta P = P_a - P_x. \quad (2)$$

The bigger the pore, the smaller ΔP_{crit} becomes.

The largest pores in a conduit wall are in the pit membranes between conduits. Therefore, whenever there are any gas-filled conduits in the vascular system (e.g. from leaf abscission or fine-root death), the ΔP_{crit} of xylem conduits will be determined by the structure of these pit membranes (Fig. 1). The P_a in embolized conduits will be near atmospheric pressure, so any changes in ΔP between gas- and water-filled conduits are determined by changes in P_x. As water stress increases and P_x becomes more negative, ΔP will increase and eventually reach the critical value where air is pulled into the conduit through the pit membrane and 'seeds' cavitation. Figure 1 ('dehydrated stem') illustrates this for a typical inter-vessel pit membrane. When a torus is present in the membrane (as in conifers), the evidence suggests that air-seeding occurs when the torus becomes displaced from its sealing position over the pit aperture and air passes around it through pores in the margo (Sperry & Tyree 1990).

The air-seeding hypothesis has been supported by work showing the dependence of the negative pressure required to cause cavitation on T (Crombie, Hipkins & Milburn 1985; Sperry & Tyree 1988), d for pit membrane pores (Sperry & Tyree 1988; Lewis 1988; Jarbeau, Ewers & Davis 1994), and pit membrane flexibility, which determines the pressure required to displace the torus from the pit aperture in conifers (Sperry & Tyree 1990).

The most direct evidence for the hypothesis comes from testing its corollary: that when ΔP across pit membranes between air- and water-filled conduits is increased by raising P_a while keeping $P_x = 0$ (atmospheric), embolism will occur over the same range of ΔP as when $P_a = 0$ and P_x is negative (Fig. 1: compare dehydrated and hydrated stems). In other words, whether air is pushed or pulled across the membranes, ΔP_{crit} will be the same. In these experiments, embolism was quantified from the loss of hydraulic conductivity in the stem xylem caused by either elevated air pressure or lowered xylem pressure. Air pressure was raised in a portion of the xylem conduits of a detached branch by injecting exposed xylem at cut petiole and/or stem ends with compressed air; the rest of the xylem was connected

to tubing filled with water at atmospheric pressure. Negative xylem pressure was induced by dehydrating detached branches, and pressures were measured with the pressure chamber on leaves removed from the branch. In every case, loss of hydraulic conductivity caused by injection of air or lowering of xylem pressure occurred over the same range of ΔP (Sperry & Tyree 1990; Sperry, Perry & Sullivan 1991; Cochard, Cruiziat & Tyree 1992; Jarbeau *et al.* 1994; Sperry & Saliendra 1994; Alder, Sperry & Pockman 1996).

While the above experiment was done to evaluate the air-seeding hypothesis, it also provides a means of determining xylem pressure that is completely independent of existing techniques. As such, the results can be used to evaluate these techniques. This is the aspect of these experiments that we emphasise in this paper. If one assumes at the outset that cavitation occurs by air-seeding, the ΔP_{crit} causing a given loss of xylem conductivity in dehydrated stems when $P_a = 0$ will be equal to the P_a causing the same loss of conductivity in hydrated stems when $P_x = 0$. Knowledge of both ΔP_{crit} and P_a allows Eqn 2 to be solved for P_x in the dehydrated stems; it will be equal and opposite to the air pressure causing the same loss of conductivity in the hydrated stems. We compared xylem pressures deduced in this manner with pressure chamber measurements to evaluate their validity. We also used this approach to deduce xylem pressures in a transpiring tree in the field for comparison with pressure chamber readings.

If the assumption that cavitation occurs by air-seeding is incorrect, cavitation would have to occur at a higher (less negative) xylem pressure than predicted for air-seeding. The xylem pressure deduced from the air-seeding assumption would then be too negative. If the pressure chamber was accurate it would consistently give less negative pressures than predicted. If the pressure chamber was inaccurate it might read pressures below and/or above the deduced value, but not necessarily show a relationship to the deduced pressure. The same would be true if cavitation does occur by air seeding but the pressure chamber was inaccurate. A one-to-one correspondence between the deduced and pressure chamber xylem pressures would indicate the validity of the air-seeding hypothesis *and* the validity of the pressure chamber method for measuring xylem pressure.

MATERIALS AND METHODS

The species analysed represented woody dicotyledons and gymnosperms from mesic to arid habitats (Table 1). The readers should consult the sources listed in Table 1 for details concerning the methods used to dehydrate stems or inject them with air, and to measure the loss of hydraulic conductivity. Only a general description is provided below.

Hydraulic conductivity measurements

Hydraulic conductivity was calculated for stem segments between 4 and 80cm long by measuring the mass flow rate of water through the segment and dividing it by the associated pressure gradient in the segment (usually between 30 and 100kPam^{-1}).

Two methods were used to measure the percentage loss of hydraulic conductivity from cavitation and embolism in response to air injection and dehydration. In the *double-segment* method (DS in Table 1), the hydraulic

Table 1. Species, vulnerability curve methods, and sources. DS = double segment method; SS = single segment method; BT = bench-top dehydration; PC = pressure chamber dehydration; SE = single-ended pressure chamber; DE = double-ended pressure chamber. See text for explanation of methods

Species	Vulnerability curve method		Source
	Dehydration	Air injection	
Gymnosperms:			
Abies balsamifera (L.) Mill.	DS-BT	DS	Sperry & Tyree (1990)
Abies concolor (Gord. & Glend.) Lindl.	DS-BT	SS	Ikeda & Sperry (unpublished results)
Juniperus virginiana L.	DS-BT	DS-SE	Sperry & Tyree (1990)
Picea rubens Sarg.	DS-BT	DS-SE	Sperry & Tyree (1990)
Psuedotsuga menziesii Franco	DS-PC	SS-DE	Ikeda & Sperry (unpublished results)
Dicotyledons:			
Acer grandidentatum Nutt.	SS-PC	SS-DE	Alder *et al.* (1996)
Betula occidentalis Hook.	SS-BT	SS-DE	Sperry & Saliendra (1994)
Heteromeles arbutifolia Lindley	SS-BT	SS-SE	Jarbeau *et al.* (1994)
Malosma laurina (Nutt.) Abrams	SS-BT	SS-SE	Jarbeau *et al.* (1994)
Populus deltoides Bartr.ex Marsch.	SS-BT,PC	SS-DE	Cochard *et al.* (1992)
Populus tremuloides Michx.	DS-BT	DS-SE	Sperry *et al.* (1991)
Salix alba L.	SS-BT,PC	SS-DE	Cochard *et al.* (1992)

conductivity of a stem segment after an air injection or dehydration treatment was compared to that of an adjacent control segment from the same branch that was not treated. In the *single-segment* method (SS in Table 1), hydraulic conductivity was measured before and after embolism in the branch by repeated 10–60 min high-pressure (*c.* 100 kPa) flushes of measuring solution through the stem (Sperry, Donnelly & Tyree 1988).

In the double-segment method, the decrease in conductivity in the treated segment relative to the control segment results only from the treatment. In the single-segment technique, the lower conductivity before the flush represents embolism caused by the treatment in addition to any reversible embolism in the segment prior to the treatment. In most cases, this 'background' or native embolism accounted for less than 20% of the loss of conductivity in the segment (40% in *Heteromeles arbutifolia*; Jarbeau *et al.* 1994).

Air injection treatment

Two methods were used to raise the air pressure in air-filled xylem conduits relative to the water pressure in water-filled conduits. In the *single-ended* pressure chamber technique (SE in Table 1), a length of branch several times longer than the longest conduit was inserted into a pressure chamber with one end of the branch protruding. The stem was wrapped in wet towelling and supplied with water at the protruding end to avoid dehydration during and after injection. Air was injected into the branch at a selected pressure. After a 20 min pressure injection, the air pressure was gradually released and the loss of hydraulic conductivity measured by one of the methods described above. Several separate stems were injected at different pressures to determine the full range of ΔP_{crit} required to eliminate all water transport. We refer to the relationship between the air-injection pressure and the loss of hydraulic conductivity as a 'vulnerability curve'.

In the *double-ended* pressure chamber method (DE in Table 1), a branch segment was sealed in a pressure chamber with *both* ends protruding. This was done either by using a double-ended pressure chamber, or by bending a pliable branch so that both ends exited the same end of a conventional pressure chamber with a modified lid. Hydraulic conductivity was measured through the branch as air pressure around the branch was increased. Air entered the vascular system through cut petioles, cut side-branches and, in some cases, notches cut into the main stem. The complete vulnerability curve was obtained from single branch segments. Several separate branches were averaged to obtain a composite curve representative of the material. An example is shown in Fig. 2a (solid symbols) for *B. occidentalis*.

Two control experiments ruled out loss of conductivity during air injection occurring from: (1) air coming out of solution as stems were depressurized ('bends' effects), and (2) cavitation arising from dehydration of the tissue during pressurizing. Xylem blockage caused by air coming out of solution was ruled out by enclosing some stems entirely within the pressure chamber and pressurizing at the highest test pressure. These stems showed no significant loss of conductivity, even when pressure was released more rapidly than normal (Sperry & Tyree 1990; Sperry *et al.* 1991; Jarbeau *et al.* 1994). Dehydration effects were ruled out by measuring the xylem pressure following injection of stems at the highest test pressure. For the single-ended method, these stems had xylem pressures above −0·35 MPa after injection, which was not different from values obtained for non-injected controls. Xylem pressures were estimated from pressure chamber measurements of bagged leaves (Jarbeau *et al.* 1994) or stem psychrometer measurements (Sperry & Tyree 1990). In the double-ended method, xylem pressures were always slightly above atmospheric because water was flowing through the xylem by gravity head during air injection.

Dehydration treatment and pressure chamber measurements of xylem pressure

Two methods were used to dehydrate stems. In the *bench top* method (BT in Table 1), segments were dried on the laboratory bench. Minimum xylem pressure was estimated from the average of at least three pressure chamber measurements of excised leaves or shoot tips after branches were enclosed in plastic bags for at least 30 min to eliminate transpiration and associated pressure gradients in the branch. In the *pressure chamber* method (PC in Table 1), intact branch tips were enclosed in a pressure chamber with the cut end protruding. Water was squeezed from the shoot by increasing the chamber pressure and the stem end blotted dry with absorbent paper. Balancing pressures (= opposite of the xylem pressure) were then measured on the entire branch. When the desired xylem pressure was reached, air pressure was released and the branch held in a plastic bag for at least 2 h to allow air to diffuse into cavitated vessels. Loss of conductivity was then measured by one of the techniques described above.

The possibility existed that loss of conductivity in the pressure chamber method arose from air injection rather than the subsequent dehydration. Use of the loss of hydraulic conductivity caused by pressure chamber dehydration to deduce xylem pressures from the air-injection data would be valid only if the air-injection phase caused no greater loss of conductivity than the following dehydration phase.

Deduction of xylem pressure

As explained in the Introduction, if the loss of conductivity in dehydrated stems was caused by air-seeding, the xylem pressure would be equal and opposite to the air pressure causing the same loss of conductivity in

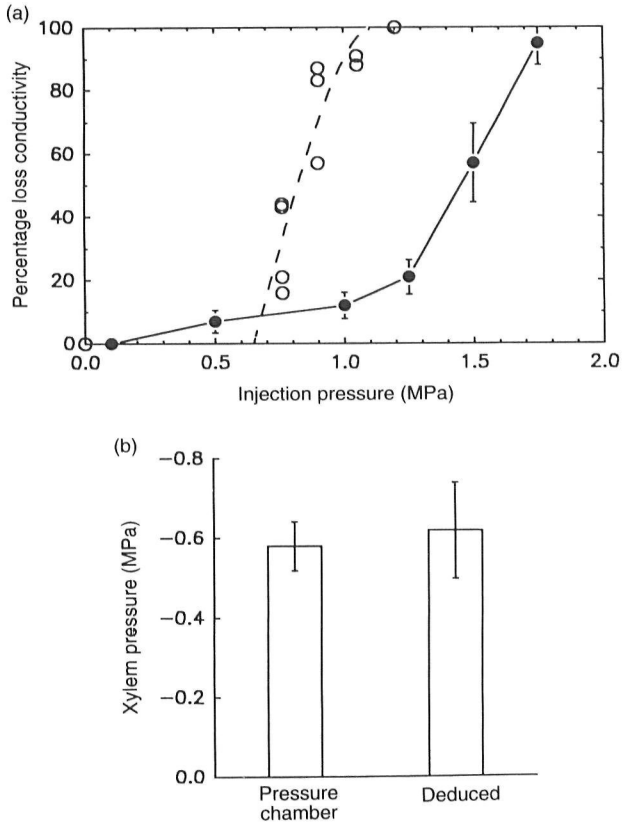

Figure 2. (a) Percentage loss in xylem conductivity versus air-injection pressure for stems of a single individual of *Betula occidentalis*. Solid symbols represent atmospheric xylem pressure in laboratory experiments. Open symbols represent field conditions. Less air pressure was required to embolize xylem in the field because xylem pressures were negative. Deduced xylem pressure in the field was the difference in air-injection pressure for the same loss of conductivity. (b) Deduced ($n = 11$) versus pressure chamber ($n = 13$) xylem pressures for the same tree as in (a). Error bars are ± 1 SD.

hydrated stems injected with air (Fig. 1). In practice, xylem pressure in dehydrated stems was determined graphically from air-injection data and the loss of conductivity measured in dehydrated stems. The method is best explained by an example. Figure 2a (solid symbols) shows the loss of hydraulic conductivity in stem xylem of *Betula occidentalis* as a function of air pressure (xylem pressure = atmospheric). Note that it required +1·5 MPa of air pressure (ΔP = 1·5 MPa) to cause a 60% loss of hydraulic conductivity. If a stem of this species was dehydrated under atmospheric pressure and developed a 60% loss of hydraulic conductivity, it must have experienced a minimum xylem pressure of −1·5 MPa (ΔP = 1·5 MPa) if the loss of conductivity was caused by air-seeding. Xylem pressures determined in this way were compared to the minimum xylem pressure measured during dehydration with the pressure chamber method to test for a one-to-one relationship.

Xylem pressures can only be deduced in this manner if the loss of conductivity is a sensitive function of air pressure, i.e. if the slope of the air-injection curve was relatively steep. Otherwise, a single loss of conductivity could correspond with a wide range of xylem pressures. Furthermore, the loss of conductivity must be above the 'background' or native value in the absence of a dehydration treatment, at least when the single-segment method was used to measure loss of conductivity. Both of these considerations limited our prediction of xylem pressures for dehydration-induced losses of conductivity above 20% (40% for *Heteromeles arbutifolia*; Jarbeau *et al.* 1994).

In situ air injection and deduction of xylem pressures in the field

In an extension of earlier work reported in Sperry & Pockman (1993), we also deduced xylem pressures in a transpiring individual of *Betula occidentalis* in the field from *in situ* air-injection experiments. A laboratory air-injection curve was already available for stems of this individual (Sperry & Saliendra 1994; Fig. 2a, solid symbols). This curve was obtained in the usual double-ended manner for xylem pressures at atmospheric pressure. A second curve was obtained during a single sunny afternoon by injecting air into side-branches of the transpiring tree in the field. According to the air-seeding hypothesis, it would require less air pressure to cause the same loss of hydraulic conductivity in the field than in the laboratory if xylem pressures were negative in the field. Furthermore, the negative pressure in the field would equal the difference in air pressure causing the same loss of hydraulic conductivity under field and laboratory conditions. For example, if it required a P_a of 2·0 MPa to cause a 30% loss of conductivity in hydrated stems when P_x = 0 in the laboratory, the associated ΔP_{crit} would be 2 MPa. If it took a P_a of only 0·3 MPa to cause a 30% loss of conductivity in the field, ΔP_{crit} would still be 2 MPa, and therefore *in situ* P_x would have to equal −1·7 MPa (from Eqn 2). This is the field-minus-laboratory P_a causing a 30% loss in conductivity. Xylem pressure deduced in this manner was compared with independent pressure chamber measurements.

The distal 30 cm of the side-branches to be injected with air was covered with aluminium foil the evening prior to the experiment to eliminate transpiration and prevent changes of xylem pressure upon excision. Leaves subjacent to the chosen point of excision were covered with foil at this time to allow equilibration of their xylem pressure with the stem below. The following afternoon, the covered branch tips were excised, and xylem pressures of the bagged leaves measured with the pressure chamber. Each attached branch stub (4·3–6·3 mm diameter) was then inserted in a steel fitting designed for injecting branches with compressed air (see Sperry & Pockman 1993). Branches were injected at a

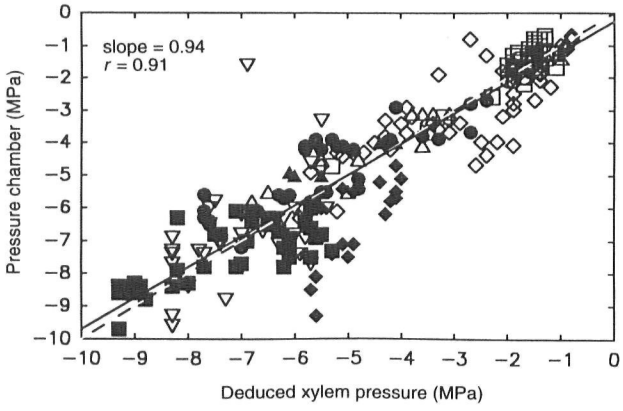

Figure 3. Deduced versus pressure chamber xylem pressures in 12 species of woody angiosperms (open symbols) and gymnosperms (solid symbols). The solid line is a linear regression with a slope not significantly different from 1 (dashed line). ● two species of *Abies, A. balsmea,* and *A. concolor*; ◆ *Juniperus virginiana*; ■ *Picea rubens*; ▲ *Psuedotsuga menziesii*; △ *Acer grandidentatum*; ○ *Betula occidentalis*; ▽ *Heteromeles arbutifolia*; ◇ *Malosma laurina*; □ three species of Salicaceae: *Populus tremuloides, P. deltoides,* and *Salix alba.* See Table 1 for sources.

specific air pressure for 10 min. Following injection, the injected branch was cut from the tree and brought into the laboratory in a plastic bag. A segment located 10 cm (longer than 95% of the vessels) from the injected end was cut from each branch underwater and measured for its loss of conductivity using the single-segment method. Native loss of conductivity in this species averaged less than 10% (Sperry & Pockman 1993).

RESULTS

Figure 3 shows the xylem pressure deduced from conductivity and air-injection data versus the xylem pressure measured with the pressure chamber for the species listed in Table 1. The slope is not significantly different from 1·0 (Student's *t*-test, $t_s = 0{\cdot}301$, $P > 0{\cdot}5$) indicating that negative pressures were accurately measured by the pressure chamber and that cavitation occurred by air-seeding.

Figure 2 shows the results of the *in situ* air injection of *B. occidentalis*. As predicted by the air-seeding hypothesis, less air pressure was required to cause the same loss of conductivity in the field when xylem pressures should have been negative (Fig. 2a, open symbols) than in the laboratory when xylem pressures were atmospheric (Fig. 2a, solid symbols). The difference in air pressure for a given loss of conductivity gave the negative *in situ* xylem pressure. The average xylem pressure deduced from this experiment was $-0{\cdot}62 \pm 0{\cdot}12$ MPa ($n = 11$; Fig. 2b). The average xylem pressure measured with the pressure chamber was not significantly different ($t_s = 0{\cdot}76$, $P > 0{\cdot}4$): $-0{\cdot}59 \pm 0{\cdot}07$ MPa ($n = 13$; Fig. 2b).

DISCUSSION

The one-to-one correspondence between the xylem pressure predicted from air-injection data and that measured with the pressure chamber in the laboratory (Fig. 3) and in the field (Fig. 2) provides strong support for: (1) the existence of large negative pressures in the xylem (i.e. below −0·4 MPa), (2) the accuracy of the pressure chamber method, and (3) the occurrence of cavitation by air-seeding.

It is difficult to explain the results shown in Figs 2 and 3 if xylem pressures were limited to above −0·4 MPa as claimed by Zimmermann and colleagues (see, e.g. Zimmermann *et al.* 1994). It is certainly possible that cavitation may occur before negative pressures reach the magnitude required to pull air across the interconduit membranes. If that were the case, xylem pressures deduced from the air-injection experiments would be much too negative. In addition, the pressure chamber method would be guilty of giving grossly inaccurate and overly negative estimates. What makes the probability of this scenario vanishingly small, however, is that there is no logical reason why these two highly inaccurate yet independent determinations of xylem pressure would give the *same value* when compared across a dozen diverse species (Fig. 3). Our opinion is that the explanation for this correspondence is that cavitation occurs by air-seeding at negative pressures correctly estimated by the pressure chamber and air-injection methods.

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 chamber method can give apparent xylem pressures that indicate overly negative tissue water potentials because the balance pressure is not achieved until at least part of the xylem path from mesophyll to cut surface is refilled (Sperry, Alder & Eastlack 1993; Sperry & Saliendra 1994). It has been said that the same error occurs even if there is partial cavitation in the xylem (Balling & Zimmermann 1990). Based on the data in Figs 2 and 3, such an error is undetectably small. Cavitated conduits will remain completely gas-filled until xylem pressures rise to within 10 to 20 kPa below atmospheric. When xylem pressures reach atmospheric at the balance point, only a small amount of water is pulled into the gas-filled conduits as a meniscus forms across the tapered ends of the conduit lumens. Only if the xylem is held at atmospheric pressure for an extended period will the cavitated conduits completely refill with water as the air in them slowly dissolves (Yang & Tyree 1992). Balancing points made on extensively cavitated material are marked by wetting of only a part of the cut surface that is still conductive (J. S. Sperry, personal observation).

Our results add to the already considerable evidence in support of the pressure chamber method. There is

generally close agreement between pressure chamber and psychrometer methods of estimating xylem pressure (Brown & Tanner 1981; Dixon & Tyree 1984) despite the fact they are based on very different principles. Pressures measured with the pressure chamber are also consistent with those predicted from models of water transport based on independent measurements of hydraulic conductivity and leaf transpiration (Heine 1970; Tyree *et al.* 1983; Gibson, Calkin & Nobel 1985; Tyree 1988; Ewers, Fisher & Chiu 1989). Recently, Holbrook, Burns & Field (1995) have shown a 1:1 correspondence between the xylem pressure imposed by centrifugal force on rotating stems and pressure chamber readings. Similarly, two of the authors have shown that centrifugal force induced cavitation at the same pressures as estimated by the pressure chamber on air-dehydrated stems and predicted from air-injection experiments (Pockman, Sperry & O'Leary 1995). Significantly, measurements obtained using the *turgor* pressure probe have been shown to agree with turgor pressures predicted from pressure chamber measurements of xylem pressure and determinations of osmotic pressure in xylem and cell saps (Murphy & Smith 1994).

How can these results be reconciled with the xylem pressure probe data? First, we give a brief summary of comparisons between probe and pressure chamber measurements (see Zimmermann *et al.* 1994 and references cited therein). The cavitation pressure of a conduit penetrated by a pressure probe is variable, but generally between vacuum (*c.* −0·1 MPa) and −0·5 MPa (pressure relative to atmospheric). Under well-watered conditions when transpiration is low, probe measurements can average between near-atmospheric and *c.* −0·4 MPa. Simultaneous pressure chamber estimates on transpiring leaves read *c.* 0·3 MPa below the pressure probe value. When stems were excised and dehydrated, or when transpiration was high, probe measurements typically gave an average reading between atmospheric and vacuum. Thus, xylem pressures appeared to *increase* (become closer to atmospheric) under stress. In contrast, pressure chamber estimates showed a *decrease* in xylem pressure during water stress (e.g. between −1 and −3 MPa) as predicted by the cohesion-tension theory. Therefore, the discrepancy between pressure chamber and pressure probe readings was increased by water stress.

While the discrepancies between the probe and pressure chamber estimates of xylem pressure could mean the cohesion-tension theory is invalid and the pressure chamber is inaccurate, there are at least two alternative explanations. The first is that valid comparisons between the probe and pressure chamber have not yet been made under non-stressful conditions when xylem pressures would be above (less negative than) the cavitation threshold for a probed conduit. The probe measures *in situ* pressure in a single conduit; this is typically a stem vessel, or a vessel in the leaf midrib. The pressure chamber measures a bulk-average xylem pressure for all the detached shoot tissue enclosed in the chamber. *A priori*, the pressure chamber will give a lower (more negative) value for the xylem pressure than the pressure probe whenever there is any transpiration. Even a low rate of transpiration will cause detectable gradients in xylem pressure between leaf midrib or stem xylem and bulk leaf tissue because of the high resistance to flow in the extra-xylary flow path in the mesophyll of the leaf (Boyer 1985; Tyree *et al.* 1991). This is why the pressure chamber measurements reported in this paper were made on bagged shoots or leaves: to measure xylem pressure in the stem with a pressure chamber measurement of a leaf, transpiration must be eliminated. Unfortunately, none of the comparisons between probe and pressure chamber have been made under conditions where there was a confirmed absence of transpiration. The same criticism was made in an earlier review of xylem pressure probe results (Passioura 1991).

The second explanation concerns conditions where plants were dehydrated and/or transpiring rapidly and the probe and pressure chamber measurements showed the greatest discrepancy. Perhaps the probe is incapable of measuring negative xylem pressures below *c.* −0·4 MPa because its insertion into the xylem conduit nucleates cavitation. Notably, cavitation within the probe system limits its calibration with a 'Hepp-type' osmometer to pressures above *c.* −0·6 MPa (Balling & Zimmermann 1990; Zimmermann *et al.* 1993a). Using the osmometer to generate known pressures in vascular tissue, the insertion of the probe into a conduit did not cause cavitation 'as long as the pressure was not more negative than −0·5 MPa' (Balling & Zimmermann 1990). When conduits were probed at less negative pressures and pressures subsequently decreased, cavitation was always observed in the approximate range of −0·3 to −0·5 MPa (e.g. Zimmermann *et al.* 1993b). Is this cavitation pressure an artifact of the measurement system, or does it reflect the true cavitation pressure of intact xylem conduits? Our results suggest the former conclusion because xylem pressures as low as −10 MPa were indicated in intact xylem conduits (Fig. 3).

The probe data themselves are also consistent with the artifact interpretation. If the probe cannot reliably measure beyond the −0·4 to −0·6 MPa range, its insertion into a conduit holding water at lower pressures will cause cavitation. The probe will register the gas pressure in cavitated conduits. The gas pressure will be between atmospheric and vapour pressure (*c.* vacuum) depending on the mix of water vapour and air. In fact, as mentioned above, this is the 'xylem' pressure range for most probe readings when water-stressed material is measured (Zimmermann *et al.* 1994). The more negative probe readings seen in well-watered and slowly transpiring plants are expected if these pressures were not negative enough to cause cavitation during measurement.

The difficulty of knowing whether a probed vessel is

gas-filled or not increases the likelihood of gas pressure being taken for water pressure. The uptake of dyes injected into vessels does not prove a probed vessel is water-filled (as claimed by Balling & Zimmermann 1990) because, even if the injected vessel was embolized, the dye would be wicked through the vessel wall to surrounding functional vessels and be swept up the transpiration stream. Similarly, pressure pulses resulting from brief injections of water into the punctured vessel (Zimmermann *et al.* 1993b, Fig. 7) would also be found in gas-filled conduits if the probe mouth was obstructed by the vessel wall (e.g. the wall opposite the entry point). If a dependable test were devised, it would have to be applied for every measurement showing pressures between atmospheric and vacuum to avoid mistaking gas for water pressure.

The possibility that the pressure probe may often be measuring gas rather than liquid pressure can explain a number of superficially puzzling observations made with the probe in addition to the basic disagreement between probe and pressure bomb measurements. For example, much has been made of the unresponsiveness of the probe to changes in transpiration rate or to squeezing or water injection of tissue in a pressure chamber (Balling & Zimmermann 1990). However, this would be expected if the probe were reading gas rather than liquid pressure because of the much greater compressibility of gas than water. Importantly, the pressure chamber experiments showed that the probe *did* respond to chamber pressure once a threshold pressure had been exceeded (Balling & Zimmermann 1990). This makes sense if the threshold pressure refilled the gas-filled conduit with water and established hydraulic contact with the probe. Notably, when tissue was infiltrated with water to prevent cavitation during root pressure probe measurements, xylem pressures responded immediately to changes in transpiration *unless* pressures were negative enough to cause cavitation (Heydt & Steudle 1991).

To claim that the probe data demonstrate the non-existence of large negative xylem pressures, it is necessary to assume that the cavitation pressure of a xylem conduit is the same whether impaled by an 8 μm diameter glass probe or not. This is a remarkably weak assumption given the well-known fact that the negative pressure developed by water is strongly dependent on the container in which it is held (Zimmermann 1983; Zimmerman *et al.* 1994; Smith 1994). And the change in the container is drastic: from an intact conduit developed from living cells to a conduit punctured with a glass probe attached to a plexi-glass reservoir. On the basis of the cohesion-tension theory itself, it is expected that direct measurements of xylem pressure involving rupture of the conduit wall would cause cavitation with increasing probability as xylem pressures dropped.

The insertion of the probe into a xylem conduit creates two obvious potential nucleating sites for cavitation that are absent in the intact conduit. One is the integrity of the seal between the glass probe and the ruptured conduit wall. Unlike the self-sealing properties of a cell membrane that minimizes leaks from a living cell during probe insertion for turgor pressure determinations, the xylem conduit wall will not necessarily seal tightly around the glass probe. The gaps between glass and cell wall are undoubtedly larger than the pore sizes in the original wall and will lower the air-seeding pressure (ΔP_{crit}) of the conduit below its value when the conduit was intact.

The second possible nucleating site is at the interface between the xylem sap and the inner wall of the probe. Adhesion between water and the glass and plastic surfaces inside the probe apparatus may be less than between water and the inner cell wall surface of the conduit, which is primarily cellulose with a low lignin content (see Pickard 1981; Tyree *et al.* 1994 for detailed discussions of cavitation nucleation at wall–water interfaces). Variability in surface features and length of wetting period probably accounts for the large variation in negative pressures generated in glass and plastic Bertholot tubes and spinning 'Z' tubes (Dixon 1914; Briggs 1950; Pickard 1981; Smith 1994). The stability of water in these artificial containers is of limited use for inferring the cavitation pressure of an intact xylem conduit for the simple reason that they are not intact xylem conduits. With regard to the Z-tube experiments (Smith 1994), the relevant experiment for determining stability of water in *xylem* is to spin *stems* rather than glass tubes (Holbrook, Burns & Field 1995; Pockman *et al.* 1995).

While it is not the purpose of this paper to give a comprehensive evaluation of evidence concerning the cohesion-tension theory, we must point out that recent objections to the theory that were not based on pressure probe data (Zimmermann *et al.* 1994) also have alternative explanations. It has been said that the ability of insects to extract water from xylem conduits indicates that xylem pressures more than *c.* 0·3 MPa below atmospheric would be impossible because this is the maximum suction that insects can generate (Zimmermann *et al.* 1994). In fact, insects could extract water from the xylem regardless of how negative the original pressure was if they caused cavitation during insertion of their mouthparts into the conduit. Cavitation instantly causes the water pressure in the conduit to rise to the vapour pressure of water or above. This occurs with minimal change in conduit water volume because of the incompressibility of water. The insect could then readily suck water from the conduit as it is also drained by the surrounding transpiration stream. The moderation of xylem tensions in the surrounding vessels by the release of water from the cavitated conduit would also favour the ability of the insect to compete with the transpiration stream for the water. Ironically, it could be *easier* for an insect to feed from xylem sap when initial xylem pressures were lower rather than higher.

Lower initial xylem pressures would increase the likelihood of cavitation during mouthpiece insertion which in turn allows the water to be sucked from the conduit. If no cavitation occurred (as during penetration at modest negative pressures), the insect would have to pull against the negative pressure in the xylem. However, if pressures were extremely low the water might be drained from the vessels too fast for the insect to obtain much water. This may explain why leaf-hopper feeding rates increased with decreasing xylem pressure before falling off at the lowest pressures (Anderson, Brodbeck & Mizell 1992).

Another objection to the cohesion theory raised by Zimmermann *et al.* (1994) was the absence of a vertical pressure gradient required to pull water against friction and gravity in transpiring trees. In some cases, pressures can be less negative at the top of a tree than in lower branches (Hinckley & Ritchie 1970). In fact, as has been pointed out several times (Richter 1973; Zimmermann 1983; Tyree 1988; Tyree & Ewers 1991), these observations are consistent with the cohesion-tension theory. A number of studies have predicted pressure gradients in trees from direct measurements of the hydraulic conductivity of xylem and transpiration rates. Most of the appreciable drop in pressure required to overcome frictional drag in the shoot xylem occurs in minor branches, petioles, and leaf veins rather than in the main stem (Zimmermann 1978; Ewers & Zimmermann 1984a, b; Tyree *et al.* 1983). Therefore, the pressure drop from the root collar to the end of a lower branch can be equal to or greater than the pressure drop from the root collar to the top of the tree during transpiration. Xylem pressures measured at the tips of separate branches (or even on transpiring leaves of a single branch) will not necessarily show a gradient with height or distance.

When an observation appears at odds with an established theory, the theory may be incorrect, or the observation may have been misinterpreted and is actually neutral or even supportive with respect to the theory. While the pressure probe results are superficially at odds with the cohesion-tension theory, there are very reasonable alternative explanations for them that have not been adequately considered. To summarize our arguments above, the failure of the pressure probe to measure large negative xylem pressures is in fact *predicted* by the cohesion-tension theory. Without data that convincingly rule out these alternative explanations and that undermine the many lines of evidence for large negative pressures in xylem, the recent crop of 'new' theories of water transport (Zimmermann *et al.* 1994; Canny 1995) will wither as they did during their previous incarnation 100 years ago when the cohesion-tension theory was proposed.

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REFERENCES

Alder N.N., Sperry J.S. & Pockman W.T. (1996) Root and stem xylem embolism, stomatal conductance, and leaf turgor in *Acer grandidentatum* populations along a soil moisture gradient. *Oecologia*, in press.

Anderson P.C., Brodbeck B.V. & Mizell R.F. (1992) Feeding by the leafhopper, *Homalodisca coagulata*, in relation to xylem fluid chemistry and tension. *Journal of Insect Physiology* **38,** 611–622.

Balling A. & Zimmermann U. (1990) Comparative measurements of the xylem pressure of *Nicotiana* plants by means of the pressure bomb and pressure probe. *Planta* **182,** 325–338.

Benkert R., Balling A. & Zimmermann U. (1991) Direct measurements of the pressure and flow in the xylem vessels of *Nicotiana tobacum* and their dependence on flow resistance and transpiration rate. *Botanica Acta* **104,** 423–432.

Briggs L.J. (1950) Limiting negative pressure of water. *Journal of Applied Physics* **21,** 721–722.

Brown P.W. & Tanner C.B. (1981) Alfalfa water potential measurement: a comparison of the pressure chamber and leaf dewpoint hygrometers. *Crop Science* **21,** 240–244.

Boyer J.S. (1985) Water transport in plants. *Annual Review of Plant Physiology* **36,** 473–516.

Canny M.J. (1995) A new theory for the ascent of sap–cohesion supported by tissue pressure. *Annals of Botany* **75,** 343–357.

Cochard H., Cruiziat P. & Tyree M.T. (1992) Use of positive pressures to establish vulnerability curves. *Plant Physiology* **100,** 205–209.

Crombie D.S., Hipkins H.F. & Milburn J.A. (1985) Gas penetration of pit membranes in the xylem of *Rhododendron* as the cause of acoustically detectable sap cavitation. *Australian Journal of Plant Physiology* **12,** 445–453.

Dixon H.H. (1914) *Transpiration and the Ascent of Sap in Plants.* Macmillan, London.

Dixon M.A. & Tyree M.T. (1984) A new stem hygrometer, corrected for temperature gradients and calibrated against the pressure bomb. *Plant, Cell and Environment* **7,** 693–697.

Ewers F.W. & Zimmermann M.H. (1984a) The hydraulic architecture of balsam fir (*Abies balsamea*). *Physiologia Plantarum* **60,** 453–458.

Ewers F.W. & Zimmermann M.H. (1984b) The hydraulic architecture of eastern hemlock (*Tsuga canadensis*). *Canadian Journal of Botany* **62,** 940–946.

Ewers F.W., Fisher J.B. & Chiu S.T. (1989) Water transport in the liana *Bauhinia fassoglensis* (Fabaceae). *Plant Physiology* **91,** 1625–1631.

Gibson A.C., Calkin H.W. & Nobel P.S. (1985) hydraulic conductance and xylem structure in tracheid-bearing plants. *IAWA Bulletin* **6,** 293–302.

Heine R.W. (1970) Estimation of conductivity and conducting area of poplar stems using a radioactive tracer. *Annals of Botany* **34,** 1019–1024.

Heydt H. & Steudle E. (1991) Measurement of negative pressure in the xylem of excised roots. Effects on water and solute relations. *Planta* **184,** 389–396.

Hickley T.M. & Ritchie G.A. (1970) Within-crown patterns of transpiration, water stress, and stomatal activity in *Abies amabilis*. *Forest Science* **16,** 490–492.

Holbrook N.M., Burns M.J. & Field C.B. (1995) Negative xylem pressures in plants: a test of the balancing pressure technique. *Science* **270,** 1183–1192.

Jarbeau J.A., Ewers F.W. & Davis S.D. (1994) The mechanism of water stress-induced embolism in two species of chaparral shrubs. *Plant, Cell and Environment* **18,** 189–196.

Lewis A.M. (1988) A test of the air-seedling hypothesis using *Sphagnum* hyalocysts. *Plant Physiology* **87,** 577–582.

Lewis A.M., Harnden V.D. & Tyree M.T. (1994) Collapse of water-stress emboli in the tracheids of *Thuja occidentalis* L. *Plant Physiology* **106,** 1639–1646.

Murphy R. & Smith J.A.C. (1994) A critical comparison of the pressure-probe and pressure chamber techniques for estimating leaf-cell turgor pressure in *Kalanchöe diagremontiana*. *Plant, Cell and Environment* **17,** 15–29.

Passioura J.B. (1991) An impasse in plant water relations? *Botanica Acta* **104,** 405–411.

Pickard W.F. (1981) The ascent of sap in plants. *Progress in Biophysical and Molecular Biology* **37,** 181–229.

Pockman W.T., Sperry J.S. & O'Leary J.W. (1995) Sustained and significant negative water pressure in xylem. *Nature* **378,** 715–716.

Richter H. (1973) Frictional potential losses and total water potential in plants: a re-evaluation. *Journal of Experimental Botany* **24,** 983–994.

Smith A.M. (1994) Xylem transport and the negative pressure sustainable by water. *Annals of Botany* **74,** 647–651.

Sperry J.S. & Tyree M.T. (1988) Mechanism of water stress induced xylem embolism. *Plant Physiology* **88,** 581–587.

Sperry J.S., Donnelly J.R. & Tyree M.T. (1988) A method for measuring hydraulic conductivity and embolism in xylem. *Plant, Cell and Environment* **11,** 35–40.

Sperry J.S. & Tyree M.T. (1990) Water stress induced xylem embolism in three species of conifers. *Plant, Cell and Environment* **13,** 427–436.

Sperry J.S., Perry A. & Sullivan J.E.M. (1991) Pit membrane degradation and air-embolism formation in ageing xylem vessels of *Populus tremuloides* Michx. *Journal of Experimental Botany* **42,** 1399–1406.

Sperry J.S. & Pockman W.T. (1993) Limitation of transpiration by hydraulic conductance and xylem cavitation in *Betula occidentalis*. *Plant, Cell and Environment* **16,** 279–288.

Sperry J.S., Alder N.N. & Eastlack S.E. (1993) The effect of reduced hydraulic conductance and xylem cavitation in *Betula occidentalis*. *Plant, Cell and Environment* **16,** 279–288.

Sperry J.S. & Saliendra N.Z. (1994) Intra- and inter-plant variation in xylem cavitation in *Betula occidentalis*. *Plant, Cell and Environment* **17,** 1233–1241.

Tyree M.T. (1988) A dynamic model for water flow in a single tree: evidence that models must account for hydraulic architecture. *Tree Physiology* **4,** 195–217.

Tyree M.T. & Sperry J.S. (1989) Vulnerability of xylem to cavitation and embolism. *Annual Review of Plant Physiology and Molecular Biology* **40,** 19–38.

Tyree M.T. & Ewers F.W. (1991) The hydraulic architecture of trees and other woody plants. *New Phytologist* **11,** 345–360.

Tyree M.T., Snyderman D.A., Wilmot T.R. & Machado J.L. (1991) Water relations and hydraulic architecture of a tropical tree (*Schefflera morototoni*). *Plant Physiology* **96,** 1105–1113.

Tyree M.T., Graham M.E.D., Cooper K.E. & Bazos L.J. (1983) The hydraulic architecture of *Thuja occidentalis*. *Canadian Journal of Botany* **61,** 2105–2111.

Tyree M.T., Davis S.D. & Cochard H. (1994) Biophysical perspectives of xylem evolution: is there a tradeoff of hydraulic efficiency for vulnerability to dysfunction? *IAWA Bulletin* **15,** 335–360.

Yang S. & Tyree M.T. (1992) A theoretical model of hydraulic conductivity recovery from embolism with comparison to experimental data on *Acer saccharum*. *Plant, Cell and Environment* **15,** 633–643.

Zimmermann M.H. (1978) Hydraulic architecture of some diffuse porous trees. *Canadian Journal of Botany* **56,** 2286–2295.

Zimmermann M.H. (1983) *Xylem Structure and the Ascent of Sap*. Springer Verlag, Berlin.

Zimmermann U., Benkert R., Schneider H., Rygol J., Zhu J.J. & Zimmermann G. (1993a) Xylem pressure and transport in higher plants and tall trees. In *Water Deficits: Plant Responses from Cell to Community* (eds J.A.C. Smith & H. Griffiths), pp. 87–108. Bios Scientific Publishers Ltd, Oxford.

Zimmermann U., Haase A., Langbein D. & Meinzer F.C. (1993b) Mechanisms of long-distance water transport in plants: a re-examination of some paradigms in the light of new evidence. *Philosophical Transactions of the Royal Society of London B* **341,** 19–31.

Zimmermann U., Meinzer F.C., Benkert R., Zhu J.J., Schneider H., Goldstein G., Kuchenbrod E. & Haase A. (1994) Xylem water transport: is the available evidence consistent with the cohesion theory? *Plant, Cell and Environment* **17,** 1169–1181.

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