

# The essentials of direct xylem pressure measurement

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## ABSTRACT

**This paper discusses the essentials of the oil-filled pressure probe technique in the measurement of negative xylem pressures, focusing in particular on the technique and physics underlying our recent, successful experiment which has rekindled the debate on the validity of the Cohesion–Tension theory. We illustrate a number of general problems associated with the cell pressure probe and xylem pressure probe techniques, and propose appropriate criteria for micropipette construction. We enumerate factors dealing with the cavitation problem and suggest methods for eliminating air seeds in the system. We introduce reliable criteria for the successful measurement of xylem pressure, and emphasize the importance of the probe pressure relaxation test. Several problems regarding the controversy over the Cohesion–Tension theory are also discussed. We discuss the correlation between xylem pressure and the transpiration rate, the existence of absolute negative xylem pressure in intact plants, the most negative values of xylem pressure measured by the pressure probe, the agreement between the pressure probe and pressure bomb techniques, and the vulnerability to cavitation (tensile strength) of pressure probes.**

*Key-words:* cavitation; cell pressure probe; Cohesion–Tension theory; negative pressure; Scholander pressure bomb.

## INTRODUCTION

The hot debate on the validity of the Cohesion–Tension (C–T) theory and the Scholander pressure bomb method has recently resurfaced (Wei, Steudle & Tyree 2000; Zimmermann *et al.* 2000). During the past decade, the C–T theory has been called into question (Balling & Zimmermann 1990; Benkert, Balling & Zimmermann 1991; Zimmermann *et al.* 1993; Zimmermann, Meinzer & Bentrup 1995). The most serious challenge has come from pressure probe work which failed to confirm the presence of absolute negative xylem pressures consistent with the predictions of the C–T theory. Furthermore, the Scholander pressure bomb method (Scholander *et al.* 1965), which has traditionally been used to measure xylem pressure, was

also questioned because conflicting pressure probe results indicated that xylem pressures were much less negative than those measured with the pressure bomb method.

According to the pressure probe experiments carried out by Zimmermann *et al.*, xylem pressures are commonly in the range of –0.1 MPa to 0 MPa, and can never be lower than approximately –0.65 MPa (Note: all pressures in this paper are relative to atmospheric pressure. Thus, –0.1 MPa represents a vacuum. Accordingly, pressures between –0.1 MPa and 0 MPa are referred to as subatmospheric pressures, whereas, xylem pressures below –0.1 MPa are referred to as absolute negative pressures, which means that water in the xylem vessel is under tension). These pressure values suggest that the pressure bomb method overestimates the tension in the xylem. Although the validity of the C–T theory does not depend solely on the accuracy of the pressure bomb method, so far much of the evidence for the C–T theory depends upon this balance pressure measurement. Hence, the above arguments about the pressure bomb have cast doubt on the validity of the C–T theory.

Our recent work with the pressure probe, however, gave a very different story (Wei, Steudle & Tyree 1999a; Wei, Tyree & Steudle 1999b). We maintained ongoing real time measurement of negative xylem pressures for hours while changing the environmental conditions. We have measured negative xylem pressures down to –1 MPa, the most negative value ever recorded, and this has enabled us to confirm the pressure bomb method over a much wider range of pressures. This paper describes our probing experience for people who have expressed interest in the method.

## THE CELL PRESSURE PROBE

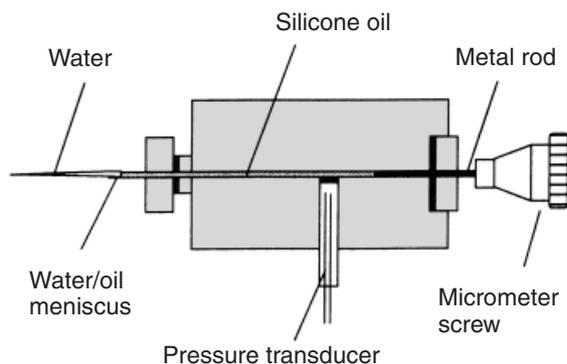
The process of using a tapered capillary to measure negative xylem pressure can be traced back to 1965, when Dr Franz Floto in Copenhagen, Denmark demonstrated his probe to John A. Milburn (Milburn 1996). Floto's capillary contained water and a small bead of mercury. When the capillary penetrated a xylem vessel, the mercury resisted being drawn into the tip, so that the extent of its travel could be used to gauge negative pressures. Another pioneer was Paul Green who used a similar technique to measure positive cell turgor pressures. He inserted a partially water-filled micropipette into a *Nitella* cell (Green and Stanton 1967, Green 1968). The capillary was sealed at the other end so that the compression of the air bubble trapped within the capillary allowed him to calculate the cell turgor pressure.

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The first version of the contemporary cell pressure probe was introduced in 1969 to monitor turgor pressure in giant-celled algae such as *Valonia* or in other characean internodes (Zimmermann, Rade & Steudle 1969). The second version (Steudle & Zimmermann 1971) differed from the first in the further reduction of the internal volume and the use of a moveable rod to quantitatively manipulate the internal volume. The search for a pressure probe applicable to higher plant cells led to the construction of the present version. Besides a further reduction of pressure chamber volume, the current version has reduced the effective dead volume of the probe by using the meniscus formed in the tip of the micropipette as a reference (Hüsken, Steudle & Zimmermann 1978).

The current cell pressure probe (Fig. 1) consists of a methacrylate block with a drilled 'T'-shaped internal channel, a surface-mounted pressure transducer, and a compression fitting for a pulled micropipette. The system is filled with liquid media (silicone oil and a small amount of water in the tip of the micropipette). When the micropipette tip is inserted into either a cell or a xylem vessel, the cell turgor pressure or xylem pressure is transmitted through the media and is measured by the pressure transducer. During measurements when the volume or pressure of the system has to be controlled, a movable metal rod (micropiston) inserted into the chamber is advanced or retracted by means of a micrometer screw. The pressure probe assembly is mounted on a manual micro-manipulator, and the tip of micropipette is viewed through a dissecting microscope during probing.

In 1990, the so-called xylem pressure probe was developed to measure pressures in xylem vessels (Balling & Zimmermann 1990). The only difference between the cell pressure probe and the xylem pressure probe is that the



**Figure 1.** Cell pressure probe for measuring xylem pressures in intact plants. The probe consists of a micropipette and a methacrylate plastic chamber. A pressure transducer is mounted on the chamber. The system is filled with silicone oil and a small amount of water in the tip of micropipette. When the tip is inserted into a xylem vessel, the xylem pressure is transmitted through the liquid medium and measured by the pressure transducer. In some cases, the pressure of the system has to be controlled. This can be achieved by means of a movable metal rod (controlled by a micrometer) inserted into the chamber.

latter uses only water to fill the chamber and the micropipette. It was considered that 'For measurement of tensions in the xylem vessels the oil must be replaced by de-gassed water in order to avoid bubble formation which occurs readily at the sap-oil boundary' (quoted from Balling & Zimmermann 1990). The assumption underlying this statement has proved to be false, however. We have shown that oil-water-filled cell pressure probes are also capable of measuring negative pressures (Wei *et al.* 1999a,b). The pressure probe discussed in this paper is a cell pressure probe, i.e. it is filled with silicone oil and water.

It should be noted that there is a fundamental difference between probing a living cell with positive turgor and probing a dead xylem vessel under negative pressure. The positioning of the oil-water meniscus is critical in probing a positively pressurized cell as the meniscus position is used as a reference. On the other hand its position is not a factor at all in measuring xylem pressure because the on-going transpiration of the plant quickly restores the entire system to its full negative pressure. Silicone oil is used in xylem pressure measurements because of its higher affinity for the chamber walls than water, thereby allowing the probe to withstand a higher tension before cavitation occurs.

When recording pressures in living cells, a small amount of water is introduced into the micropipette tip, simply to provide a visible meniscus whose position serves as a reference point for full pressurization of the cell (Hüsken *et al.* 1978). In xylem pressure measurements, however, a large amount of water (approximately 4  $\mu\text{L}$ ) must be introduced into the capillary tip to ensure that no silicone oil will be sucked into the xylem vessel.

## THE MICROPIPETTE

The accepted terminology for micropipette tip morphology is as follows. The 'shaft' of a micropipette is the straight portion of capillary tubing; the 'shoulder' is the tapering segment that is formed during pulling; the 'tip' refers to a micropipette's terminal end, and the 'tip diameter' refers to the outer diameter. In some cases the term 'tip' refers to the entire concave tapered portion of the micropipette. The 'bevel angle' of the tip is the angle between the long axis of the micropipette and the bevelled surface.

Our micropipettes were made by pulling borosilicate glass capillaries (outside diameter = 1.0 mm, inside diameter = 0.5 mm; HILGENBERG, Malsfeld, Germany) and the tips were polished with a bevelling machine (bevel angle = 40° to 45°). The micropipette puller (List-Medical-Electronic L/M-3P-A, Darmstadt, Germany) was a common type of vertical puller designed for the fabrication of moderate taper lengths. With careful adjustment of the pulling speeds, it could consistently produce pipettes of the desired form. The bevelling was carried out on a rotating wet stone beveler (Bachofer-462, Reutlingen, Germany). It has been reported that some researchers break the tip, instead of bevelling it. We have found that although occasionally such breaks produce tips of a form similar to that produced by bevelling, it is very difficult to obtain an acute

bevel angle by breaking. Moreover, the tip diameter produced by breaking is usually too large. Therefore, we believe that bevelling is important for successful probing since the resulting fine and smooth tip can facilitate penetration through the xylem wall.

Ideally, it would be nice to have capillaries with very consistent tip sizes and shapes, but unfortunately this is not always the case. Variability results from inhomogeneities in the chemical composition and molecular structure of borosilicate glass, which have been discussed in detail by Brown & Flaming (1995). In our case, the tip diameter of our micropipettes varied between 4 and 5  $\mu\text{m}$ ; the lengths of the shoulder and the shaft were about 8 and 45 mm, respectively; the bevel angle was 40–45°.

Smaller tip diameters are less likely to cause leaking and cavitation during penetration of the xylem vessel, however, they do cause a number of problems of their own. Small tips are difficult to fill with water and frequently lack the strength to penetrate xylem walls without breaking. In order to produce good tips efficiently it is essential to have a reliable method of estimating tip diameter. Although tip diameters can be measured directly under the microscope, it is time consuming and potentially risky to the tips. We have found a simple way of estimating tip diameter when it is mounted directly on the pressure probe block. The method relies on the variable pressures transmitted through the probe system originating in the surface tension produced by the meniscus or water drop at the tip whose size depends upon tip diameter. The smaller the tip diameter, the higher the pressure recorded by the probe. A tip diameter larger than 8  $\mu\text{m}$  shows no pressure on the pressure transducer, whereas a tip diameter of 4–5  $\mu\text{m}$  will show pressure of about 0.03 MPa. Indeed 0.03 MPa is approximately the pressure generated by the water droplet at a tip of 5  $\mu\text{m}$  diameter. In practice, it is found that while tightening a pipette to the probe chamber, pressure builds up in the probe. Once the tightening is done the pressure relaxes gradually until it is balanced by the hydrostatic pressure of the water droplet at the tip. This indication of tip diameter turns out to be quite reliable. Whenever the pressure transducer does not show pressures higher than 0.03 MPa, we replace the pipette.

The relative merits of small versus large tip diameters are as follows: a large tip diameter with a corresponding small time constant will more immediately reflect the insertion of the tip into a xylem vessel, as the pressure drop will be more obvious (see below). On the other hand, a smaller tip will increase the chance of a successful probing, since the chance that the tip only partly penetrates the xylem wall will be lower.

Micropipettes with long shoulders seem to have fewer cavitation problems. Note that with our method we maintain a probe pressure of 0.03–0.05 MPa during penetration, by advancing the micropiston of the pressure probe (Wei *et al.* 1999b). This means that we always have an outward flow from the tip of the micropipette. The flow velocity at the tip is much higher than that in the shaft due to the incompressibility of water and silicone oil. The hydraulic

dynamics analysis of this flow shows that a gradual and smooth tapering of the micropipette will help to generate a uniform exit velocity profile of the water; on the other hand an abrupt change in capillary taper will generate a steep adverse pressure gradient which can increase the chance of cavitation when the tip penetrates into the xylem vessel (for details see Morel 1975, 1977). As cavitation is the single most significant problem in probing negative pressures in xylem vessels, we prefer microcapillaries with a long shoulder.

## PROBE PRESSURE RELAXATION AND THE HALF-TIME

When measuring negative xylem pressure, it is helpful to take note of a property of the probe: the half-time of the probe pressure relaxation,  $\tau_p$ . By this half-time we can judge (1) whether the tip has penetrated into the xylem vessel; (2) whether the tip has broken; or (3) whether the system is leaking.

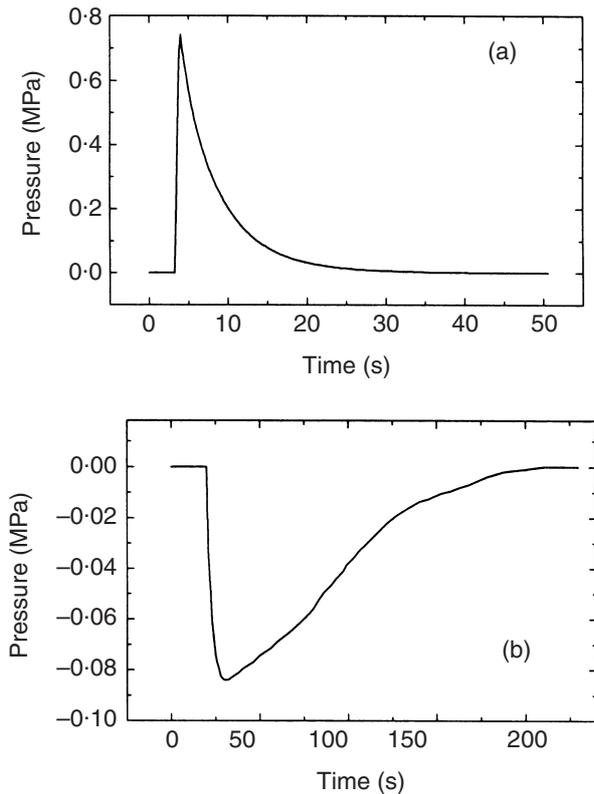
The set-up to determine probe pressure relaxation involves no more than a pressure probe and a small beaker of water. The micromanipulator is set at an angle allowing the capillary (previously filled with silicone oil and water) to extend into the beaker. A positive pressure pulse is then given to the probe by driving the micropiston forward. The typical time course of the pressure relaxation curve is shown in Fig. 2a. Similarly, a negative pressure pulse is generated by pulling back the micropiston, and its typical relaxation curve is shown in Fig. 2b. In both positive and negative pressure relaxations, the  $\tau_p$  is the time needed in order to reach half of the initial pressure value. The larger the tip diameter is, the faster the pressure returns to zero, and therefore the  $\tau_p$  value is smaller (Table 1, Fig. 3). The values of  $\tau_p$  in the table deviate somewhat from the classic Hagen–Poiseuille's law (Nobel 1991) as we are dealing with ducts of changing cross-sectional area (for a discussion on flow in non-uniform ducts cf. Langlois 1972).

The pressure outside the capillary tip is  $\rho gh$ , where  $\rho$  is the density of water,  $g$  is the gravitational constant, and  $h$  is the distance between the tip and the surface of the water. This pressure can be considered constant because the tiny amount of water entering or leaving the probe causes prac-

**Table 1.** The half-time of positive pressure relaxation of the cell pressure probe for different microcapillary tip diameters, reported in mean  $\pm$  standard deviation. They were calculated from the time course of the pressure relaxation, which was recorded using a computerized system (resolution = 0.01 s)

Tip O.D. ( $\mu\text{m}$ )	Half-time (s)
4	3.64 $\pm$ 0.39 ( $n = 8$ )
5	2.65 $\pm$ 0.88 ( $n = 18$ )
7	0.61 $\pm$ 0.15 ( $n = 10$ )
10	0.32 $\pm$ 0.12 ( $n = 6$ )

O.D. = outside diameter.



**Figure 2.** Pressure relaxations of a positive pressure pulse (a) and a negative pressure pulse (b). These pressure pulses were induced by advancing or retracting the metal piston of the probe, while keeping the micropipette tip in a water reservoir. The diameter of the micropipette tip was  $5\ \mu\text{m}$ .

tically no change to the water level,  $h$ , in the beaker. In fact  $\rho gh$  can be considered to be zero because it is much smaller than the probe pressures we are dealing with ( $\rho gh$  is about  $0.001\ \text{MPa}$ , but the probe pressure is  $0.1\text{--}0.8\ \text{MPa}$ ).

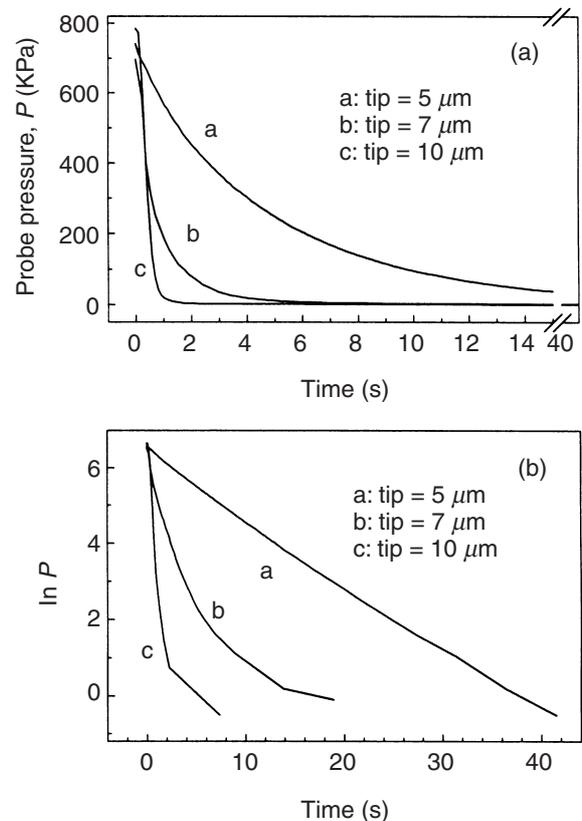
The relaxation half-time,  $\tau_p$ , for positive pressure provides a way of determining whether the micropipette tip has penetrated a xylem vessel. When the tip penetrates a xylem vessel, the probe pressure shows a precipitous drop. However, there are some other situations which also show a steep drop in probe pressure, for example, when the tip breaks, when the system leaks, or when we are not advancing the micropiston while maintaining the  $0.03\text{--}0.05\ \text{MPa}$  probe pressure. The problem is that without knowing the characteristic  $\tau_p$  of the probe, it is hard to distinguish between the different causes of pressure drop.

The recent controversies over the C–T theory and the Scholander pressure bomb technique concern the response of xylem pressure to changes in pneumatic pressure applied to the plant tissue. Balling and Zimmermann pressurized the shoot apex of a *Nicotiana* plant; at the same time they probed a petiole or stem maintained at atmospheric pressure (Balling & Zimmermann 1990; Fig. 5a, b). They failed to record a xylem pressure response that agrees with the theories. In contrast, in an experiment in which we probed

leaf xylem pressure of a maize plant while simultaneously pressurizing the root system, we successfully recorded the response of xylem pressure to the changes in root-bomb pressure (Wei *et al.* 1999a, 1999b). This response nicely coincides with the C–T theory. This experiment demonstrates the benefit of knowing the  $\tau_p$  of the pressure probe. For instance, the pressure transducer shows an increase in xylem pressure when the root-bomb pressure is increased. However, the probe pressure could also increase because of leaking, temperature changes, or cavitation. Indeed, pressurization of the plant tissue could easily cause leaking at the probing site. Without knowing the relaxation half-time (for negative pressure) of the probe, it would be difficult to distinguish between leaking and a true xylem pressure response.

### AIR BUBBLES IN THE SYSTEM

In xylem vessels of transpiring plants, air seeds are considered to be an important cause of cavitation and embolism (Tyree 1999). It is believed that the small diameter of xylem vessels and the properties of their wall help to maintain the continuity of the xylem water column (Tyree *et al.* 1984).



**Figure 3.** (a) The effect of tip diameter on the positive pressure relaxation of the cell pressure probe. Notice that the speed of pressure relaxation is greater for larger tips. (b) The linear relationship between  $\ln(\text{pressure})$  and time. This is especially pronounced for micropipettes with small tip diameters ( $\sim 5\ \mu\text{m}$ ).

Similarly, any air bubbles trapped in a probe will make it impossible to record absolute negative pressures because the silicon oil and water will not be able to reach a tensile state. However, because the diameter of the water column in the pressure probe channels is much larger than that of xylem vessels, air bubbles in the pressure probe are an even more significant factor.

For any air bubbles in a liquid medium the pressure inside the bubbles must be higher than that in the surrounding liquid by  $2\tau/R$ , where  $\tau$  is the surface tension and  $R$  is the radius. The  $\tau$  of water at 20 °C is  $0.073 \text{ N m}^{-1}$ , which gives  $2\tau/R = 0.15 \text{ MPa}$  for a bubble of  $1 \text{ }\mu\text{m}$  radius. This means that for any negative pressure it is possible to calculate a minimum bubble diameter which will cause cavitation. For instance, if a pressure probe is designed to measure  $1.5 \text{ MPa}$  negative pressure, any air bubbles of radius larger than  $100 \text{ nm}$  will cavitate the system and make the measurement impossible. The above consideration may not apply to a gas bubble that sticks to a crevice of a hydrophobic wall because the hydrophobic properties of the wall enable the liquid tension to reduce the gas pressure in the bubble (Pickard 1981). Nevertheless, it is clear that air bubbles are capable of rendering any given probe set-up ineffective in measuring negative pressures.

It is essential therefore to ensure that there are no air bubbles in the probe chamber before attaching a pipette to the chamber. A reliable check is to look at the speeds of pressure rise and drop during a pressure relaxation test. Any air bubbles present in the system will slow the speed of pressure relaxation. Again, this requires a familiarity with the  $\tau_p$  of the probe. The check is reliable, but it is time consuming. As the micropipette often breaks during penetration of the xylem wall (we needed to replace about 15 micropipettes each day in the experiment Wei *et al.* 1999b), a repeated check can be very frustrating. Therefore, we have an alternative way to assess quickly the presence of an air bubble. This 'rule of thumb' is accomplished by pressing one's thumb on the outlet of the probe chamber. A tight chamber (i.e. presumably no air bubbles) should show an obvious pressure pulse. The underlying physics is that any air bubbles present in the liquid would be pressurized and thus damp out the transmission of liquid pressure. Of course, even if there were no air bubbles in the chamber, the magnitude of the pulse could vary from time to time, depending on how successfully the thumb compressed the liquid. Our experience shows that if the pressure pulse reads at least  $0.5 \text{ MPa}$ , the probe will usually be ready for measurement.

Visible air bubbles can be removed by mechanically dislodging them with a fine wire, but this may not be sufficient to ensure negative pressure readings. This is because at a certain negative pressure, air dissolved in either the water or the silicone oil under ambient temperature and pressure will become gaseous again leading to cavitation. The problem can be overcome by vacuum degassing the liquid over a magnetic stirrer before it is injected into the probe.

The same principle is used to keep the probe free from gaseous air. We attach a micropipette whose tip is sealed

with glue to the probe body, increase the probe pressure (using the micropiston of the probe) to about  $0.5 \text{ MPa}$ , and let the system equilibrate overnight. Any air bubbles in the system will dissolve in the water/silicone oil and can be removed by replacing with freshly degassed liquids the next day. This method can also be used to remove air spaces in the pressure probe resulting from cavitation. After a cavitation event, even 1 min of pressurization with the tip sealed turns out to be very helpful for successive measurements.

## PROBING TECHNIQUE

Probing negative pressure in xylem vessels requires considerable attention to detail. Most essential for repeated success is a clear understanding of the behaviour of the micropipette tip as it approaches, touches, and penetrates the xylem vessel. Much of the controversy regarding the presence or absence of negative pressure in actively transpiring xylem vessels may result from an inadequate understanding of the behaviour of the probe during the probing process.

The measurements made by Zimmermann *et al.* in recent years used water-filled xylem pressure probes. They inserted their micropipettes into the tissue and stopped the insertion instantly (by an automatic feedback mechanism, or manually) once their probe sensed a pressure below atmospheric, believing that the tip of the capillary was in a xylem vessel (Benkert *et al.* 1991; Zimmermann *et al.* 1993, 1994a, 1994b, 1995).

In contrast, we fill the pressure probe with low viscosity silicone oil (WACKER AS4, Munchen, Germany) because testing has showed that it will sustain greater negative pressures than a water-filled probe. Furthermore, the probe is kept at an overpressure of about  $0.03 \text{ MPa}$ , by slowly advancing the micropiston, during insertion of the micropipette. Keeping this overpressure during the insertion is very important both in preventing the formation of air bubbles in the tip and in ensuring that the tip is not blocked during penetration. As we have noted previously, the presence of any air bubbles in the system will make it impossible to make negative pressure measurements and will result only in subatmospheric pressure readings.

There are two key indicators that we rely upon for successful insertion of the tip into a xylem vessel. The first of these is the flexing of the micropipette tip against the outside of the xylem vessel wall, which can be seen under the microscope. Using the micromanipulator the flexed tip must be straightened before it is advanced to penetrate the cell wall. The second indicator of successful probing is a precipitous drop in probe pressure.

Clearly, there are substantial differences between Zimmermann's probing technique and our own. This may explain why we have significantly different findings regarding the existence of absolute negative xylem pressures in actively transpiring plants. We probed 131 maize plants in the experiment Wei *et al.* 1999b, and we recorded 107 absolute negative xylem pressures. Thus, we measured

xylem pressures in the subatmospheric range (i.e. between vacuum and atmospheric pressure) in no more than 20% of the maize plants. In contrast, Zimmermann *et al.* claimed that 'measurements on more than two hundred plants showed that the xylem pressure was far more commonly between vacuum and ambient pressure' (Benkert *et al.* 1991).

We believe that a steep drop in probe pressure cannot solely be considered an indication of complete penetration into a xylem vessel. Indeed, such a drop often happens without observing the bending of the micropipette tip, which may occur when the tip is only partly inserted into the xylem vessel. In this case, it was not surprising that the probe transducer gave a subatmospheric pressure value. Moreover, neither transpiration, nor changes in the pneumatic pressure applied to the plant tissue correlatively affected this pressure reading.

Other criteria have also proved to be reliable indicators of successful probing of xylem pressure. They are: (a) the response of xylem pressure to changes in transpiration rate (e.g. application of higher light intensity to the plants can result in a drop in xylem pressure); (b) the translocation of dye-labelled water from the tip into the xylem vessel; (c) the response of xylem pressure to changes in soil water potential (e.g. Wei *et al.* 1999b; in which the application of air pressure to the root system resulted in an increase in xylem pressure).

## CONCLUSION

One could conclude that much of the controversy over the Cohesion–Tension theory is a result of artifacts resulting from improper probing technique. For instance, if either the micropipette tip is not completely inserted into a xylem vessel or an air bubble is trapped in the tip during the advance of the micropipette, the probe will not be able to record an absolute negative pressure. Of course, the reading shown by the pressure transducer will not represent the true xylem pressure. Clearly one cannot expect to record the response of this reading to changes in transpiration rate.

Xylem pressures down to  $-1$  MPa have been found in maize plants (Wei *et al.* 1999a). Nevertheless, they are far less than the tensions predicted by the C–T theory, which raises the additional question: is the pressure probe capable of measuring high tension without cavitation? We evaluated both water-filled and oil–water-filled pressure probes for their vulnerability to cavitation. It turned out that the latter could sustain more negative pressure than the former, but its maximum tensile strength is only about  $-1.6$  MPa (Wei *et al.* 1999b). This is far less negative than that proposed to exist in the xylem of some transpiring plants ( $-10$  MPa according to measurements with the pressure bomb method; Kolb & Davis 1994). Obviously if pressures down to  $-10$  MPa are going to be measured, probes should not cavitate at pressures higher than  $-10$  MPa. Unfortunately, this point has been overlooked for almost a decade, and the limitation of the pressure probe has led to claims that the C–T theory is invalid.

Comparison between the pressure probe and pressure bomb methods has been made in recent years by several researchers (Melcher *et al.* 1998; Wei *et al.* 1999a, 1999b). To correctly compare the two methods, the xylem pressure value obtained by the pressure probe has to be a result of stable measurement. Quite often, when the micropipette tip just penetrates into a xylem vessel, cavitation occurs before the pressure transducer gives a stable reading (i.e. during the precipitous drop of probe pressure). We should not include these data in the comparison. With this in mind, we found good agreement between the pressure probe and pressure bomb methods through a range of 0–0.8 MPa.

The oil–water-filled pressure probe will probably continue to be the instrument of choice for the measurement of negative pressures in the transpiration stream. No other method, at least at this time, offers the possibility of direct measurement of absolute negative xylem pressures. The measurement poses unique technical challenges, foremost among which is the probability of inducing cavitation within the system, although improvement of the device may enhance the rate of success. In general terms, the handling and methodology of use of the probe is critical to success.

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