

SHORT COMMUNICATION

# Comparative measurements of xylem pressure in transpiring and non-transpiring leaves by means of the pressure chamber and the xylem pressure probe

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## Abstract

Simultaneous measurements were made with the xylem pressure probe on exposed, transpiring leaves and with the Scholander pressure chamber on both transpiring and covered, non-transpiring leaves of sugarcane and maize plants. Xylem tensions inferred from pressure chamber balancing pressures on non-transpiring leaves were similar to those measured directly with the xylem pressure probe in transpiring leaves. However, tensions inferred with the pressure chamber on transpiring leaves that were placed in plastic bags just prior to excision were up to 0.6 MPa greater than those measured concurrently with the xylem pressure probe. These findings suggest that relatively large differences in water potential between the xylem and bulk leaf tissue can exist during periods of rapid transpiration, and they confirm that the balance pressure of an excised, previously transpiring leaf is only a measure of the bulk average equilibrium leaf water potential and not of the true xylem pressure that existed prior to excision.

Key words: Cohesion-Tension theory, xylem pressure probe, pressure chamber, xylem tension.

## Introduction

The current understanding of long-distance water transport in plants is based on the Cohesion-Tension theory

proposed more than 100 years ago by Dixon and Joly (1894). According to this theory, long-distance water transport in xylem is driven by tension (negative pressure) gradients generated by the evaporation of water from leaves and transmitted through continuous water columns from the leaf apoplast to the roots. Evidence supporting the Cohesion-Tension theory consists largely of thousands of indirect estimates of xylem pressure obtained in excised leaves and branches with the Scholander pressure chamber (Scholander *et al.*, 1965). The recent development of the xylem pressure probe (xylem probe) by Balling and Zimmermann (1990) has allowed the *direct* measurement of xylem pressure in single vessels of intact plants.

Measurements with the xylem probe have confirmed the existence of tensions in the xylem of both transpiring and non-transpiring plants, but the values obtained are often substantially smaller than those inferred from indirect techniques, such as the pressure chamber and psychrometer (Balling and Zimmermann, 1990; Zimmermann *et al.*, 1991, 1994a, b). This has led some researchers to question the validity of estimates of xylem tension obtained with the pressure chamber and to postulate that the currently accepted hundred-year-old Cohesion-Tension theory may not account for all the driving forces involved in long-distance water transport in plants (Balling and Zimmermann, 1990; Benkert *et al.*, 1995; Canny, 1995; Kuchenbrod *et al.*, 1996; Zimmermann *et al.*, 1991, 1994a, b). Respondents to these challenges argue that the theory is secure and the xylem probe is underestimating the true operating tension in xylem sap

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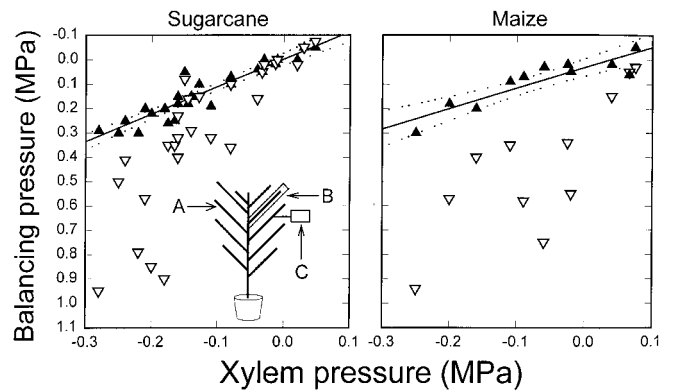
(Tyree *et al.*, 1998; Tyree, 1997; Holbrook *et al.*, 1995; Milburn, 1996; Pockman *et al.*, 1995; Sperry *et al.*, 1996; Steudle, 1995).

The controversy surrounding the validity of the xylem probe and pressure chamber techniques remains unsettled, in part because the two techniques have rarely been employed concurrently in an effort to gain insight concerning the basis of the apparent discrepancy. In this study, simultaneous measurements were made with the xylem probe inserted into xylem vessels of intact, transpiring leaves, and with the pressure chamber in both previously-transpiring and previously-non-transpiring, covered leaves that were rapidly detached from the same plant. This experiment was performed on both sugarcane and maize plants. The hypotheses are (1) that balancing pressures obtained with the pressure chamber on non-transpiring leaves would be similar in absolute magnitude to xylem pressures determined with the xylem probe on nearby leaves regardless of whether they were transpiring or not, and (2) that large deviations between the two techniques would be observed only when balancing pressures were obtained from transpiring leaves.

## Materials and methods

The design and principle of the xylem pressure probe are described in detail elsewhere (Balling and Zimmermann, 1990). Basically, the probe consists of a conventional cell turgor pressure probe modified by fitting it with a water-wettable pressure transducer and filling it with deionized, degassed water instead of silicone oil. Because the probe's glass microcapillary tip can be inserted into particular vessels, this is a 'fine' technique capable of measuring the pressure in individual xylem vessels directly. The pressure chamber, on the other hand, is a 'gross' technique that measures average pressures across all tissues. It involves pressurizing the gas phase surrounding an excised leaf or twig until water is forced from the relatively elastic-walled living cells back into the rigid xylem elements. It is assumed that the balancing pressure, indicated by the appearance of water at the cut surface, is reached when the distribution of water within the leaf or twig is restored to the condition existing before excision. At this point the pressure applied externally to a whole leaf or twig is considered to be equal in magnitude to the tension existing within the individual xylem vessels prior to application of pressure (Tyree, 1997; Holbrook *et al.*, 1995; Scholander *et al.*, 1965).

All measurements in this study were carried out on 1.5–2 m tall, well-irrigated sugarcane (*Saccharum* spp. hybrid H65–7052) and maize (*Zea mays* inbred HI27) plants growing in pots in a glasshouse. The experimental design (Fig. 1, inset) involved covering one or two leaves of each plant with aluminium foil prior to dawn to prevent substantial transpiration during the day. After successfully inserting the xylem probe into a xylem vessel in an exposed, transpiring leaf, balancing pressures were determined with a pressure chamber on both covered and exposed leaves that were excised simultaneously from the same plant. It should be noted that the leaves were covered with plastic bags just before excision to prevent dehydration after excision. The excised leaves, still in plastic bags, except for the cut end, were sealed in a pressure chamber containing



**Fig. 1.** The relationship between direct (xylem-probe) measurements of xylem pressure in transpiring leaves and balancing pressures measured with the pressure chamber on covered, non-transpiring leaves (solid symbols) and exposed, transpiring leaves (open symbols) of sugarcane and maize are shown. The  $y$ -axis is the balancing pressure minus 0.1 MPa to make it equivalent to the absolute scale used on the  $x$ -axis. The 95% confidence intervals (dotted lines) and linear regressions (solid lines) for the relationship between xylem probe and pressure chamber measurements on covered leaves are shown for both species. Inset: A diagram indicating locations of simultaneous measurements made on the 1.5–2-m-tall plants. (A) the exposed transpiring leaf measured with the pressure chamber, (B) the covered non-transpiring leaf measured with the pressure chamber, and (C) the xylem probe inserted into an exposed, transpiring leaf.

moist paper towels to reduce further tissue dehydration during the measurement. The rate of pressurization was about  $0.005 \text{ MPa s}^{-1}$ . Multiple plants were used through the course of the experiments to obtain a range of transpiration rates and balancing pressures.

## Results

A nearly 1:1 correspondence was observed between absolute values of xylem pressure measured directly with the xylem probe and balancing pressures determined concurrently with the pressure chamber on non-transpiring leaves of both sugarcane and maize plants (Fig. 1, closed symbols). When leaves were allowed to transpire, however, the balancing pressure required to exude sap with the pressure chamber was up to 0.6 MPa greater than the absolute value of the tension measured directly with the xylem probe (Fig. 1, open symbols).

## Discussion

Agreement between the two techniques was excellent when the leaves used in the pressure chamber had been covered before dawn with aluminium foil to prevent transpiration while the leaves were still attached to the plants (Fig. 1A, B, closed symbols). When leaves had been allowed to transpire prior to excision, large discrepancies were often observed between xylem tensions inferred from their balancing pressures and actual operating xylem pressures obtained directly with the xylem-probe in individual vessels (Fig. 1, open symbols). This

supports the large body of evidence that *in situ* xylem tensions inferred from balancing pressures determined on excised, previously transpiring leaves are not equal to the previously existing xylem pressure, but rather to some bulk average value of leaf water potential.

The inability of the pressure chamber to measure xylem tension in transpiring leaves was recognized many years ago by Janes and Gee (1973). They obtained a balancing pressure of 0.3 MPa in leaves of transpiring pepper plants that had their roots pressurized to 0.6 MPa to produce a positive pressure in the xylem. The existence of positive xylem pressure in the intact plant was confirmed by observation of water exuding from the stump of the petiole when the leaf was excised. We suggest that the presence of a significant hydraulic resistance between the xylem and remaining leaf tissue (possibly associated with air-filled spaces) is a likely contributor to the disparity between estimates of leaf xylem pressure obtained with the xylem probe and with the pressure chamber. A substantial hydraulic resistance within the leaf could cause steep water potential gradients to develop in response to transpirational water movement. Indeed, it has long been recognized that large differences in pressure chamber balancing pressure can be observed between adjacent transpiring leaves and covered, non-transpiring leaves (Begg and Turner, 1970; Ritchie and Hinckley, 1971; Turner and Long, 1980; Turner, 1981; Saliendra and Meinzer, 1989). The data presented here are consistent with this and support Passioura's (1982) prediction that if the water columns are continuous throughout the plant and if local variations in xylem tension are negligible, then the covered, non-transpiring leaf should function as a tensiometer, permitting the xylem pressure of the stem and remaining nearby leaves to be estimated from its balancing pressure.

Despite early recognition of the inability of the pressure chamber to measure xylem pressure under non-equilibrium conditions, a number of more recent studies have relied on balance pressures of previously transpiring leaves and shoots to infer the *in situ* xylem pressure (Lamhamedi *et al.*, 1992; Sperry and Pockman, 1993; Sun *et al.*, 1995; Kavanagh and Zaerr, 1997; Tognetti *et al.*, 1997). However, Tyree (1997) stated that by definition, when the equilibrium balance pressure is established, gradients in water potential between the xylem and the leaf evaporating surfaces that existed prior to excision have disappeared. The equilibrium xylem pressure, inferred with the pressure chamber on leaves that were transpiring, is thus likely to be more negative than that originally present at the time of excision.

Recently, Tyree and colleagues (Yang and Tyree, 1994; Tyree *et al.*, 1998) have estimated that leaf hydraulic resistance in some species is sufficient to generate differences in water potential of 2.0 MPa between the xylem and surrounding leaf tissue at midday transpiration rates. This implies that if a rapidly transpiring leaf of such a

plant is excised, the tension in the xylem will re-equilibrate to a value between that of the water potential of the bulk leaf tissue and the original *in situ* value of tension. Because typically less than 10% of the leaf water is contained in the xylem, the post-excision value of xylem tension deduced with the pressure chamber will approximate the water potential of the bulk leaf tissue rather than the pre-excision xylem tension. An exhaustive evaluation of the preceding hypothesis will require concurrent measurements of xylem pressures, leaf balancing pressures, transpiration rates, and leaf hydraulic properties.

Balancing pressures obtained from covered and exposed leaves of plants that have been slowly dehydrated over time tend to converge eventually (Turner and Long, 1980; Saliendra and Meinzer, 1989). This convergence of balancing pressures is associated with stomatal closure and the cessation of significant transpiration in exposed leaves and presumably with dissipation of transpiration-induced water potential gradients. Nevertheless, the balancing pressures of leaves experiencing this degree of dehydration may not necessarily reflect *in situ* xylem pressures. The increased proportion of gas-filled relative to water-filled spaces in severely dehydrated leaves may lead to substantial overpressures being required to re-establish hydraulic continuity and move water to the cut surface. Furthermore, much of the xylem water may already have cavitated, yet application of sufficient pressure will cause water to re-enter the xylem and appear at the cut surface.

The inability of the pressure chamber to measure the xylem pressure reliably in transpiring leaves could lead to errors in characterizing the driving forces for, and in analysing the mechanisms involved in long-distance water transport. Because widely-held beliefs about operating ranges of xylem pressure in plants are based in part on balancing pressures obtained with previously transpiring leaves, *in situ* xylem pressures should be re-evaluated, preferably with the xylem probe.

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