

# Diurnal variation in the radial reflection coefficient of intact maize roots determined with the xylem pressure probe

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## Abstract

Xylem pressure and its relative response to the imposition of an external osmotic stress (the so-called radial reflection coefficient) were recorded in roots of intact maize plants using the xylem pressure probe technique. Consecutive insertion of two probes into the same xylem vessel or into adjacent vessels of intact roots of plants exposed to high light intensity and salt stress under laboratory conditions showed that the xylem tension was not changed by vessel probing. It was also shown by using the double probe approach that the plants were capable of overcoming artificially induced leakages. This and other evidence reported in the literature convincingly demonstrated that the probe accurately reads xylem pressure and xylem pressure responses to osmotic stress. Additional experiments were performed on plants grown in a greenhouse at a subtropical latitude. Under these conditions the plants were exposed to strong diurnal fluctuations in light intensity, relative humidity and temperature. The results showed that the absolute xylem pressure in the roots of untreated plants decreased with increasing transpiration rate from positive values in the early morning to negative values around noon (average value  $-0.15$  MPa; maximum negative value  $-0.57$  MPa). As the day progressed and the transpiration rate decreased, xylem pressure increased again to positive values. Correspondingly, the radial reflection coefficient for NaCl increased from about zero in the early morning to about unity at noon when transpiration reached its highest value and decreased again to very low values towards the evening.

**The data raise questions concerning conclusions about the mechanism of water transport in intact roots drawn from the low radial reflection coefficients measured on excised roots using the root pressure probe.**

Key words: Xylem pressure probe, osmotic stress, reflection coefficient, transpiration, diurnal changes.

## Introduction

Root pressure probe measurements on excised plant roots have suggested (Steudle, 1989, 1992, 1993) that the radial reflection coefficients ( $\sigma_r$ ) of roots are very low (0.2–0.5). This means that the change in xylem pressure ( $\Delta P$ ) in response to external osmotic stress ( $\Delta\pi$ ) is much less than that expected for an ideal osmometer. Several models have been developed to explain this finding (Taura *et al.*, 1988; Katou and Taura, 1989; Steudle, 1992, 1993). However, the question that remains is whether these results and conclusions are in accord with what might be expected for the intact root. It is now becoming clear that stationary turgor and osmotic pressure gradients in the cortex collapse when transpiration is eliminated by root excision (Ryggol and Zimmermann, 1990; Zimmermann *et al.*, 1992; Ryggol *et al.*, 1993). This leads to unpredictable changes in the distribution of osmotically active solutes within the root tissue. Therefore, among other things, pronounced concentration polarization effects can be envisaged. Such effects may influence calculations of the flow-force pattern and of radial reflection coefficients from the osmotically induced hydrostatic pressure change ( $\Delta P$ ) when using the parameters of the surrounding bulk media (Nobel, 1983; Dainty, 1985).

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Furthermore, since the net water flow through the root is zero, an excised root represents (more or less) an osmometer. However, in the case of an intact plant, the root must be treated as a 'water transport device' (operating under steady-state conditions at constant transpiration rates). This means that the response of xylem pressure to osmotic stress may depend on the transpiration rate and the availability of water (i.e. the turgescence) from the living tissue cells near the xylem.

In fact, xylem probe measurements have recently shown (Schneider *et al.*, 1997) that the pressure response of the xylem to osmotic stress in intact roots of glycophytes depended strongly on the light intensity and thus on the transpiration rate. At light intensities greater than 400–700  $\mu\text{mol m}^{-2} \text{s}^{-1}$  intact roots of maize, wheat and barley exhibited  $\sigma_r$ -values of unity or even higher. In contrast, in the dark (when transpiration was reduced) the xylem pressure response was very small under comparable osmotic conditions (and corresponded to that measured in excised roots by means of the root pressure probe; Zhu *et al.*, 1995; Schneider *et al.*, 1997). The experiments mentioned above were performed under laboratory conditions at room temperature and the remaining question is, therefore, whether the larger diurnal changes in transpiration expected under greenhouse or field conditions are also reflected in changes of the radial reflection coefficients of intact roots. In this communication, this question was addressed by measuring the  $\sigma_r$ -values of intact maize roots for NaCl in relation to the diurnal changes in transpiration in a greenhouse near Honolulu, Hawaii. At this subtropical latitude, the plants were exposed to strong diurnal fluctuations in light intensity, relative humidity and temperature.

In agreement with the previous laboratory data, the results clearly showed that the pressure response of the root xylem to salt stress, and thus the radial reflection coefficients of the root, depended strongly on the time of day and the environmental conditions.

## Materials and methods

### Plant material

Seeds of maize (*Zea mays* cv. Buras and Green, respectively) were germinated for 2–4 d in the dark on moist filter paper. The seedlings were then transferred to aerated hydroculture solutions (Johnson's solution modified according to Rygol and Zimmermann, 1990; osmolality about 8 mosmol  $\text{kg}^{-1}$  = 0.02 MPa). The culture solutions were renewed every 3 d. For double-probe experiments, plants were cultivated under constant laboratory conditions (for further details, see Zhu *et al.*, 1995; Schneider *et al.*, 1997). For investigations on diurnal variations in xylem pressure, plants were grown in a glasshouse near Honolulu, Hawaii. The maximum light intensity in the glasshouse was about 1500  $\mu\text{mol m}^{-2} \text{s}^{-1}$  and the temperature and relative humidity varied between 21 °C and 41 °C and 31% and 100%, respectively. Plants 6–19-d-old were used in all experiments.

### Xylem pressure probe

The principle, calibration and possible pitfalls of the xylem pressure probe have been described in detail elsewhere (Balling *et al.*, 1988; Balling and Zimmermann, 1990; Zimmermann *et al.*, 1993a, b, 1994; Zhu *et al.*, 1995; see also below). Because of the large temperature variations in the greenhouse during the experiments, the accuracy of the probe was checked regularly. To this end, the microcapillary of the probe was inserted into nutrient solution prior to the measurements in order to test if the atmospheric pressure was read. Additionally, cavitations occurring during the experiments (see below) yielded correct probe readings corresponding to saturation water vapour pressure at the prevailing temperature.

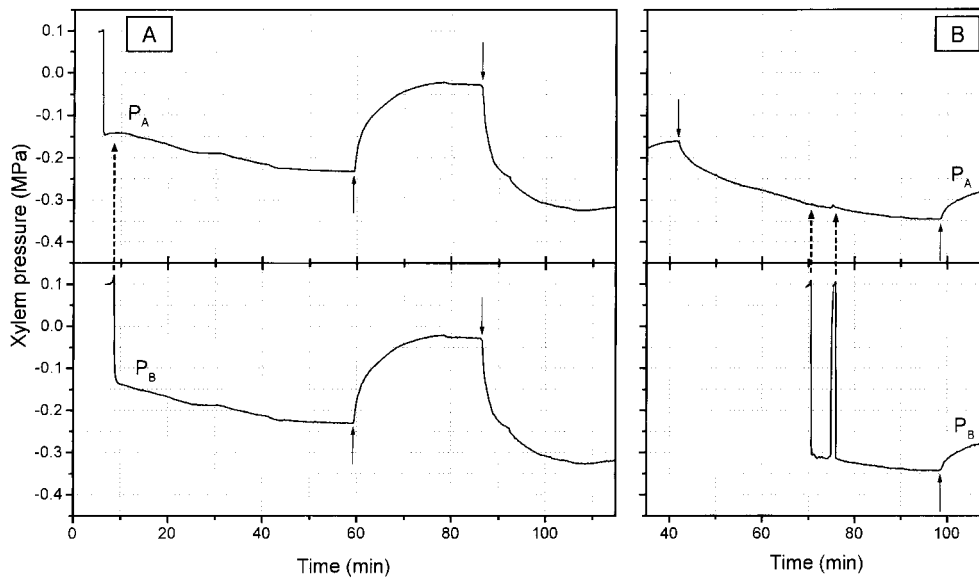
For insertion of the probe into a single xylem vessel, a primary seminal root of an intact maize plant was gently clamped into the plastic stage of the measuring chamber which was filled with nutrient solution. In order to avoid vibrations, the bathing solution was not aerated. Vessels were probed about 200 mm from the root tip. If not otherwise stated, the experiments were performed in the greenhouse. During the experiments, the changes in temperature, relative humidity and light intensity (see above) were recorded continuously by means of a datalogger (21X Micrologger, Campbell Scientific Inc., Logan, UT). The transpiration rate of the plants was determined by continuous weighing of pots on an electronic balance. Salt stress was induced by replacement of the nutrient medium by solutions containing different concentrations of NaCl. The osmolality of the solutions was determined cryoscopically (Osmomat 030, Gonotec, Berlin, Germany). The radial reflection coefficients ( $\sigma_r$ ) were calculated from the pressure change ( $\Delta P$ ) in the probed vessel in relation to the change in osmotic pressure of the bulk solution ( $\Delta\pi$ ).

## Results

### Evaluation of the method

The ability of the xylem pressure probe to measure negative pressure values in conducting vessels accurately has been tested in many model and plant experiments (Balling *et al.*, 1988; Balling and Zimmermann, 1990; Benkert *et al.*, 1991, 1995; Zimmermann *et al.*, 1993a, b, 1994, 1995a, b). Despite these many results, it has been argued (Holbrook *et al.*, 1995; Pockman *et al.*, 1995; Steudle, 1995; Sperry *et al.*, 1996; Milburn, 1996) that tension would be eliminated immediately upon piercing of the vessel by the capillary tip. Therefore, further evidence is presented on the ability of the probe to measure xylem pressure correctly in intact maize roots in order to support the conclusions drawn from the  $\sigma_r$ -data obtained under greenhouse conditions.

Figure 1A shows an experiment in which two probes ( $P_A$  and  $P_B$ , respectively) were inserted consecutively into two nearby vessels of the same intact maize root under laboratory conditions at a light intensity of about 600  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . Under these conditions, the average absolute xylem pressure of maize roots bathed in nutrient solution was (in agreement with previous data, see Schneider *et al.*, 1997) in the slightly negative range (data not shown). In order to establish more negative pressure



**Fig. 1.** Two experiments, showing responses to the consecutive insertion of two pressure probes ( $P_A$  and  $P_B$ , respectively) into adjacent xylem vessels of the roots of 2-week-old maize plants. The measurements were performed under laboratory conditions ( $T=22^\circ\text{C}$ , relative humidity 30%). (A) The roots were exposed to a  $50\text{ mol m}^{-3}$  NaCl solution at a light intensity of  $c. 600\ \mu\text{mol m}^{-2}\text{ s}^{-1}$  7 min prior to the insertion of the first probe ( $P_A$ ). The second probe ( $P_B$ ) was inserted about 3 min later (the instant of insertion is indicated by the dashed arrow). After about 60 min, the saline solution was replaced by nutrient solution (upwardly directed arrows). About 25 min later, the root was exposed to a  $100\text{ mol m}^{-3}$  NaCl solution (downwardly directed arrows). (B) Part of a similar experiment in which an absolute xylem pressure of about  $-0.3\text{ MPa}$  was established at a light intensity of about  $700\ \mu\text{mol m}^{-2}\text{ s}^{-1}$  after addition of  $25\text{ mol m}^{-3}$  NaCl (downwardly directed arrow) before the second probe ( $P_B$ ) was inserted (see first dashed arrow). After recording a nearly stable pressure value, the probe was gently removed from the vessel and subsequently reinserted a few microns away (second dashed arrow). Upwardly directed arrows: Replacement of the saline solution by nutrient medium. Note that in both experiments,  $P_A$  and  $P_B$  read nearly identical values of xylem pressure and responded identically to saline/nutrient regimes, even after generation of a small leak.

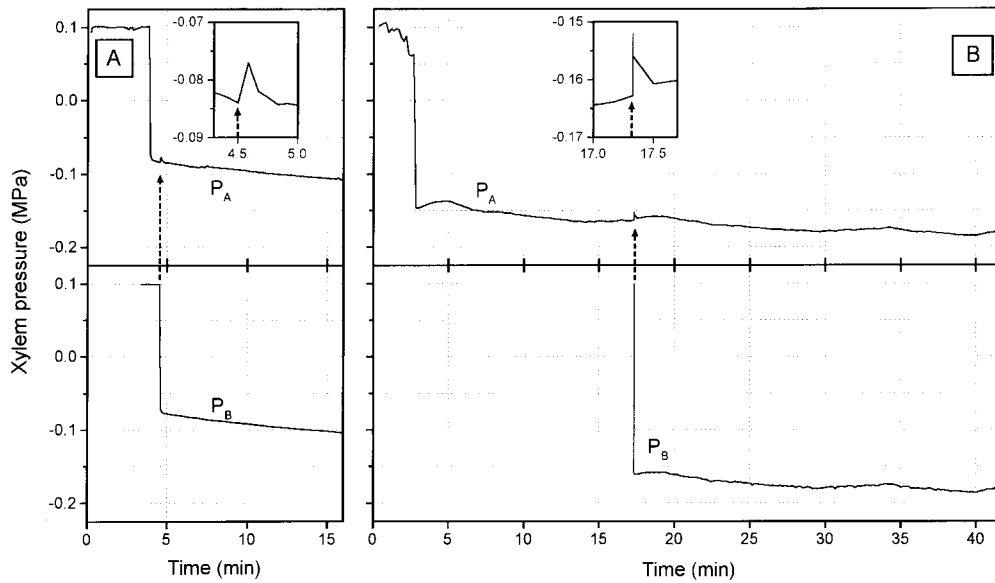
values, the root was exposed to  $50\text{ mol m}^{-3}$  NaCl (corresponding to an osmotic pressure of  $0.25\text{ MPa}$ ) 7 min prior to the insertion of the first probe. After insertion of the first probe ( $P_A$ ), an absolute xylem pressure of about  $-0.15\text{ MPa}$ <sup>1</sup> was recorded. About 3 min later the second probe ( $P_B$ ) was positioned in a vessel about 3 cm away from the first probe (the instant of insertion of  $P_B$  is marked by the dashed arrow). It is evident that the second probe ( $P_B$ ) read the same negative pressure value as the first one ( $P_A$ ), even when the saline medium was replaced by nutrient solution (upwardly directed solid arrows) and when the root was then re-subjected to saline solution ( $100\text{ mol m}^{-3}$ ; downwardly directed arrows). A similar experiment is shown in Fig. 1B. In the root of this plant, a xylem pressure of  $-0.33\text{ MPa}$  was recorded by the first probe ( $P_A$ ) after addition of appropriate concentrations of NaCl (downwardly directed solid arrow) at a light intensity of about  $700\ \mu\text{mol m}^{-2}\text{ s}^{-1}$ . Insertion of the second probe ( $P_B$ ; the instant of insertion is indicated by the first dashed arrow) about 0.5 cm away from the first insertion point after 70 min did not change the xylem pressure (Fig. 1B), even when  $P_B$  was gently removed after  $c. 4$  min and subsequently reinserted several microns away from the first insertion site (marked by the second

dashed arrow). Both probes read and responded identically to subsequent osmotically induced changes.

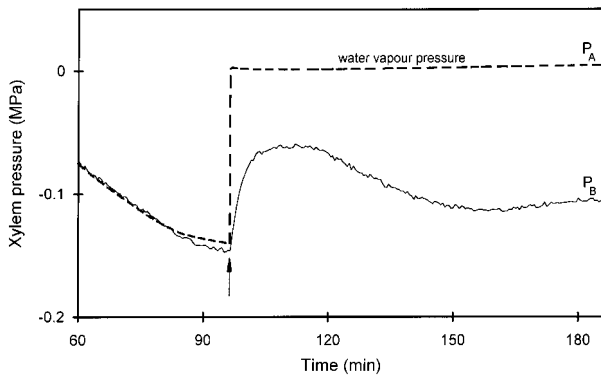
When the second probe was inserted into the same vessel as the first probe, a more or less pronounced 'pressure spike' was recorded by the latter (see insets in Fig. 2). Figure 2 shows typical experiments performed on roots bathed in  $50\text{ mol m}^{-3}$  NaCl solution at a relatively low light intensity (about  $10\ \mu\text{mol m}^{-2}\text{ s}^{-1}$ ; Fig. 2A) or in nutrient medium at a relatively high light intensity (about  $700\ \mu\text{mol m}^{-2}\text{ s}^{-1}$ ; Fig. 2B). It is evident that pressure changes induced during insertion are small and are dissipated very rapidly.

Cavitation induced artificially in one of two probes inserted into adjacent vessels or the same vessel immediately resulted in a xylem pressure increase as recorded by the other probe (Fig. 3). Nevertheless, the pressure in the non-cavitated probe remained in the negative range and gradually relaxed back to more negative values (Fig. 3). During this relaxation process, withdrawal of water from the cavitated probe into the penetrated xylem vessel was detected by observing the continuous extension of the gas bubble in its microcapillary. The extension rate of the bubble and the final xylem pressure depended on the degree of clogging of the tip of the cavitated probe. When

<sup>1</sup> Note that xylem pressure values are quoted as absolute pressures; atmospheric pressure =  $+0.1\text{ MPa}$ .



**Fig. 2.** An experiment similar to the one described in Fig. 1 except that the second probe ( $P_B$ ) was introduced into the same vessel as the first probe ( $P_A$ ), causing a small pressure spike to be recorded in  $P_A$  (insets). The measurements were performed in  $50 \text{ mol m}^{-3}$  NaCl solution at a relatively low light intensity (about  $10 \mu\text{mol m}^{-2} \text{ s}^{-1}$ ; A) and in nutrient solution at a relatively high light intensity (about  $700 \mu\text{mol m}^{-2} \text{ s}^{-1}$ ; B).



**Fig. 3.** Part of an experiment in which two adjacent vessels of the same root were probed under laboratory conditions ( $T = 22^\circ\text{C}$ , relative humidity 30%, light intensity  $c. 10 \mu\text{mol m}^{-2} \text{ s}^{-1}$ ). About 95 min after the insertion of the first probe ( $P_A$ ), cavitation occurred in this probe (arrow), indicated by an abrupt increase in the pressure in the cavitated probe to the pressure of water vapour. Note that the pressure read by the second probe ( $P_B$ ) temporarily increased, but remained in the negative range.

the probe was completely clogged, as indicated by a stationary water/gas interface, the final xylem pressures recorded were about as negative as those observed before cavitation (data not shown). Measurements of the radial reflection coefficients after cavitation in one of the probes demonstrated that the values were significantly lower than under non-cavitated conditions (Fig. 4). After removal of the cavitated probe, the original radial reflection coefficients could be read (Fig. 4).

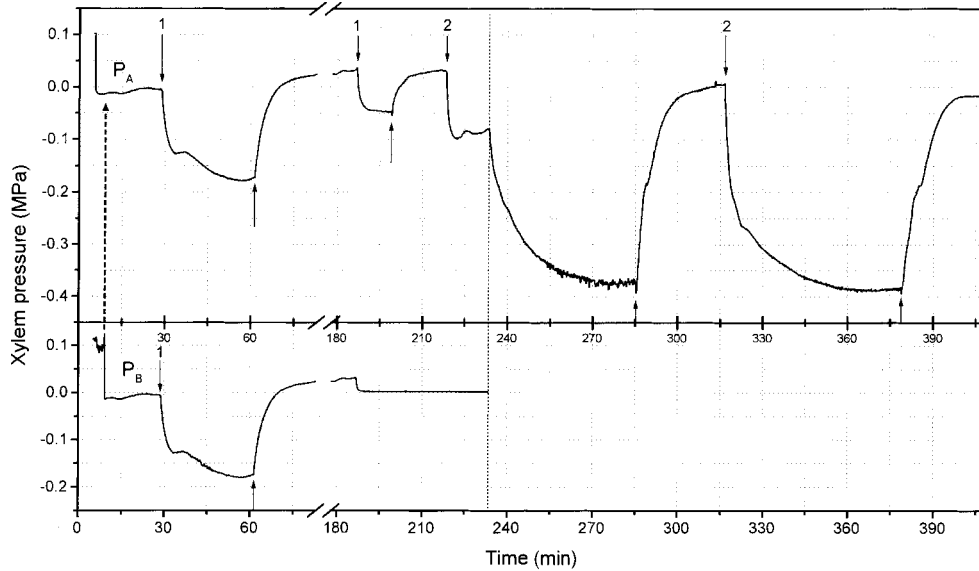
When the two probes were inserted into the same vessel, removal of one of them resulted in an increase in xylem pressure to positive, sub-atmospheric values as recorded by the remaining probe (Fig. 5). However,

tension developed again and the original value was re-established after some time ( $> 30$  min; data not shown). The recovery of negative xylem pressure values could be accelerated by a large increase in the light intensity ( $700 \mu\text{mol m}^{-2} \text{ s}^{-1}$ ) and/or by addition of salt (Fig. 5). The type of xylem pressure response described in Fig. 5 was observed only when the leakage was induced in the same vessel or when a rather large leakage was created by rapid removal of the second probe from an adjacent vessel. When the second probe was gently removed to create a very small leak, immediate reinsertion at a slightly different location showed that the pressure in the vessel remained unaltered (Fig. 1B).

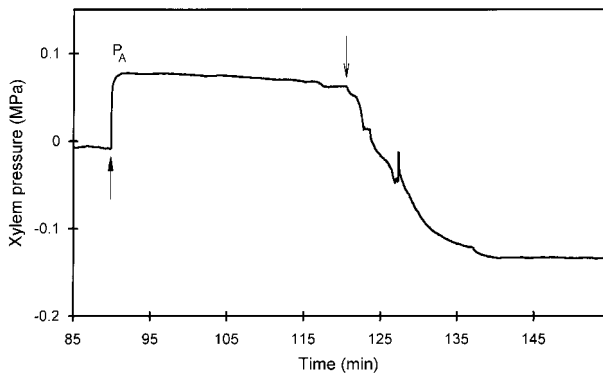
From the foregoing results it seems clear that the probe accurately read the pressure which existed in the vessel before penetration and that the plants were capable of overcoming the effects of local cavitations and leakages.

#### *Diurnal changes in xylem pressure in response to salt stress*

Long-term xylem pressure probe investigations on the roots of intact maize plants in the greenhouse revealed a characteristic diurnal pattern closely correlated with changes in the transpiration rate (Fig. 6A). During the night, the xylem pressure of roots bathed in nutrition medium (Fig. 6B, open symbols) was in the positive, sub-atmospheric range and often exceeded the atmospheric value before sunrise (Fig. 6B). Guttation was observed under these circumstances. After daybreak (around 06.00 h), transpiration increased (Fig. 6A) and the xylem pressure decreased accordingly (Fig. 6B). Around noon, when the transpiration rate reached a maximum (Fig. 6A), the xylem pressure attained average absolute



**Fig. 4.** Results of a double-probe insertion into two different xylem vessels of the same root at a light intensity of about  $700 \mu\text{mol m}^{-2} \text{s}^{-1}$  in the course of which radial reflection coefficients were determined before and after cavitation occurred in probe  $P_B$  (indicated by the constant water vapour pressure measured with this probe in contrast to the actual negative xylem pressure measured by means of  $P_A$ ). While the gas bubble continuously expanded within the cavitated probe, addition of NaCl solution (downwardly directed arrows: 1 =  $50 \text{ mol m}^{-3}$ , 2 =  $100 \text{ mol m}^{-3}$ ; the upwardly directed arrows indicate exchange of the salt solution with nutrition medium) resulted in very small xylem pressure drops corresponding to  $\sigma_r$ -values of *c.* 0.3 detected in the intact probe. When the probe capillary was completely emptied (dashed vertical line), xylem tension immediately increased in the intact probe and the  $\sigma_r$ -values determined were of the order of 0.9.

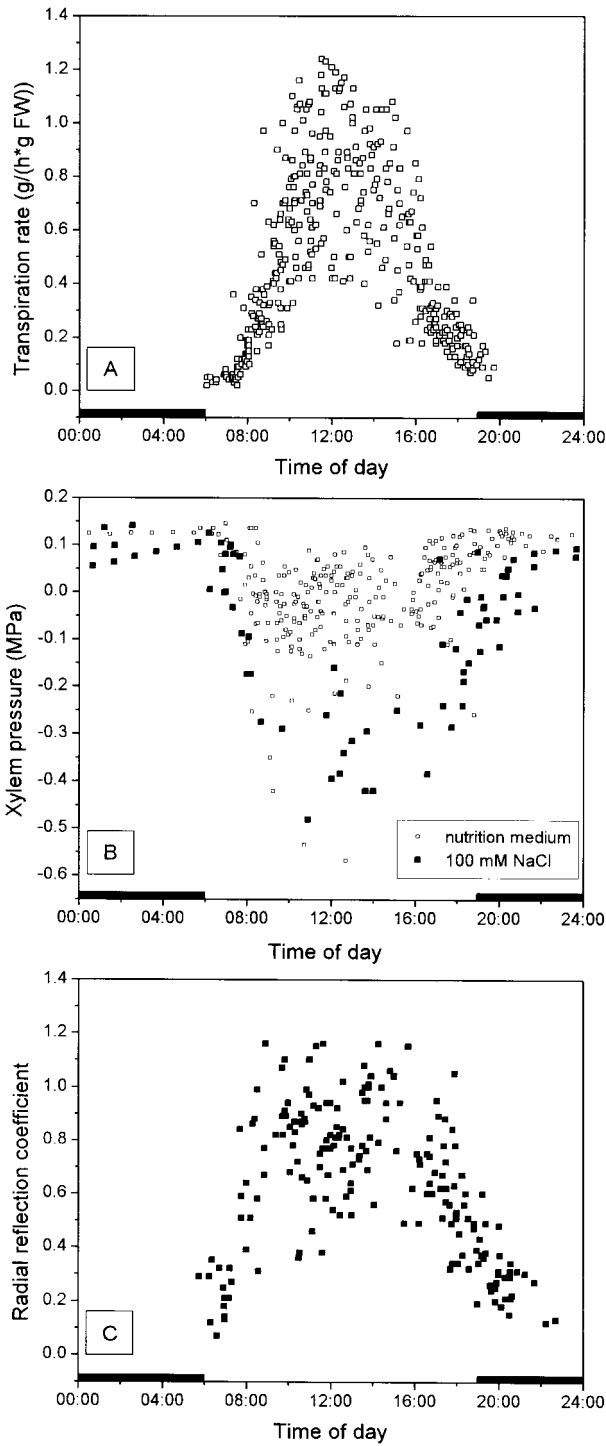


**Fig. 5.** Part of an experiment in which two xylem pressure probes had been inserted into the same root vessel. One of the probes ( $P_B$ ) was rapidly removed (upwardly directed arrow) after cavitation had occurred. The recording of the remaining probe ( $P_A$ ; experimental conditions: root bathed in nutrient solution, light intensity about  $700 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) shows that the xylem pressure increased to nearly +0.1 MPa. Addition of  $25 \text{ mol m}^{-3}$  salt (downwardly directed arrow) about 30 min later resulted in a decrease in the xylem pressure. The final value corresponded to that measured in the absence of a leakage.

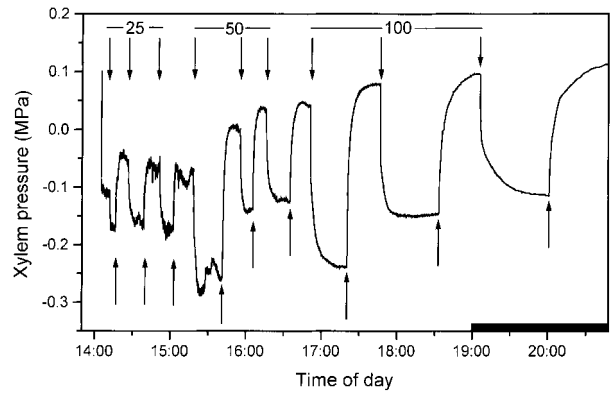
values between vacuum and  $-0.15 \text{ MPa}$ . Occasionally, when the plants were exposed to bright sunlight, pressures down to  $-0.57 \text{ MPa}$  were recorded (Fig. 6B). Under these conditions, cavitation occurred frequently. Cavitated vessels apparently refilled by around 14.00 to 15.00 h because negative pressure values could then be recorded again. Towards the late afternoon and evening the root xylem pressure increased again to positive, sub-

atmospheric values (Fig. 6B; the night began around 19.00 h).

Figure 7 shows a long-term xylem probe reading recorded in the intact root of a 2-week-old plant. In this particular experiment, a vessel was penetrated around 14.00 h. After establishment of a fairly constant pressure, the root was subjected to repeated exchanges of saline and nutrient solutions. As indicated in the figure, the salt concentration was increased from  $25 \text{ mol m}^{-3}$  to  $100 \text{ mol m}^{-3}$  during the course of the experiment. Addition of salt caused the xylem pressure to decrease to more negative values in agreement with laboratory experiments (Zhu *et al.*, 1995; Schneider *et al.*, 1997). At a given time of the day, the decrease in xylem pressure depended on the salt concentration (data not shown). After salt addition or replacement of the saline solution by nutrient solution, the pressure changed immediately. In many cases, a stable final pressure value could be recorded after up to 30 min. However, fluctuations (i.e. upward and downward regulations in the minute range) of the xylem pressure were also frequently observed (Fig. 7). These fluctuations were associated with large variations in the transpiration rate caused by rapidly alternating sunny and partly cloudy periods during the experiment. Changes in light intensity and temperature were thus reflected immediately in the xylem pressure (as already found previously under laboratory conditions). Repeated salt/nutrient solution exchanges did not change the xylem pressure response provided that the environ-



**Fig. 6.** Diurnal courses of (A) transpiration rates, (B) absolute xylem pressure values in the presence of nutrition medium (open squares) and after addition of  $100 \text{ mol m}^{-3}$  NaCl (filled squares), respectively, and (C) radial reflection coefficients, i.e. the ratio of the xylem pressure changes ( $\Delta P$ ) and the osmotic pressure changes ( $\Delta \pi$ ) upon addition of  $25 \text{ mol m}^{-3}$ ,  $50 \text{ mol m}^{-3}$  and  $100 \text{ mol m}^{-3}$  NaCl. Note that the diurnal changes in the response of xylem pressure to the addition of  $100 \text{ mol m}^{-3}$  NaCl (B) and of the radial reflection coefficients (C) closely followed the diurnal changes in transpiration rate (A). The black sections of the time axes represent the night period.



**Fig. 7.** A typical long-term recording of xylem pressure and xylem pressure changes in response to saline/nutrient solution exchanges in the root of a 2-week-old maize plant under greenhouse conditions. During the course of the experiments the NaCl concentration was increased from  $25 \text{ mol m}^{-3}$  to  $100 \text{ mol m}^{-3}$  NaCl as indicated (downwardly directed arrows: application of saline solution to the root, upwardly directed arrows: exchange of the saline solution with nutrition medium). Note that the small fluctuations with a time resolution of a few minutes in the final xylem pressure established after addition of saline solution or replacement by nutrient solution reflected rapid changes in temperature, light intensity and relative humidity due to the sunny/cloudy weather conditions between 14.00 and 16.00 h. The black section of the time axis represents the night period.

mental conditions were nearly constant (Fig. 7). This was also confirmed by vessel probing at various times of the day and subsequent single salt treatment of the roots which always resulted in pressure changes comparable to those recorded after long-term salt/nutrient solution regimes.

A plot of the absolute xylem pressure values in nutrition medium and the final xylem pressure values established after addition of  $100 \text{ mol m}^{-3}$  NaCl (osmotic pressure *c.* 0.5 MPa) is shown in Fig. 6B (open and filled squares, respectively). It is obvious that the absolute root xylem pressure values in the presence of saline solution depended strongly on the time of day. During the night, the xylem pressure did not respond to the addition of saline solution. After sunrise, the xylem tension increased continuously and the pressure change after addition of the salt solution became more pronounced. The xylem tension increase was closely coupled to the increase in transpiration rate (Fig. 6A). Around noon, when the transpiration rate reached a maximum value, xylem pressure values of  $-0.3$  to  $-0.5$  MPa were recorded on average in the presence of  $100 \text{ mol m}^{-3}$  NaCl (Fig. 6B). As the day progressed, the salt-induced xylem pressure changes decreased again until the tension reached very low values in the late evening and the pressure change upon salt addition became minimal.

Similar diurnal changes of the pressure response on the transpiration rate were obtained when  $25 \text{ mol m}^{-3}$  or  $50 \text{ mol m}^{-3}$  NaCl solutions were used (Fig. 7). A cumulative plot of the radial reflection coefficients (i.e. the ratio of the xylem pressure response to the change in the

external osmotic pressure) is given in Fig. 6C. Since the magnitude of the  $\sigma_r$ -values was independent of the salt concentration, the data were pooled. It is clear from this figure that the diurnal course of the  $\sigma_r$ -values closely followed that of the transpiration rates. The  $\sigma_r$ -values increased during the morning and reached or even slightly exceeded unity at around noon (on very sunny days) and decreased again to values of about 0.2 in the late evening.

## Discussion

The results presented here demonstrate that diurnal variation in the transpiration rate of maize plants leads to variation in the magnitude of the response of root xylem pressure to salt stress and, therefore, to large diurnal variation in the radial reflection coefficient. This important phenomenon is obviously obscured when excised roots are used because no transpirational water movement occurs. The results are consistent with xylem probe measurements on mangroves at low transpiration rates in which the xylem pressure did not respond to changes in the osmolality of the external medium over a wide range of salinity (Zimmermann *et al.*, 1994).

There is much less certainty about what cellular and/or tissue transport properties of the root lead to the dependence of the radial reflection coefficients on the transpiration rate. The increase of the apparent hydraulic conductivity of roots with the increase of the driving force reported by other authors (Nulsen and Thurtell, 1978; Weatherley, 1982) cannot explain the observed increase of the radial reflection coefficients with increasing transpiration rate, since the reflection coefficients depend inversely on the hydraulic conductivity. As discussed elsewhere (Schneider *et al.*, 1997), it is conceivable that concentration polarization effects in the root tissue due to unstirred layers are responsible for the observed phenomenon. Such effects decrease the actual osmotic concentration within the root, resulting in an apparently low  $\sigma_r$ -value since the value of the bulk solution is used for calculation (Dainty, 1985). Concentration polarization effects decrease with the 'stirring rate' of the compartment and should, therefore, be less significant when the transpirational flow increases<sup>2</sup>.

It is also conceivable that changes in xylem pressure induced by treatment with osmoticum were offset by compensatory changes in the hydrostatic component of xylem pressure associated with alterations in the transpiration rate. It is known (Cowan, 1977) that osmotic stress applied to transpiring plants can cause rapid changes in stomatal aperture and thus in the transpiration rate. However, it has to be noted that the 'fluctuations' (pres-

sure changes in the minute range) were more pronounced at low salinity than at high salinity.

Although the causes for the dependence of the radial reflection coefficient on transpiration are not known, the data presented here raise questions concerning conclusions about the mechanism of water transport in intact roots drawn by Steudle and many other authors from excised root experiments (Steudle, 1989, 1992, 1993). Steudle's hypothesis is based on the assumption that the radial reflection coefficients of intact roots are much less than unity. Clarkson's (1993) statement that the behaviour of excised root systems may not always be a reliable guide to that of an intact plant thus appears to be correct.

Nevertheless, the finding of transpiration-dependent  $\sigma_r$ -values will almost certainly reinitiate the debate about the validity of xylem pressure measurements using the probe technique. Further experiments (in addition to the published evidence cited above) have been presented here which clearly show that the probe accurately reads xylem pressure. Consecutive insertion of two probes into the same root has convincingly demonstrated (Figs 1–3) that the initial probe's xylem pressure reading is not changed by penetration of the xylem wall by the second probe (provided that the insertion was performed properly). Only in the case of large leakages or leakages induced in the impaled vessel did the xylem pressure increase to positive, but sub-atmospheric values. However, such leakages are readily detected and it has been shown that they can be easily 'repaired' if the tension is increased by salt stress or an increase in the transpiration rate. This agrees with frequent observations made in previous experiments that the likelihood of leakages induced by the insertion of the microcapillary into a xylem vessel is much less when the tension in the vessels is fairly high ( $>0.1$  MPa). The 'seal of the tissue cells' around the inserted probe apparently depends on the pressure difference between the xylem vessel and the environment. Cavitations occurring near the measuring point influenced—at least temporarily (see Figs 3, 4)—xylem pressure and salt-induced xylem pressure response, but it has been shown that recovery of the original xylem tension occurred after some time, depending on the water supply. The double probe experiments under laboratory conditions have shown (see above) that after cavitation, the xylem pressure response upon salt treatment was less than under non-cavitated conditions. Therefore, it is conceivable that the scatter of the  $\sigma_r$ -values at noon (Fig. 6C) was associated not only with changes in the transpiration rates (Fig. 6A) but also with cavitations in the vessels at this time of the day. If this was true, measurements of  $\sigma_r$ -values would be an elegant way to record cavitation. This interesting conclusion deserves more attention in future work.

<sup>2</sup> Concentration polarization effects increase with increasing volume flow. However, they decrease if the thickness of the unstirred layer decreases (see for details Barry and Diamond, 1984). If the latter effect overcompensates the first one under our experimental conditions, a decrease of concentration polarization effects with increasing transpiration can be expected.

The finding that the xylem pressure of roots bathed in nutrient solution changed with the transpiration rate as expected and responded reproducibly to different saline/nutrient solution regimes (Fig. 7) is also a convincing demonstration that the probe was measuring the pressure correctly and that very large negative pressures did not develop in the root xylem of maize plants. The most negative (stable) values which could be recorded with the probe were about  $-0.57$  MPa around noon when transpiration was at a maximum. This value agrees well with probe measurements in the xylem of other higher plants (Zimmermann *et al.*, 1993a, b, 1995b; Benkert *et al.*, 1995), but is not consistent with the values obtained by means of the indirect methods (e.g. the pressure chamber of Scholander *et al.*, 1965, or the centrifugation methods of Holbrook *et al.*, 1995 and Sperry *et al.*, 1996) whose physical basis is uncertain (Zimmermann *et al.*, 1993a, b; forthcoming paper).

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### References

- 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**, 325–38.
- Balling A, Zimmermann U, Büchner K-H, Lange OL.** 1988. Direct measurement of negative pressure in artificial-biological systems. *Naturwissenschaften* **75**, 409–11.
- Barry PH, Diamond JM.** 1984. Effects of unstirred layers on membrane phenomena. *Physiological Reviews* **64**, 763–872.
- Benkert R, Balling A, Zimmermann U.** 1991. Direct measurements of the pressure and flow in the xylem vessels of *Nicotiana tabacum* and their dependence on flow resistance and transpiration rate. *Botanica Acta* **104**, 423–32.
- Benkert R, Zhu J-J, Zimmermann G, Türk R, Bentrup F-W, Zimmermann U.** 1995. Long-term xylem pressure measurements in the liana *Tetrastigma voimierianum* by means of the xylem pressure probe. *Planta* **196**, 804–13.
- Clarkson DT.** 1993. Roots and the delivery of solutes to the xylem. *Philosophical Transactions of the Royal Society of London Series B* **341**, 5–17.
- Cowan IR.** 1977. Stomatal behavior and environment. *Advances in Botanical Research* **4**, 117–228.
- Dainty J.** 1985. Water transport through the root. *Acta Horticulturae* **171**, 21–31.
- Holbrook NM, Burns MJ, Field CB.** 1995. Negative xylem pressures in plants: a test of the balancing pressure technique. *Science* **270**, 1193–4.
- Katou K, Taura T.** 1989. Mechanism of pressure-induced water flow across plant roots. *Protoplasma* **150**, 124–30.
- Milburn JA.** 1996. Sap ascent in vascular plants: challengers to the Cohesion theory ignore the significance of immature xylem and the recycling of Münch water. *Annals of Botany* **78**, 399–407.
- Nobel PS.** 1983. *Biophysical plant physiology and ecology*. New York: WH Freeman and Company.
- Nulsen RA, Thurtell GW.** 1978. Osmotically induced changes in the pressure-flow relationship of maize root systems. *Australian Journal of Plant Physiology* **5**, 469–76.
- Pockman WT, Sperry JS, O'Leary JW.** 1995. Sustained and significant negative water pressure in xylem. *Nature* **378**, 715–16.
- Rygol J, Pritchard J, Zhu JJ, Tomos AD, Zimmermann U.** 1993. Transpiration induces radial turgor pressure gradients in wheat and maize roots. *Plant Physiology* **103**, 493–500.
- Rygol J, Zimmermann U.** 1990. Radial and axial turgor pressure measurements in individual root cells of *Mesembryanthemum crystallinum* grown under various saline conditions. *Plant, Cell and Environment* **13**, 15–26.
- Schneider H, Zhu JJ, Zimmermann U.** 1997. Xylem and cell turgor pressure probe measurements in intact roots of glycophytes: transpiration induces a change in the radial and cellular reflection coefficients. *Plant, Cell and Environment* **20**, 221–9.
- Scholander P, Hammel HT, Bradstreet ED, Hemmingen EA.** 1965. Sap pressures in vascular plants. *Science* **148**, 339–46.
- Sperry JS, Saliendra NZ, Pockman WT, Cochard H, Cruiziat P, Davis SD, Ewers FW, Tyree MT.** 1996. New evidence for large negative xylem pressures and their measurement by the pressure chamber method. *Plant, Cell and Environment* **19**, 427–36.
- Steudle E.** 1989. Water flow in plants and its coupling to other processes; an overview. *Methods in Enzymology* **174**, 183–225.
- Steudle E.** 1992. The biophysics of plant water: compartmentation, coupling with metabolic processes, and flow of water in plant roots. In: Somero GN, Osmond CB, Bolis CL, eds. *Water and life: comparative analysis of water relationships at the organismic, cellular, and molecular levels*. Heidelberg: Springer-Verlag, 173–204.
- Steudle E.** 1993. Pressure probe techniques: basic principles and application to studies of water and solute relations at the cell, tissue and organ level. In: Smith JAC, Griffiths H, eds. *Water deficits: plant responses from cell to community*. Oxford: Bios Scientific Publishers, 5–36.
- Steudle E.** 1995. Trees under tension. *Nature* **378**, 663–4.
- Taura T, Iwaikawa Y, Furomoto M, Katou K.** 1988. A model for radial water transport across plant roots. *Protoplasma* **144**, 170–9.
- Weatherley PE.** 1982. Water uptake and flow in roots. In: Lange OL, Nobel PS, Osmond CB, Ziegler H, eds. *Encyclopedia of plant physiology*, Vol. 12B. Berlin, Heidelberg, New York: Springer-Verlag, 79–109.
- Zhu JJ, Zimmermann U, Thürmer F, Haase A.** 1995. Xylem pressure response in maize roots subjected to osmotic stress: determination of radial reflection coefficients by use of the xylem pressure probe. *Plant, Cell and Environment* **18**, 906–12.
- Zimmermann G, Zhu JJ, Benkert R, Schneider H, Thürmer F, Zimmermann U.** 1995a. Xylem pressure measurements in intact laboratory plants and excised organs: a critical evaluation of methods in the literature and the xylem pressure probe. In: Terazawa M, McLeod CA, Tamia Y, eds. *Tree sap*. Hokkaido University Press, 59–70.
- Zimmermann U, Benkert R, Schneider H, Rygol J, Zhu JJ, Zimmermann G.** 1993a. Xylem pressure and transport in higher plants and tall trees. In: Smith JAC, Griffiths H, eds. *Water deficits: plant responses from cell to community*. Oxford: Bios Scientific Publishers, 87–108.
- Zimmermann U, Haase A, Langbein D, Meinzer F.** 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 Series B* **341**, 19–31.
- Zimmermann U, Meinzer FC, Bentrup F-W.** 1995b. How does water ascend in tall trees and other vascular plants? *Annals of Botany* **76**, 545–51.
- Zimmermann U, Rygol J, Balling A, Klöck G, Metzler A, Haase A.** 1992. Radial turgor and osmotic pressure profiles in intact and excised roots of *Aster tripolium*. Pressure probe measurements and nuclear magnetic resonance-imaging analysis. *Plant Physiology* **99**, 186–96.
- Zimmermann U, Zhu JJ, Meinzer FC, Goldstein G, Schneider H, Zimmermann G, Benkert R, Thürmer F, Melcher P, Webb D, Haase A.** 1994. High molecular weight organic compounds in the xylem sap of mangroves: implications for long-distance water transport. *Botanica Acta* **107**, 218–29.