Root hydraulic conductivity measured by pressure clamp is substantially affected by internal unstirred layers

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Abstract
Using the root pressure probe in the pressure clamping (PC) mode, the impact of internal unstirred layers (USLs) was quantified for young corn roots, both in experiments and in computer simulations applying the convection/diffusion model of Knipfer et al. In the experiments, water flows (J_Vr) during PC were analysed in great detail, showing that J_Vr (and the apparent root hydraulic conductivity) were high during early stages of PC and declined rapidly during the first 80 s of clamping to a steady-state value of 40–30% of the original. The comparison of experimental results with simulations showed that, during PC, internal USLs at the inner surface of the endodermis substantially modify the overall force driving the water. As a consequence, J_Vr, and Lp_r were inhibited. Effects of internal USLs were minimized when using the pressure relaxation mode, when internal USLs had not yet developed. Additional stop-clamp experiments and experiments where the endodermis was punctured to reduce the effect of internal USLs verified the existence of internal USLs during PC. Data indicated that the role of pressure propagation along the root xylem for both PC and pressure relaxation modes should be small, as should the effects of filling of the capacities during root pressure probe experiments, which are discussed as an alternative model. The results supported the idea that concentration polarization effects at the endodermis (internal USLs) cause a serious problem whenever relatively large amounts of water (xylem sap) are radially moved across the root, such as during PC or when using the high-pressure flow meter technique.

Key words: Convection/diffusion model, high-pressure flowmeter (HPFM), pressure clamp, pressure propagation, pressure relaxations, root pressure probe, simulations, storage capacity, unstirred layers (USLs), Zea mays L.

Introduction
The water balance of higher plants is provided by the difference between the water uptake across roots and the loss by stomata during transpiration. For the movement of water in the soil/plant/air continuum, the root hydraulic conductivity (Lp_r) is a key parameter contributing to the limitation of the rate of water flow (Steudle et al., 1987). Next to stomata, the water status of the shoot will be largely determined by Lp_r. Based on root anatomy, the composite transport model best describes the flow of water across roots (Steudle and Peterson, 1998). Usually, the endodermis with its Caspian bands and suberin lamellae represents the most critical boundary for the radial transport of water and solutes, and there is experimental evidence that the endodermis acts as an osmotic barrier (Steudle et al., 1993). Due to the osmotic properties of the endodermis, it is assumed that the radial transport of water across the root should result in solute accumulation at the stelar side of the endodermis, due to sweep-away effects (Dainty, 1963; Knipfer et al., 2007).

In the past, only a few techniques have been used to measure root hydraulics, such as the root-pressure chamber, the root pressure probe (RPP), and the high-pressure flowmeter (HPFM; Fiscus, 1975, 1977; Steudle, 1993; Tyree et al., 1995). Using the RPP, detailed measurements have been performed, which have been combined with cell pressure probe experiments in order to quantify the amounts of water moving along different pathways (Steudle and Jeschke, 1983; Steudle and Freensch, 1989; Zhu and Steudle, 1991; Steudle et al., 1993; Ye and Steudle, 2006). Usually with the RPP, pressure relaxations...
(PRs) are performed in that a certain amount of water is injected into the xylem of an excised root which then moves out radially. As an alternative to the PR method, pressure clamping (PC) techniques have been used. When PC techniques were applied to excised roots, constant step changes of root pressure were applied and responses in water fluxes measured with the time period of clamping (Magnani et al., 1996; Murphy, 1999; Bramley, 2006; Bramley et al., 2007). During high pressure-flow measurements, the PC technique was modified by applying a ramp of pressure tendency to move much larger amounts of water across roots in a direction opposite to that during transpiration (Tyree et al., 1994, 1995). This should increase the osmotic concentration in the root xylem and stelar apoplast, and induce the build-up of concentration gradients (unstirred layers, USLs) at the inner surface of the endodermis (concentration polarization; Knipfer et al., 2007). The build-up of internal USLs should tend to underestimate the real \( L_p \) due to an overestimation of the force driving the water. To date, this was neglected in the literature of root PC and HPFM techniques. To quantify the role of internal USLs, Knipfer et al. (2007) applied a convection/diffusion (C/D) model to young roots of corn. Both experiments and simulations provided consistent evidence that, during PC, effects of internal USLs may be dominating and can only be minimized using a technique where initial water flows are measured, such as during PRs. The data indicated that some caution is required when comparing the \( L_p \) of entire roots measured by PC or HPFM techniques with those obtained from the cell level, in order to work out the contribution of aquaporins to the overall water flow across roots (Henzler et al., 1999; Javot and Maurel, 2002; Wan et al., 2004; Lee et al., 2005).

In the recent experiments of Knipfer et al. (2007), roots were subjected to PC of different durations, and rates of subsequent PRs analysed. As theoretically expected, there was a substantial increase in half time \( (T_{1/2}) \) due to the build-up of internal USLs, i.e. root \( L_p \), decreased \( (L_p \propto 1/T_{1/2}) \). In other ‘stop-clamp’ experiments, the existence of USLs could be verified when the step change of pressure applied during PC techniques was taken back to the original root pressure right after the clamp. This resulted in a transient increase of root pressure due to the osmotic gradient established at the endodermis, as predicted by the C/D model. During PRs, effects of internal USLs resulted in splitting up into two phases, which had already been observed in the literature (e.g. Steudle and Frensch, 1989; Hose et al., 2000; Ye and Steudle, 2006). Hose et al. (2000) state different possible reasons for the occurrence of two different phases during PRs obtained with young corn roots, which could be affected by a treatment with abscisic acid (CABA). One reason could be that ABA enhanced the role of the cell-to-cell as compared with the apoplastic water transport. However, there should also be effects of ‘concentration polarization at the osmotic barriers in the root (endodermis)’. During RPP experiments with corn roots, the first fast component was usually referred to the radial transfer of water across the root and the second slow component to the build-up of internal USLs. Hence, when the short first phase was used, the effect of such USLs could be minimized.

In contrast to the results of Knipfer et al. (2007), Bramley (2006) and Bramley et al. (2007) recently claimed that osmotic effects during PC can be neglected. During their experiments with RPPs, they used both PR and PC modes. In some experiments, end segments of roots were cut open at both ends and connected between two RPPs to see how changes in pressure applied on one side would propagate across the root segments. For two reasons, these authors concluded that PC rather than PR should be used to measure the correct \( L_p \). (i) When using RPPs in either the PR or PC mode, there should be a ‘propagation of pressure’ along the elastic tubes of xylem tending to result in a pressure gradient from the basal to the apical part of the root. (ii) There should be substantial ‘water capacities’ in the stele and cortex, which are filled in the short term with water and contribute to the overall \( T_{1/2} \) of radial water flow measured during PRs. In their leaky-elastic pipe model of the root xylem, Bramley (2006) and Bramley et al. (2007) did not consider osmotic changes within the root during either PC or PR modes. They claim that they could exclude that internal USLs played a significant role during their experiments.

The main purpose of the present paper is a detailed experimental analysis of water flows during PC in roots. Different from PC with cells (Wendler and Zimmermann, 1982), this type of analysis has never been carried out before, although steady-state techniques with roots have been in use for some time (for references, see above). Different from cells, PC (or HPFM) techniques are based on the injection of pure water into basal ends of roots, which leaves by radial outflow. Hence, there should be no effect of changes in the absolute amount of solutes in the xylem or stele, but there could be transient displacements of solutes within the root, i.e. a build-up of internal USLs. This effect should increase as water flows increase and the mobility of solutes within the root cylinder decreases (C/D model of root; Knipfer et al., 2007). Experimental data are provided in this paper indicating that the model may apply. Alternatives are discussed. The recent paper by Knipfer et al. (2007) focused on the role of internal USLs during PRs. Here, evidence is provided on the contribution of the effects of internal USLs on measured \( L_p \) during PC at different step changes of applied pressures and at different durations of clamps. The present results indicated that effects of USLs can be substantial and even dominating. Time constants of changes in water flows as
measured during PC agreed with those expected for the build-up of internal osmotic gradients. They were much longer than those of PRs. Experimental results were compared with results derived from the C/D model of Knipfer et al. (2007). However, independent of the model used to interpret transient changes of water flow across roots during PC, the experimental results of the present paper have to be taken into account when analysing results from PC or HPFM techniques. The present data indicate that the recent alternative interpretation in terms of the leaky-elastic pipe model should be treated with some caution. It is concluded that, in order to minimize effects of internal USLs, PR rather than PC techniques should be used to measure root $L_p$.

**Materials and methods**

**Plant material**

Maize seeds (Zea mays L. cv. Helix; Kleinwanzlebener Saatzucht AG, Kleinwanzleben, Germany) were germinated for 3–4 d in the dark on wetted filter paper soaked with 0.1 mM CaCl$_2$. When seedlings had a root length of 20–30 mm, they were transferred to hydroponics containing a nutrient solution of (in mM) KH$_2$PO$_4$ (1.5), KNO$_3$ (2.0), CaCl$_2$ (1.0), MgSO$_4$ (1.0), and (in μM) FeNaEDTA (18.0), H$_3$BO$_3$ (8.1), MnCl$_2$ (1.5). Further growth was maintained in a growth chamber at a day/night rhythm of 14 h/10 h at 20 °C/17 °C. After 2–6 d in the medium, plants were used for RPP experiments. Including the time for germination, the overall age of roots used was 5–11 d. Depending on age, excised end segments attached to the RPP (see below) had a length of 98–215 mm and a diameter of 0.85–1.3 mm.

**RPP experiments: PR, PC, and stop-clamp**

Excised root segments were tightly connected to the RPP by silicone seals prepared from silicone material (Xantopren plus, Bayer, Leverkusen, Germany). To minimize the thickness of external USLs, the nutrient solution used for growth was circulated around the fixed roots (Ye and Steudle, 2006). Steady root pressures ($P_{ro}$) were usually obtained after 1–2 h. After each experiment, the proper function of the seal was checked by cutting the roots close to the seal. This should have resulted in a rapid decline in $P_{ro}$, and in monophasic PRs with at least 5-fold shorter half times than before the cut. If this did not occur, experiments with that root were discarded. The elasticity ($β$) of the measuring device ($ΔP_δ$/Δ$V_δ$) was determined by moving the metal rod in the RPP instantaneously and recording the change in root pressure ($ΔP_r$). The change in volume of the measuring system ($ΔV_δ$) was calculated from the shift of the oil–water meniscus within the capillary (diameter: 280 μm or 360 μm) as observed with a stereo-microscope. The elasticity coefficient ranged from 1 to 2×10$^7$ MPa m$^{-3}$. The root hydraulic conductivity ($L_p$) was evaluated from half times ($T_{1/2}$) of PRs, according to equation 1 (e.g. Steudle and Jeschke, 1983; Frensch and Steudle, 1989; Azaizeh and Steudle, 1991; Steudle et al., 1993):

$$k_t = \frac{\ln(2)}{T_{1/2}} = \frac{ΔP_{ro}}{ΔV_{δ}} \cdot L_p \quad (1)$$

where the effective root surface area ($A_r$) for water transport was estimated from the length and diameter of the root (considered as a cylinder) and corrected for the immature xylem region, which was 20 mm from the tip (Steudle et al., 1993). The region of immature xylem was identified in sectioning experiments using the technique of Frensch and Steudle (1989). The rate constant ($k_t$) was obtained from the first rapid phase of the biphasic PRs, where the contribution of USLs is minimized (Knipfer et al., 2007). During PC experiments, water flows were induced by moving the meniscus rapidly either forward (exosmotic water flow) or backward to reverse the flow (endosmotic water flow) by rapid step changes of pressure of around 0.05 MPa. During each PR, the position of the meniscus was kept constant.

During exosmotic PC, the steady-state root pressure was increased by a step change of $ΔP_{ro,0}$=0.05 MPa, and was then kept constant, in some experiments up to 195 s. Step changes of around 0.05 MPa had to be used to resolve changes in the kinetics of water flow adequately (see below). Effects of concentration polarization at the endodermis during pressure clamping were investigated by measuring half times of PRs just following clamps ($T_{1/2}$). According to Knipfer et al. (2007), PR and PC experiments differed in the amount of displacement of solutes within the stele resulting in USL effects at the endodermis.

To demonstrate the build-up of internal USLs at the endodermis during PC, exosmotic stop-clamp experiments were performed (Knipfer et al., 2007). For stop-clamps, the time period of clamping was 60 s, and step changes of pressure ($ΔP_{ro}$) were either $ΔP_{ro}=0.01$ MPa or $ΔP_{ro}=0.05$ MPa. At the end of clamping periods, root pressure was instantaneously taken back to the original steady $P_{ro}$ by turning the metal rod of the RPP rapidly in the reverse direction. At this point, the meniscus in the capillary of the RPP was kept constant, and the response in pressure with time was observed [$P_{r}(t)$]. According to theory, transients of $P_{r}(t)$ were due to a water influx just following an osmotic gradient. These transients in $P_{r}(t)$ should always occur, even when there was a significant drop in pressure along the root xylem causing a decrease in radial water flow from the base to the tip, and a reduction in the concentration polarization effect along the root. However, small effects of USLs at the endodermis should result in small transient changes of $P_{r}(t)$. Large transients should be observed when sweep-away effects of solutes to the endodermis were more pronounced, i.e. at prolonged times of clamping or higher increments of clamped pressure.

**Puncturing of the endodermis**

To disturb the build-up of USLs during PC, the endodermis of roots was punctured with the tip of a microcapillary (diameter: 50 μm) at various distances from the root tip. Holes in the endoderm were made after the tip of the microcapillary was carefully driven radially into the root and then withdrawn, as described in detail by Steudle et al. (1993). After puncturing, there was a drop in root pressure to a new $P_{ro}$, which was an indication of the hole in the endoderm. A new steady $P_{ro}$ was attained, when the loss of solutes through the hole was compensated by the active uptake of solutes (pump-leak model; see equation 6 of Steudle et al., 1993). At the steady $P_{ro}$, effects of solute losses on internal concentration polarization effects were investigated by measuring half times of (i) exosmotic PRs at the endodermis during pressure clamping were investigated by measuring half times of PRs just following clamps ($T_{1/2}$). Measurements were first done on the intact root and repeated after puncturing, when the declining root pressure reached the new $P_{ro}$ (Steudle et al., 1993). In some experiments the endodermis was punctured twice.

**Kinetics of water flow ($\dot{V}_w$) during PC**

To check for the build-up of internal USLs during PC with the RPP and how this would affect the apparent $L_p$, the rate of water flow
was determined during PC lasting 180–195 s (ΔPc ~0.065 MPa). Changes in water volume during PC were measured by following the movement of the meniscus within the glass capillary of the probe with time using a stereo-microscope. Due to the limited resolution of measuring changes of water flow with sufficient accuracy, ΔPc had to be taken as ~0.05 MPa (diameter of measuring capillary of 280 μm or 360 μm). When the meniscus passed a specific AV (6.2, 5.1, or 3.1×10⁻⁷ m² depending on the experimental set-up) on the ocular scale, a signal was set on the measuring protocol of the recording computer to calculate the corresponding Δt. The corresponding volume flow (Jv(t)) during the clamp was calculated by

\[ J_v(t) = (1/A_r) \cdot (\Delta V/\Delta t) \]  

(2)

Due to an initial exponential decline in measured Jv with the time period of clamping, where values ended up in a steady state, data points were plotted over the middle of time intervals, Δt. An exponential equation was fitted to calculate Jv(t) at any time step during the clamping period, i.e.

\[ J_v(t) = J_{v_0} + \Delta J_v \cdot e^{(-k_1 \cdot t)} \]  

(3)

According to equation 3, Jv referred to the steady flow of water obtained after sufficient time of clamping, and the exponential term characterized initial phases, which were presumably due to the build-up of internal USLs or other processes (see Discussion). The rate constant k1 was then a measure of the exponential decline of water flow, which was initially rapid and then approached a steady value. When simulated and experimental water flows were compared, the experimental Jv(t) was corrected for the surface area of the endodermis \[ J_{v_0}(t) = J_{v_0}(t) \times R/R_{e} \], where R is the radius of the whole root and Re is the radius of the endodermis; see equation 10 of Knipfer et al. (2007)]. Due to the fact that water flows are usually referred to unit root surface area to obtain \( L_p \), the water flow density \( L_{p_{0}}^{ac} \) across the endodermis was larger by the ratio of \( R/R_{e} \). According to Jv(t) obtained from equation 3, an apparent root hydraulic conductivity from PC \( (L_{p_{0}}^{PC}) \), could be determined at any time period of clamping by:

\[ L_{p_{0}}^{PC}(t) = [J_{v_0}(t)]/(\Delta P_r) \]  

(4)

It should be noted that the apparent \( L_{p_{0}}^{PC} \) was not corrected for the build-up of USL at the endodermis, i.e. concentration polarization effects at the endodermis were not taken into account, which should have tended to reduce the water flow. Changes of water volume V(t) measured within the capillary at any time period of clamping were obtained by integration of equation 3:

\[ V(t) = A_r \cdot J_{v_0} \cdot t + \frac{1}{k_1} \cdot A_r \cdot J_{v_0} \cdot \left(1 - e^{(-k_1 \cdot t)}\right) \]  

(5)

When substituting \( [A_r, J_{v_0}] = a \), and \( [(1/k_1) \cdot A_r \cdot J_{v_0}] = b \), the generalized equation 6 was fitted to the measured Δv(t) curve, i.e.

\[ V(t) = a \cdot t + b \cdot \left(1 - e^{(-k_1 \cdot t)}\right) \]  

(6)

Equation 6 consists of an exponential component to describe V(t) during earlier stages of clamping and a linear component for the steady state of V(t).

**Simulations of concentration profiles with the C/D model**

Using the C/D model of Knipfer et al. (2007), concentration profiles between xylem and endodermis were simulated during PC. The C/D model simulated effects of radial transport of water across the stele on the distribution of solutes during PC and PRs. Simulations were performed for corn (authors’ own experiments) and wheat roots (data taken from Bramley, 2006). The distances (d) of simulated transport between mature xylem vessels and the inner surface of the endodermis were estimated from cross-sections \( [[d=0.5\mu m\ for\ corn,\ and\ d=30\mu m\ for\ wheat,\ the\ latter\ value\ taken\ from\ Bramley\ (2006)]]\). For corn, \( d=60\mu m \) represented the physical distance between endodermis and mature early metaxylem vessels, which was estimated from cross-sections. In wheat, according to fig. 4.8b of Bramley (2006), \( d=30\mu m \) represented the distance between endodermis and the mature central late metaxylem. However, when tortuosity effects of solute and water flow through the stelar apoplast were considered in the C/D model, \( d \) could be substantially larger and USL effects much more pronounced. This means that, using the physical rather than the effective path length tended to underestimate C/D effects. For young corn roots, the tortuosity factor in the stelar apoplast has been estimated to be about 2 (Jarvis and House, 1969).

To restrict water flow to the apoplastic space available for water and solute flow for both species, a constriction factor of \( \phi=0.05 \) was introduced for the stelar tissue (Knipfer et al., 2007), which was estimated from cross-sections of corn (Steudle and Peterson, 1998). Again, this factor would tend to underestimate rather than overestimate the effects. This is so because the cross-sectional area of pores available for transport in the apoplast should be smaller than the geometric. In the literature, factors of \( \phi \) of between 0.01 and 0.025 (1% and 2.5%) have been used to quantify the area available for apoplastic transport (as a fraction of total cross-sectional area including both the cell-to-cell and the apoplastic path, e.g. Tyree, 1969, 2003; Molz and Ikenberry, 1974). The C/D model simplified convection versus diffusion processes in the stele, assuming that the mature vessels of early metaxylem were forming a rigid ring (EMX ring). For the sake of simplicity, the endodermis was assumed to have a reflection coefficient of \( \sigma_{end}=1 \). In view of earlier results (e.g. Steudle and Frensch, 1989; Steudle and Peterson, 1998), the assumption may be questioned. However, values of \( \sigma_{end} <1 \) would mean that the concentrations of solutes in the stele (and the concentration profiles) would be just larger by a factor \( 1/\sigma_{end} \) without changing the basic facts. Hence, for a given positive change of pressure (\( \Delta P_c \)), the volumetric water flow at the endodermis (\( V_{end}^{C/D} \)) was calculated according to equation 7, where it caused a convective flow of solutes to the endodermis tending to increase the concentration at the endodermis (\( C_{e} \)), i.e.

\[ J_{v_{end}}^{C/D} = L_{p_{0}}^{end} \left[(P_{w} + \Delta P_{r}) - R T C_{e}\right] \]  

(7)

For modelling, the measured \( L_{p} \) from RPP experiments (usually referred to the outer surface of the root) was referred to the reduced surface area of the endodermis \( (L_{p_{0}}^{end}=L_{p} \times R/R_{e}; \ see\ above) \). Concentration profiles between the EMX ring and the endodermis as they develop with time were simulated according to the partial differential equation (Knipfer et al., 2007), i.e.

\[ \frac{\partial C}{\partial t} = \frac{1}{r} \frac{\partial}{\partial r} \left(r \cdot D \frac{\partial C}{\partial r}\right) - \frac{1}{r} \frac{\partial}{\partial r} (r \cdot V \cdot C) \]  

(8)

In cylindrical coordinates, this equation describes the solute diffusion in the stele (first term on the right side) in the presence of an opposing solute convection (second term on the right side). According to equation 8, changes in concentration at certain distances (r) from the root centre could be calculated for different time intervals, i.e. concentration profiles were provided (Knipfer et al., 2007). The diffusion coefficient (D) of solutes within the stelar apoplast should have been somewhat reduced as...
compared with bulk solution. According to literature data, it was taken as (4–9)×10⁻¹¹ m² s⁻¹ (Walker and Pitman, 1976; Touchard et al., 1989; Michael and Ehwald, 1996). The velocity (v) of the volumetric flow resulted in a certain solute concentration (C) at a certain position r. At t=0, the concentration of the stelar apoplast was \( C(r) = C_{\text{stelar}} \), whereas the concentration in the xylem was maintained constant throughout the experiment. In order to work out concentration profiles, the distances of \( \delta = 60 \, \mu m \) and 30 \( \mu m \) between mature xylem and the endodermis were subdivided by \( n = 100 \) shells for corn, and \( n = 50 \) shells for wheat, i.e. for both species the thickness of shells was 0.6 \( \mu m \). The time interval used during numerical integration was \( \Delta t = 0.005 \, s \), which was sufficient to provide the resolution required in space and time (Knipfer et al., 2007). For the simulations with corn, \( L_p^{\text{end}} \) at the endodermis ranged between 20 and 26×10⁻⁷ m s⁻¹ MPa⁻¹ (\( R/R_E = 2.6 \)), which was derived from typical \( L_p \) values of the roots used. For wheat, \( L_p^{\text{end}} \) was 3.6×10⁻⁷ m s⁻¹ MPa⁻¹ (\( R/R_E = 3.6 \)), according to a mean \( L_p \) of 1.0×10⁻⁷ m s⁻¹ MPa⁻¹ given by Bramley (2006). Values of \( C_{\text{stelar}} \) were estimated according to van’t Hoff’s law from \( P_{\text{so}} \). In simulations, step changes of pressure of \( \Delta P_r = 0.005, 0.01, 0.03, \) and 0.05 MPa were applied, which referred to different water flow densities at the endodermis and different rates of convective solute flow.

**Results**

A typical PC experiment with a corn root with a closed apical end is shown in Fig. 1. Prior to the clamp, a PR was performed which displayed a short \( T_{1/2} \) of 1.9 s. When the equilibrium root pressure of about 0.08 MPa was reached again, the PC of 195 s was performed using a \( \Delta P_r \) of 0.065 MPa. The inset of Fig. 1 demonstrates how water flow, measured by following the movement of the meniscus within the glass capillary of the RPP, declined during the clamp. It can be seen from the inset that, according to the fit of equation 6 (\( R^2 > 0.99 \)), there was a rapid exponential increase in water volume with the time period of clamping \( [V(t)] \) during early stages of clamping (initial water flow). Because the stele of the young corn roots used did not contain measurable amounts of air spaces (see Esau, 1969, p. 547, fig. A), the initial rapid decline of pressure was not due to a rapid filling of these storage spaces (see Discussion). A linear increase of \( V(t) \) was reached after about 60 s. The total volume change at \( t=60 \, s \) to reach the steady flow (time constant \( = 20.1 \, s \)) was 1.9×10⁻¹⁰ m³ (190 nl), which was 63% of the total \( V(t) \) at the end of the clamp at \( t=195 \, s \). Since the resolution for measuring a certain \( \Delta V \) at various times, \( t \), was limited by the diameter of the measuring capillary (280 \( \mu m \)), the magnification of the stereo-microscope (×50), and the changes in the shape of the meniscus, the first \( \Delta V \) could only be resolved properly for \( \pm 3.1 \times 10^{-11} \, m^3 \) (±31 nl). Hence, the first measured data point, following the onset of the PC, was at 5 s. Right after the clamp, the subsequent PR measured at a constant position of the meniscus resulted in a \( T_{1/2} \) of 24.2 s, which was longer by a factor of 13 than that of the PR made before the PC (1.9 s).

Figure 2 demonstrates that, when simulating the PC of Fig. 1 with the aid of the C/D model of Knipfer et al. (2007), the steady state in the build-up of internal USLs due to increases in concentrations at the endodermis (\( C_E \)) was reached after about \( t > 50 \, s \) of clamping, i.e. after a time interval which was similar to that required to reach a constant increase of \( V(t) \) (\( t > 60 \, s \)). The C/D model predicted that already within 1 s of the initial clamping period, \( C_E \) increased by 41%, i.e. from the initial xylem concentration of 32.4 mol m⁻³ to 45.4 mol m⁻³, before it remained steady at \( t > 50 \, s \) at 56.4 mol m⁻³. The figure indicated that there should be changes of initial water flow during PC due to effects of concentration polarization at the endodermis, but the process was hard to resolve experimentally within the first second of clamping. Thus, water flows measured during clamping should be dominated by concentration polarization effects, especially when measuring during time intervals where flow rates are steady.

Figure 3 shows the corresponding volume flow \( [J_{VR}(t)] = 1/A_r \, dV(t)/dt \) during the PC of Fig. 1. Data were fitted according to equation 3 (\( R^2 \approx 0.99 \)) including the initial water flow for \( 0 < t < 5 \, s \), where changes in \( V(t) \) could not be resolved properly (see above). It can be seen from the figure that \( J_{VR}(t) \) exponentially declined during earlier periods of clamping, until an almost constant \( J_{VR}(t) \) of 5.2×10⁻⁹ m s⁻¹ was reached at \( t > 80 \, s \). As compared with the steady state, the extrapolation to zero time resulted in a 4-fold bigger initial \( J_{VR}(t) \) of 20×10⁻⁹ m s⁻¹. Hence, during the entire clamp of 195 s, \( J_{VR}(t) \) was reduced to 25% of the initial value. The result shows that the assumption usually made of a constant flow rate during clamping of roots does not hold. As indicated in Fig. 3, assuming a constant \( J_{VR}(t) \) at a clamping time of 120 s would result in an average \( J_{VR} \) of 8.8×10⁻⁹ m s⁻¹, which was larger by 41% than that measured in the steady state. The latter, however, would contain substantial contributions due to USLs. Hence, using the PC technique with roots without considering that the \( L_p \) is estimated from a variable \( J_{VR}(t) \) could result in erroneous data (see Discussion). When time periods of clamping were reduced, this would lead to substantial errors in getting accurate estimates of volume flows. Anyhow, when using the PC mode, changes in water flow during clamping need to be carefully considered, particularly when using relatively short time intervals of clamping to determine \( L_p \) values.

For four different roots, Fig. 4 indicates how changes in the time period of clamping (\( \Delta P_r \approx 0.065 \) MPa; time for clamping: 180–195 s) would affect the measured overall values of the apparent \( L_p^{\text{PC}(t)} \) according to \( J_{VR}(t) \) (see equation 4). It can be seen that during the entire clamping period \( J_{VR}(t) \)s were reduced by 60–69%, i.e. from the initial \( J_{VR}(0) = 1.3–2.0 \times 10^{-8} \, m^3 \, m^{-2} \, s^{-1} \) to the steady state \( J_{VR}(t) = 5.2–6.2 \times 10^{-9} \, m^3 \, m^{-2} \, s^{-1} \) at \( t > 100 \, s \).
between the measured overall was correct because they found a linear relationship phase. These authors emphasized that their interpretation the idea that effects of pressure propagation along the glass capillary (diameter = 280 μm) of the probe (see inset). The exponential fit of \( V(t) = a \cdot t + b \cdot (1 - e^{-\lambda t}) \) (equation 6) resulted in an \( R^2 > 0.99 \). An initial exponential increase of \( V(t) \) with a half time of 14.1 s was observed ending up in a linear slope of \( V(t) \) after \( t=50 \) s of clamping. By an order of magnitude, the half time measured for the \( V(t) \) curve was larger than that observed during the PR. The second PR measured right after clamping also showed a much higher \( T_{1/2} \) of 24.2 s as compared with the 1.9 s of the first PR.

(according to the fit of equation 3, \( R^2 \approx 0.99 \)). Figure 4 shows that the apparent \( Lp_{PC} \) (i) decreased in proportion to \( J_{xylem} \) from 1.7 to 0.9 \( \times 10^{-7} \) m s\(^{-1} \) MPa\(^{-1} \), when times of clamping increased from \( t=15 \) s to 150 s. The data underline the importance of knowing the time course of volume flows during PC to work out \( J_{xylem} \) and \( Lp_{xylem} \) properly in this type of experiment. To date, this has been overlooked in PC and HPFM work (Tyree et al., 1994, 1995; Magnani et al., 1996; Bramley et al., 2007).

In terms of the C/D model of Knipfer et al. (2007), initial volume changes have been interpreted as a build-up of USLs at the endodermis, as analysed in Figs 1–4 (see Introduction). This interpretation is in variance to that of Bramley (2006) and Bramley et al. (2007), who favoured the idea that effects of pressure propagation along the xylem and a rapid filling of water capacities in the stele (and perhaps also in the cortex) caused the rapid initial phase. These authors emphasized that their interpretation was correct because they found a linear relationship between the measured overall \( J_{xylem} \) and the applied step changes of pressure (\( \Delta P_r \)) during PC. Hence, ‘the slope of \( J_{xylem} \) through zero is a consequence of a constant osmotic gradient across the root barriers with applied pressure’ (Bramley et al., 2007). In addition they interpreted the result of no difference between exo- and endosmotic water flow during PC as another fact to reject osmotic effects. However, it appears that the above findings would be expected in the presence of polarization effects at the endodermis according to the C/D model of Knipfer et al. (2007). From Figs 5–7, it is evident that the experimental ‘proof’ provided by Bramley (2006) and Bramley et al. (2007) is questionable. This is so, because concentration polarization effects depend on the intensity of water flow and, therefore, on the absolute value of \( \Delta P_r \) (see equation A16 in the Appendix of Knipfer et al., 2007). In the simulated concentration profiles of Fig. 5, effects of concentration polarizations are shown for young corn (Fig. 5a–c; \( P_{xylem}=0.13 \) MPa, \( C_{xylem}=50 \) mOsmol, \( \delta=60 \) μm, \( Lp_{xylem}=7.7 \times 10^{-7} \) m s\(^{-1} \) MPa\(^{-1} \), \( Lp_{end}=20 \times 10^{-7} \) m s\(^{-1} \) MPa\(^{-1} \), \( D=9 \times 10^{-11} \) m\(^2\) s\(^{-1} \)) and wheat roots (Fig. 5d–f; \( P_{xylem}=0.12 \) MPa, \( C_{xylem}=45 \) mOsmol, \( \delta=30 \) μm, \( Lp_{xylem}=1.0 \times 10^{-7} \) m s\(^{-1} \) MPa\(^{-1} \)), when pressurizing at different \( \Delta P_{PC} \) of 0.005, 0.01, and 0.05 MPa. Profiles for wheat roots were calculated according to the data of Bramley (2006) using the same diffusion coefficient as for corn and an \( Lp_{end} \) of 3.6 \( \times 10^{-7} \) m s\(^{-1} \) MPa\(^{-1} \) (as derived from the wheat \( Lp_{xylem} \)). The results indicated that, according to the C/D model of Knipfer et al. (2007), effects of concentration polarizations increased as the clamped pressure increased. For the steady-state profiles of Fig. 5, the change in concentration at the endodermis (\( \Delta C_E \)) increased due to increasing intensities of \( \Delta P_r \), i.e. \( \Delta C_E \) (corn) = 1.6 → 16.7 mol m\(^{-3} \) and \( \Delta C_E \) (wheat) = 0.5 → 5.1 mol m\(^{-3} \). According to the data of Bramley (2006), effects on concentration profiles in the case of wheat were smaller, because \( C_{xylem} \) (according to \( P_{ro} \), \( Lp_{ro} \) and \( \delta \) were smaller as compared with corn. However, it is indicated by the inset of Fig. 6 that, when plotting \( \Delta C_E \) (according to Fig. 5) against the corresponding \( \Delta P_r \), there was a linear relationship, because effects of
concentration polarization increased in proportion to increases of water flow or $\Delta P_r$. In other words, when solution is swept to the endodermis, the amount of solutes and, hence, the concentration at the endodermis was proportional to the water flow density ($J_{V_E}$, as is also seen from equation A16 of the Appendix of Knipfer et al., 2007). On the other hand, the diffusional backflow is proportional to $C_E$. Hence, as can be seen in Fig. 6, there was also a linear relationship between the measured steady $J_{V_E}$ and $\Delta P_r$ resulting in a straight line passing through the origin. It should be noted that the maximal changes in $C_E$ for the biggest $\Delta P_r$s of 0.05 MPa were relatively small as compared with the basal level of concentration, i.e. 16.1/50.0 = 33.4% for corn and 5.1/45.0 = 11.3% for wheat. According to changes in the driving force of $\Delta P_{r_s}$ = 0.005–0.05 MPa, this should result in changes of osmotic concentrations of 2.2–20.0 mM (40 mOsmol = 0.1 MPa). However, due to USL effects, calculated reductions in the driving force ($\Delta P_r$) of 0.05 MPa could be as large as 80% (16.1 mM = 0.04 MPa) for corn and 20% (5.1 mM = 0.01 MPa) for wheat.

According to Fig. 7, the above findings of a concentration polarization at the endodermis have been experimentally verified by stop-clamp experiments. In this type of experiment, a PC was first performed for a certain time interval and the pressure then taken to the original equilibrium pressure ($P_{ro}$). This resulted in a transient change in pressure caused by the osmotic pressure built up at the endodermis (for details, see Knipfer et al., 2007). Different from the experiments provided by Knipfer et al. (2007), Fig. 7 indicates that, at the given duration of clamping of $t$ = 60 s, the transient responses in pressure linearly increased with increasing $\Delta P_r$. According to the figure, the maximal $P_r(t)$ transient for $\Delta P_r$ of 0.05 MPa [max. $P_r(t) = 0.01$ MPa] was ~5-fold higher than that of $\Delta P_r$ = 0.01 MPa (max. $P_r(t) = 0.002$ MPa). This was in line with the linear relationship between $\Delta P_r$ and $\Delta C_E$ shown in Figs 5 and 6. The inset of Fig. 7 shows that experimental and simulated stop-clamps showed similar $P_r(t)$ transients. Experimental stop-clamps gave evidence that the results of the simulation in Figs 5–7 were in accordance with the experiments.

Figure 8 shows that puncturing the endodermis with a 50-μm-diameter microcapillary reduced the effect of internal USLs, in that solutes could rapidly leak out, at least in the area where a hole was made in the endodermis. The size of the hole was a fraction of 1% of the endodermal surface area (Steudle et al., 1993). It can be seen in Fig. 8 that puncturing reduced the half time ($T^{1/2}_{p_c}$) measured right after 30 s PC, as expected from the C/D model. It can also be seen that, when the endodermis was punctured twice, $T^{1/2}_{p_c}$ tended to decrease even more. However, this second change was not significant. There was no effect of the position of the hole as long as a mature endodermis was hit, i.e. when puncturing roots at distances of between 70 mm and 120 mm from the tip. It was found that ‘tail phases’ of PR curves tended to become more rapid when the number of holes increased (not shown). When the hole in the endodermis was too large, which resulted in a $P_{ro}$ of zero, the massive loss of

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**Fig. 3.** The volume flow $J_{V_E}(t)$ during the pressure clamp of Fig. 1 ($\Delta P_r=0.06$, $t=195$ s) resulted in an exponential reduction in $J_{V_E}(t)$ ending up in an almost constant flow for $t > 80$ s of clamping ($J_{V_E}=5.2\times10^{-5}$ m$^3$ m$^{-2}$ s$^{-1}$). The fit of $J_{V_E}(t) = J_{V_E} + \Delta J_{V_E} e^{-t/\tau}$ (equation 3) resulted in $R^2 > 0.99$. Due to a limitation of the resolution of the measuring set-up, the first measuring point of $J_{V_E}(t)$ was at 3 s. After extrapolating to zero, $J_{V_E}$ was $20\times10^{-8}$ m$^3$ m$^{-2}$ s$^{-1}$. Assuming a constant flow rate of $J_{V_E}(t)$ for $t=120$ s, and the same total volume change per surface area of the root ($V/A_o$ grey area) of 10.3×10$^{-6}$ m$^3$ m$^{-2}$, the initial $J_{V_E}(0)$ was more than 2-fold (8.8×10$^{-8}$ m$^3$ m$^{-2}$ s$^{-1}$) larger and the steady state value was overestimated by 41%, where for longer time intervals of clamping the error would be even larger ($t > 60$ s).

**Fig. 4.** Exosmotic water flows $[J_{V_E}(t)]$ for four different corn roots, as measured during pressure clamp (PC) with the RPP, are summarized. The applied pressures ($\Delta P_p$) were kept constant at 0.06–0.07 MPa for $t=180–195$ s of clamping, depending on the root used. All roots showed an exponential reduction in $J_{V_E}(t)$, ending up in a constant $J_{V_E}(t)$ for $t > 80$ s ($=6\times10^{-5}$ m$^3$ m$^{-2}$ s$^{-1}$). The mean $J_{V_E}(0)$ was 15.6×10$^{-8}$ m$^3$ m$^{-2}$ s$^{-1}$; after extrapolation to zero. Due to the $J_{V_E}(0)$ curves, the apparent $L_{p_E}^{\infty}(t)$ for different clamping times was decreasing in proportion to the declining $J_{V_E}(t)$ ($t=15–150$ s, apparent $L_{p_E}^{\infty}(t) = 1.7–0.9\times10^{-7}$ m s$^{-1}$ MPa$^{-1}$).
solute resulted in PR curves showing only one rapid exponential curve, similar to PR curves measured after the roots were cut-off.

**Discussion**

For the first time, the results show that there are rapid changes in water flow during PC, which have to be taken into account when the hydraulic conductivity of roots is derived from clamps. Adjustments of water flow and of $L_{pr}$ during initial phases of PC are most probably due to effects of concentration polarization of solutes at the endodermis (internal USLs) tending to slow down the water flow and lower the apparent $L_{pr}$ obtained by the clamps. So far, this has not been taken into account by workers using PC with roots (Magnani et al., 1996; Murphy, 1999; Bramley et al., 2007). When clamping periods at constant pressure are relatively short, initial water flows may overestimate the average root $L_{pr}$ (including effects of USLs) when detailed analyses of changes of water flow during PC are missing. However, when water flows across roots are measured at constant volume, effects of USLs can be minimized, such as during the first phase of PRs (Knipfer et al., 2007). During PC, the effects of USLs of the C/D type will increase in proportion to the rates of water forced out across the root, i.e. in proportion to the $\Delta P$ applied. The same refers to the technique of high pressure flow established by Tyree et al. (1994, 1995). A consideration of internal USLs during $L_{pr}$ measurements is of great importance.
Different from the PR technique, there have been to date no rigorous studies of the effects of USLs on water flow during measurements using either PC or the HPFM. The present study provides the first evidence that effects may be substantial during PC, when using root pressure probes.

The results indicated that the contribution of initial phases to typical PC experiments with young roots could be as large as 50%. Most probably, adjustments in water flow and apparent \( Lp \) were due to the build-up of USLs at the endodermis as shown experimentally and in agreement with computer simulations. Due to the C/D model and RPP experiments, concentration polarizations at the endodermis depend on the length of clamps and the intensity of applied pressure. The assumption of a constant efflux of water as used in recent PC experiments of Bramley et al. (2007) with young roots of corn, wheat, and lupin most probably does not hold (clamping time \( t=60–120 \) s). There should always be a contribution of an initial rapid phase during early periods of clamping, which was also observed by Magnani et al. (1996).

When using the PC mode, a critical examination of the measuring set-up is required to detect changes in flow rates of water at the sensitivity required. The applied pressure steps (\( \Delta P \), driving force) and the diameter of the measuring capillary should be appropriate to follow volume changes with a stereo-microscope of sufficient resolution. For example, using a measuring capillary of diameter 300 µm and relatively small pressure steps ranging from 0.005 MPa to 0.03 MPa (fig. 2.9 in Bramley, 2006; fig. 3 in Bramley et al., 2007), this should result in overall changes in volume of 18 nl to 108 nl, respectively. According to values of \( J_{Vr} \) given by Bramley and her co-workers for 80 s PC of 0.005 MPa (\( \rightarrow J_{Vr} \approx 0.7 \times 10^{-9} \) m\(^3\) m\(^{-2}\) s\(^{-1}\)) and 0.03 MPa (\( \rightarrow J_{Vr} \approx 4.3 \times 10^{-9} \) m\(^3\) m\(^{-2}\) s\(^{-1}\)), the corresponding overall shifts in the position of the meniscus in the capillary should be 250–1500 µm (assuming root diameter = 1 mm and root length = 100 mm). For small \( \Delta P \) of 0.005 MPa, initial changes of \( J_{Vr} \) during PC, i.e. within the first 5 s out of 80 s as done here, should have been difficult to measure with the sensitivity required. This is so, because the shape of the meniscus would also change. In the experiments of Bramley (2006) and Bramley et al. (2007), the difficulties of detecting initial water flows relate to the fact that the step changes of pressure used were quite small. As a consequence, changes in water flow during PC were overlooked. The contribution of the initial water flows could have been substantial. This was overlooked by the authors.

In terms of the role of storage capacities in the stelar tissue and cortex, injected volume pulses (applied pressures) and the resulting total volume flows should be high enough so that the efflux of water is not dampened significantly (T Knipfer et al., unpublished results). The
point is of concern because absolute volumes injected into roots could have been too small to provide a steady water flow across the roots. It is most probably true that the stelar storage capacity for water (including the pith) is negligibly small, because there are virtually no gas-filled intercellular spaces in the stele. Hence, the storage capacity of the stele should be close to the compressibility of water in the stele. According to the stelar volume of young corn roots (assuming a typical diameter of 60 μm and a length of 120 mm), this would result in a storage capacity of 3.6×10⁻¹² m³ MPa⁻¹. Hence, for a typical pressure change of ΔPₛ=0.05 MPa, the amount of water stored in the stele would be 0.18 nl, which is significantly smaller than the amount of water injected into the root during a PR (50 nl) or a PC (300 nl, see below), as used here. Since the pressure within the cortex was kept at atmospheric pressure (and pressure gradients along the cortical apoplast were small), the storage of water in cortical cells during step changes of root pressure should be rather small, too. Due to the fact that most of the hydraulic resistance of the young roots used (lacking an endodermis) should reside in the endodermis and stele, the water storage in the cortex should not be significant. In contrast to the present experimental arrangement, the total volumes injected by Bramley et al. (2007) were 18–108 nl during the entire period of PC [see again fig. 3 of Bramley et al. (2007) and above], which was smaller by a factor of 3–17 than the overall volume provided in Fig. 1 of the present paper (3×10⁻¹⁰ m³=300 nl). When using ΔPₛ of ∼0.05 MPa which corresponds to a relatively large amount of injected volume, it was possible to register initial parts of PC curves, which Bramley (2006) and Bramley et al. (2007) missed. It is assumed that the overall Lₛ measured from PC by these authors originates from a mixture of initial and steady-state components of water flow.

Bramley et al. (2007) estimated a rather large storage capacity of stelar and inner cortical cells of 0.8×10⁻⁹ m³ MPa⁻¹ per 10 mm of root length. For a typical 100 mm root, this resulted in an overall storage capacity of 8×10⁻⁹ m³ MPa⁻¹ or 8000 nl MPa⁻¹ per root. When injecting water at step pressures of between 0.005 MPa and 0.03 MPa during PC (as done by the authors of this paper), this would result in a capacity of between 40 and 240 nl root⁻¹ (8000×0.005 nl or 8000×0.03 nl, respectively). This is substantially more than was injected experimentally during PC (18–108 nl, see above). As a consequence, most of the water injected would never reach the root surface, and the results derived from the steady-state experiments are meaningless with respect to the overall Lₛ. There are two possibilities, i.e. either the storage capacity estimated by the authors was too big or the conclusions drawn from the experiments assuming steady state were wrong. According to our estimates of the storage capacity and corresponding results, we think that the storage capacities in these young roots were in fact much smaller (see above). Bramley et al. (2007) did not realize the contradictions arising from their assumption of a rather high storage capacity of the tissue in the inner part of roots, which are obvious from their data.

When they treated root segments in terms of the leaky-elastic pipe model, Bramley (2006) and Bramley et al. (2007) excluded any contribution of osmotic gradients and effects of concentration polarizations at the endodermis during PC. Due to (i) a linear response in Jᵥ,r, (when ΔP_r was varied during PC) resulting in a straight line through the origin (fig. 2.9 of Bramley, 2006; fig. 3 of Bramley et al., 2007), and (ii) missing differences between exo- and endosmotic water flow, the authors concluded that there appeared to be no osmotic effects in those measurements. However, as indicated by Figs 5 and 6 of the present study, the theory of the C/D model predicts such a linearity of ΔP_r and Jᵥ,r when concentration polarization effects are included. According to the C/D model, similarities in exo- and endosmotic water flow can be easily described by sweep-away and dilution effects, respectively, which are symmetrical. Hence, the interpretations of Bramley (2006) and Bramley et al. (2007) are premature.
It may be argued that, for three reasons, PC rather than PR techniques should be used when measuring $L_P$. Due to the elastic extension of vessel walls there should be a relatively slow propagation of pressure pulses along the extendable xylem following a step change in volume, which is subsequently kept constant during PRs. According to the resulting drop in pressure along the xylem during PR experiments, the radial efflux of water should decline from the root base to the tip. Hence, the pressure measured with the RPP at the root base, where the probe is connected to the root, represents an upper limit of the real pressure along the xylem, and gradients within the xylem could be fairly steep.

During PC, the situation may be different in that a constant pressure would soon develop along the xylem giving rise to a constant and steady outflow of water. However, these assumptions have no physical basis, simply because the equilibration of pressure along the rigid vessels of mature xylem should be very fast according to the famous Moens/Korteweg equation (see equation A1 in Appendix 1). It describes the rates of propagation of pressure (i.e. the pressure-wave velocity, PWV, $c_0$ in m s\(^{-1}\)) in terms of the properties of the tubes, such as the xylem vessels ($R_{\text{xylem}}$=inner radius of xylem vessels, $d$=thickness of xylem wall, $E$ = Young’s modulus of the vessel walls), and in terms of the fluid properties ($\rho$=xylem sap density, $\kappa$=compressibility of xylem sap = $1/\alpha_{H_2O}$, where $\alpha_{H_2O}$ is the elasticity of the sap or water in MPa). When $E$ is very high, the Moens/Korteweg equation reduces to the well-known equation for the speed of the propagation of sound in liquids (or gases; $c_0=\sqrt{\frac{E\cdot d}{\rho\cdot 2\cdot R_{\text{xylem}}}}$). On the other hand, when $E$ is low (elastic extensibility of vessel walls relatively high) the following is obtained (Korteweg, 1878; Stevanov et al., 2000; Appendix 1):

$$c_0=\sqrt{\frac{E\cdot d}{\rho\cdot 2\cdot R_{\text{xylem}}}}$$

(9)

Equation 9, states that, prior to a movement of liquid in a pipe driven by a pressure gradient (such as during a step change in pressure induced by a RPP causing a pulsatile water flow into the vessels), there will be a rapid propagation of pressure ahead of that of the flow of liquid.

This theoretical prediction is in agreement with experimental findings indicating that step changes in xylem pressure (hydraulic signals), different from a steady pressure developing, rapidly propagate within plants over long distances (Malone, 1996; Stahlberg and Cosgrove, 1997; Wei et al., 1999). However, this can also be exemplified for cylindrical internodes of Chara, which represent a permeable (leaky) elastic pipe which is as long as the root segments (see Appendix 1).

Hence, the above assumption (i) that the propagation of pressure in elastic tubes such as xylem vessels is usually a slow event of a few seconds does not hold. As a consequence of the rapid PWV, the assumption of a steep gradient of pressure along the root xylem does not hold either (assumption ii). This will be true for both immature and mature xylem. Rate limitations due to blocked xylem vessels at the seal, however, may result in delays of pressure transmittance, which do not refer to effects of pressure propagation in roots.

Assuming short phases of biphasic PRs in roots are dominated by pressure propagation in the xylem, no reduction in this phase would have been observed during experiments when opening the stelar compartment by repeated puncturing, by defined steaming of roots, or by carefully opening the tips of mature xylem at the root apex (Fig. 8; Steudle and Jeschke, 1983; Peterson and Steudle, 1993). This was, however, the case. Due to the high PVW in the xylem vessels, there should be no problems with cable effects during PRs (Landsberg and Fowkes, 1978; Frenc and Steudle, 1989). Frenc and Steudle (1989) have provided data of such effects according to detailed measurements of ratios of radial to axial root resistances $(R_R/R_x=17–44)$ indicating that effects were small. Bramley et al. (2007) do not provide data of pressure gradients (or of water potential) within the xylem during their PC experiments, although they stress the point of a high hydraulic leakiness of the xylem.

The root hydraulic conductivity $(L_P)$ measured from PRs would also not be completely free of internal USLs, but the first rapid phase from PRs tends to minimize these effects (Knipfer et al., 2007). This is due to the fact that much less water is forced across the root and hence the initial state is less disturbed (Tyree et al., 1994, 1995; Knipfer et al., 2007). In the real transpiring plant, effects of USLs should usually be included, but the contribution of external USLs at the endodermis should be relatively small, even at high $\Delta P_r$ (Dalton et al., 1975; Fiscus, 1975; Sands et al., 1982). When the osmotic concentration of the soil solution is low and the water uptake sweeps away solutes from the endodermis, effects of internal USLs would be reduced to virtually zero. During transpiration and the influx of water, dilution effects instead of concentration effects of the stelar compartment would be the consequence, as shown during endosmotic PC by Knipfer et al. (2007).

In conclusion, the present results show that, due to problems with USLs, measurements of root $L_P$ using the PR technique should be taken with some caution. This has been verified by following the kinetics of water flow during PC, where the slowing down of the initial phase of water flow had a time constant which was much longer than that found during PRs, but agreed with that calculated for the build-up of USLs at the endodermis during clamps. Recent conclusions about the existence of a slow propagation of pressure and rather steep gradients in pressure along the leaky xylem of young roots during PR have no physical basis (leaky-elastic pipe model of Bramley (2006) and Bramley et al., 2007).
Besides theoretical flaws, there are experimental misconceptions in Bramley (2006) and Bramley et al., (2007). In the present paper, it was shown that the idea of a rapid equilibration of pressure along the xylem during PRs with closed end segments of roots has a sound physical basis which includes the lateral displacement of water. Pressure propagation along the elastic tubes of mature xylem is an extremely rapid process, and is even faster than pressure propagation in the cylindrical internodes of Chara, which are similar in length to the roots usually used in RPP experiments. The results indicated that the use of PC requires a careful estimation of the contribution of internal USLs. So far, this has been overlooked in root work using PC and HPFM techniques. The existence of concentration polarization effects has been further demonstrated by puncturing the endodermis in order to reduce the effect of internal USLs.

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**Appendix 1**

The propagation of pressure in elastic tubes: calculation of pressure-wave velocity (PWV)

(i) Moens/Korteweg equation

For an ideally incompressible liquid, the propagation of pressure ($c_0$ in m s$^{-1}$, and of volume changes) would be instantaneous (equation A1; i.e. changes independent of time and of the position along the tube).

When the liquid is compressible, $c_0$ is identical to that of the propagation of sound, i.e. $c_0 = \sqrt{\frac{\rho}{\kappa}} = 1400$ m s$^{-1}$ for liquid water (see textbooks of physics). This applies to a good approximation to unextendable thick-walled, narrow glass tubes filled with water. When the walls of the tubes are extendable, the extension perpendicular to the longitudinal propagation of pressure would tend to slow down $c_0$.

The famous Moens/Korteweg equation (equation A1) of the PWV has to be applied to calculate the propagation of pressure in xylem vessels such as during PR or PC experiments with the RPP (Korteweg, 1878; Stevanov et al., 2000):

$$c_0 = \frac{1}{\sqrt{\rho \left( \kappa + \frac{2 \cdot R_{xylem}}{E \cdot d} \right)}} \approx \frac{1}{\sqrt{\frac{\rho}{2} \cdot \frac{R_{xylem}}{E \cdot d}}} = \sqrt{\frac{E \cdot d}{\rho \cdot 2 \cdot R_{xylem}}}$$

(A1)

where $\rho =$ xylem sap density, $\kappa =$ compressibility of xylem sap ($= 1/\epsilon_{H_2O}$; $\epsilon_{H_2O} =$ elastic modulus of water), $R_{xylem} =$ inner radius of xylem vessels, $E =$ Young’s modulus of the vessel walls, and $d =$ xylem wall thickness. In the case of an extremely rigid tube ($E \to \infty$), the equation reduces to the relationship given above for the speed of sound.

When the fluid is transported through extendable biological vessels in the presence of a pulsatile water flow, such as in blood vessels or the xylem, the compressibility term in the denominator of equation A1 can be usually neglected, namely when vessels are rather wide compared with their thickness, and the Young’s modulus ($E$) relatively low. The elasticity term in equation A1 originates from the fact that the radial elastic extension at a given step change of pressure ($\Delta P_r$) is given by Hooke’s law:

$$\frac{\Delta R_{xylem}}{R_{xylem}} = \frac{1}{E} \cdot \left( \frac{R_{xylem}}{d} \cdot \Delta P_r \right) = \frac{T}{E}$$

(A2)

where $\left( \frac{R_{xylem}}{d} \cdot \Delta P_r \right)$ is the tensile stress ($T$ in MPa) in the wall created by a step change in pressure, which depends on the tube geometry (see, for example, Nobel, 1999).

(ii) Propagation of pressure in xylem vessels

Equation A1 states that in a liquid-filled tube there will be a rapid propagation of pressure ahead of that of the flow of liquid, when a pressure gradient is applied. For example, this is a situation which occurs in blood vessels, when the heart ejects a certain blood volume into the aorta during a beat (Asmar et al., 1995; Blacher et al., 1999; Stevanov et al., 2000), but also in engineering, when dealing with pipes transporting liquids (Spiller, 1965; Streeter, 1969; Simpson and Wylie, 1991). Rates of propagation of pressure in the rather extendable (as compared with xylem) blood vessels are still as much as 5 m s$^{-1}$ in the large vessels (aorta). According to the different $R/d$ ratios, they can be up to 15 m s$^{-1}$ in distal narrow capillaries. There are hardly any data about the Young’s moduli of xylem walls, but those of lignin have been estimated to be up to 3 GPa (Cousins et al., 1975). In this case, $\kappa$ cannot be neglected for the calculation of PWV ($c_0$) due to the rather high $E$ term in equation A1. When using this value and a ratio of $R/d$ for mature early metaxylem vessels, as present in the roots used in this paper, of around 3.3 ($= 23 \mu m/7 \mu m$; figs 6–8 of Peterson and Steudle, 1993), the rate of pressure propagation would be $c_0 = 600$ m s$^{-1}$. This is about 40% of the speed of the propagation of sound in water (1400 m s$^{-1}$). It means that, following a step change in pressure during a PR, the pressure change will reach the tip of a 100-mm-long root (such as those used) within 0.2 ms. This is much shorter than the time constants required for the radial flow of water out of xylem into surrounding tissue, even when the latter were
only fractions of a second (according to a relatively high hydraulic conductivity of xylem vessels; Peterson and Steudle, 1993). Hence, the assumption of a constant pressure along the xylem during PRs is an excellent one. It remains excellent, even when the elastic modulus of vessel walls is much less and comparable with that of cell wall material \((E=70 \text{ MPa}; \text{ Nobel, 1999})\). In this case, \(\kappa\) can be neglected according to equation A1, and \(c_o\) would be ‘only’ 100 m s\(^{-1}\), and the time required to reach the tip of a 100-mm-long root segment 1 ms. When walls of xylem vessels were as extendable as blood vessels \((E=0.7 \text{ MPa}; \text{ Stevanov et al., 2000})\), this would still result in a reasonable PWV of 10 m s\(^{-1}\). Hence, there is no doubt that, during PRs, the pressure in the xylem is virtually homogenous and the pressure measured with the RPP identical to that along the root xylem.

\[(iii) \text{ Propagation of pressure in cylindrical internodes of Chara}\]

When individual tissue cells are measured with the cell pressure probe, the propagation of pressure within the cell during PRs or PC does not significantly affect the measurement of \(T_{1/2}\) of water flow during PRs or rates of water flow during PC. This is so because cell dimensions are small and \(R/d\) ratios rather large. However, this may be different when using the long internodes of \textit{Chara} or \textit{Nitella} that are similar in length to roots and exhibit unfavourably large ratios of \(R/d\). For an internode of \textit{Chara} \((R_{\text{chara}}=0.5 \text{ mm}, d=7 \text{ \,\mu m}, E=70 \text{ MPa}; \text{ Nobel, 1999})\), we get from equation A1 a \(c_o=49 \text{ m s}^{-1}\). This means that during a cell pressure probe experiment, when a pressure/volume pulse is injected across the node at one end of a 100 mm internode, it will take 2 ms to reach the other end of the cell. By three orders of magnitude, this time is shorter than the usual \(T_{1/2}\) of \textit{Chara} cells to exchange water of 1–3 s (Steudle and Tyerman, 1983; Schütz and Tyerman, 1997). Hence, overall measured hydraulics of \textit{Chara} cells should not be affected by pressure propagation. Since the cell \(L_p\) of a \textit{Chara} internode is inversely proportional to \(T_{1/2}\) or \(\tau\) of water exchange, it is easily verified that effects of water exchange and pressure propagation would be on a similar time scale, when cell \(L_p\) would be larger by three orders of magnitude. When comparing the \textit{Chara} internode with the leaky, elastic xylem of corn roots measured in the present paper, the difference is that, most probably, the Young’s modulus \((E)\) would be somewhat larger (see above). In addition to this, the \(d/R\) ratio of the xylem vessels would be larger by a factor of \(7/23:7/500 = 22\) as compared with the \textit{Chara} internode. Hence, pressure propagation should be even faster in the root xylem than in a \textit{Chara} cell, and, in fact, not measurable with equipment such as the RPP. Volume pulses injected into the root xylem during RPP experiments would hardly be dissipated away by the elastic extension of the pipe when travelling along the xylem, as Bramley (2006) and Bramley et al., (2007) erroneously assumed. Since the overall elastic extensibility of a \textit{Chara} cell is even larger than that of root xylem, such a dampening effect should be more important for the \textit{Chara} cell. However, this was never observed in PR experiments with the internodes. It appears to be unrealistic. Even when elastic moduli are unfavourably low and the hydraulic conductivity of radial water quite high, the ‘leaky-elastic pipe model’ could hardly be used to predict significant gradients of pressure along either the xylem of young corn roots or \textit{Chara} internodes.

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