Polarity of Water Transport across Epidermal Cell Membranes in *Tradescantia virginiana*¹[W][OPEN]

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Using the automated cell pressure probe, small and highly reproducible hydrostatic pressure clamp (PC) and pressure relaxation (PR) tests (typically, applied step change in pressure = 0.02 MPa and overall change in volume = 30 pL, respectively) were applied to individual *Tradescantia virginiana* epidermal cells to determine both exosmotic and endosmotic hydraulic conductivity (*L*ₚ⁸⁰ and *L*ₚₙ, respectively). Within-cell reproducibility of measured hydraulic parameters depended on the method used, with the PR method giving a lower average coefficient of variation (15.2%, 5.8%, and 19.0% for half-time, cell volume [Vₜₚ], and hydraulic conductivity (*L*ₚ, respectively) than the PC method (25.4%, 22.0%, and 24.2%, respectively). Vₜₚ as determined from PC and PR tests was 1.1 to 2.7 nL and in the range of optically estimated Vₜₚ values of 1.5 to 4.9 nL. For the same cell, Vₜₚ and *L*ₚ estimates were significantly lower (about 15% and 30%, respectively) when determined by PC compared with PR. Both methods, however, showed significantly higher *L*ₚOutOf/LₚIn when determined by PC compared with PR. Both methods, however, showed significantly higher *L*ₚOutOf/LₚIn when determined by PC compared with PR. Both methods, however, showed significantly higher *L*ₚOutOf/LₚIn when determined by PC compared with PR.

1.20). Because these results were obtained using small and reversible hydrostatically driven flows in the same cell, the 20% outward biased polarity of water transport is most likely not due to artifacts associated with unstirred layers or to direct effects of externally applied osmotica on the membrane, as has been suggested in previous studies. The rapid reversibility of applied flow direction, particularly for the PR method, and the lack of a clear increase in *L*ₚOutOf/LₚIn over a wide range of *L*ₚ values suggest that the observed polarity is an intrinsic biophysical property of the intact membrane/protein complex.

The conductivity of membranes to water (hydraulic conductivity [*L*ₚ]) is an important property of the cells of all organisms, and whether plant cell membranes exhibit a polarity in this property has been debated for a number of decades (Dainty and Hope, 1959; Steudle, 1993). Most early evidence for polarity was based on transcellular osmotic experiments using giant algal cells in the Characeae, in which the relative areas of cell membrane exposed to conditions of osmotic inflow (endosmosis) or outflow (exosmosis) could be varied and, hence, *L*ₚ for both directions determined (Tazawa and Shimmen, 2001). Interpretation of these experiments is complicated by unstirred layer (USL) effects (Dainty, 1963), but even after accounting for these, it was concluded that inflow *L*ₚ (*L*ₚIn) was higher than outflow *L*ₚ (*L*ₚOutOf) in these cells, with *L*ₚOutOf/*L*ₚIn of about 0.65 (Dainty, 1963). When using osmotic driving forces in algal cells, *L*ₚOutOf/*L*ₚIn values of between 0.5 and 0.91 have been reported in many studies (Steudle and Zimmermann, 1974; Steudle and Tyerman, 1983; Tazawa et al., 1996), and the same direction of polarity was also reported using osmotic driving forces in whole roots of maize (*Zea mays*; Steudle et al., 1987). When applying hydrostatic driving forces in algal cells using the pressure probe (Steudle, 1993), which is less influenced by USL effects (Steudle et al., 1980), *L*ₚOutOf/*L*ₚIn has been closer to 1 (0.83–1; Steudle and Zimmermann, 1974; Steudle and Tyerman, 1983). However, in higher plant cells, an analysis of the data presented by Steudle et al. (1980, 1982) and Tomos et al. (1981) indicates the opposite polarity, with *L*ₚOutOf/*L*ₚIn averaging from 1.2 to 1.4. Moore and Cosgrove (1991) used two contrasting hydrostatic methods to measure *L*ₚ in sugarcane (*Saccharum* spp.) stem cells: (1) the most commonly used pressure relaxation (PR) method, in which cell turgor pressure (*P*ₑₑ) changes during the measurement, and (2) the more technically demanding pressure clamp (PC) method, in which *P*ₑₑ is maintained constant. Consistent with other studies in higher plant cells, Moore and Cosgrove (1991) reported average *L*ₚOutOf/*L*ₚIn from 1.15 (PC) to 1.65 (PR). Using the PR method in epidermal cells of barley (*Hordeum vulgare*), Fricke (2000) reported only a modest *L*ₚOutOf/*L*ₚIn (based on reported half-time [*T*₁/₂] of 1.08) in view of the contribution of proteins (e.g. aquaporins) to overall membrane *L*ₚ. Tyerman et al. (2002) suggested that polarity may result either from asymmetry in the pores themselves or from an active regulation of the conductive state of the pores in response to the experimental conditions.

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conditions that cause inflow or outflow. Either of these mechanisms may explain the wide range of values reported in the literature for $L_p^{\text{OUT}} / L_p^{\text{IN}}$. Cosgrove and Steudle (1981) reported that a substantial (6-fold) and rapid (within 20 s) reduction in $L_p$ could occur in the same cell, and in hindsight, this presumably reflected the influence of aquaporins. Cosgrove and Steudle (1981) did not consider the lower $L_p$ as indicative of the $L_p$ in situ, and Wan et al. (2004) reported that a reduction in $L_p$ was associated with perturbations to $P_{\text{cell}}$ on the order of 0.1 MPa. Hence, if measured membrane $L_p$ itself can exhibit substantial changes over relatively short periods of time in the same cell, then further study of systematic differences between $L_p^{\text{OUT}}$ and $L_p^{\text{IN}}$ will require a robust hydrostatic methodology (PC or PR) that can reversibly and reproducibly apply small perturbations in pressure ($P$) to individual cells over short periods of time.

For the PR method, a $T_{1/2}$ of water exchange is measured by fitting an exponential curve to the observed decay in $P_{\text{cell}}$ over time following a step change in volume, and membrane $L_p$ can be calculated if cell surface area ($A$), cell volume ($V_o$), and volumetric elastic modulus ($e$) are known (Steudle, 1993). In practice, $A$ and $V_o$ are typically calculated from optical measurements of individual cell dimensions or estimates using average values, and $e$ is calculated based on $V_o$ and an empirical change in pressure ($dP$) to change in volume ($dV$) relation for each cell (Steudle, 1993; Tomos and

![Figure 1](image-url)

**Figure 1.** Sequential measurements with depth of $P_{\text{cell}}$ in grape mesocarp tissue as conducted with a fully ACPP compared with a manually operated CPP. A, Schematic of the experimental protocol. Dashed lines indicate stationary and forward movement phases of the capillary into the tissue. In both cases, the forward movement is controlled manually using a motorized micromanipulator. For the ACPP, the oil/sap meniscus is maintained automatically at a single location by changes in $P_{\text{oil}}$. During forward movement, feedback control automatically reduces $P_{\text{oil}}$, but upon penetration into the next cell, the meniscus quickly moves away from the control point and causes a compensating response in $P_{\text{oil}}$ as well as signals the operator that forward movement should be stopped. For the same experiment using a CPP, the meniscus location is monitored through the microscope by eye and $P_{\text{oil}}$ is adjusted manually either with switches for increasing or decreasing $P_{\text{oil}}$ directly or by manual adjustments to a $P_{\text{oil}}$ controller. Prior to forward movement, the operator reduces $P_{\text{oil}}$, initiates forward movement, stops forward movement when a rapid meniscus movement is seen, and adjusts $P_{\text{oil}}$ to return the meniscus to the perceived initial point of movement, which may be closer to or farther from the epidermis than the original meniscus location. B, Typical ACPP recording of $P_{\text{oil}}$ and meniscus location and manually recorded depth of tip in the tissue for seven cells with a $P_{\text{cell}}$ of around 0.15 MPa. Peaks in meniscus location indicate the puncturing of a new cell. C, Typical CPP recording for four cells having a $P_{\text{cell}}$ of around 0.13 MPa, as in B.
Leigh, 1999). In the PC method, first developed by Wendler and Zimmermann (1982), \( V_0 \) (and, given reasonable assumptions about cell geometry, \( A \)) is estimated without the need for optical measurements, and \( L_0 \) can be measured without the need to determine \( dP/dV \) or \( e \). However, this method is technically more demanding because it requires precise \( P \) control as well as a continuous record of the volume flow of water across the cell membrane (as measured by changes in the position of the cell solution/oil meniscus within the glass capillary over time) and has rarely been used (Wendler and Zimmermann, 1982, 1985; Cosgrove et al., 1987; Moore and Cosgrove, 1991; Zhang and Tyerman, 1991; Murphy and Smith, 1998). Since volume \( V \) is continuously changing over time, this approach may also be influenced by the hydraulic conductance of the capillary tip \( (K_h) \) used to make the measurements as well as surface tension effects due to the progressive changes in capillary diameter with meniscus position, and these influences have not been quantitatively addressed.

Automation of the pressure probe operation, particularly automatic tracking of the meniscus location in the glass microcapillary tip, would address many of the above-mentioned issues, and to date, several attempts have been made to monitor the meniscus location using electrical resistance (Hüsken et al., 1978) or hardware-based image analysis (Cosgrove and Durachko, 1986; Murphy and Smith, 1998). Recently, Wong et al. (2009) redesigned the automated cell pressure probe (ACPP), originally proposed by Cosgrove and Durachko (1986), using a software-based meniscus detection system and a precise pressure control system. In the new ACPP system, both the position of the meniscus and oil pressure \( (P_{oil}) \) are recorded frequently (typically at 10 Hz), and \( P_{oil} \) is controlled with a resolution of \( \pm 0.002 \) MPa. We have combined the ACPP with a new technique to reproducibly fabricate microcapillary tips of known hydraulic properties (Wada et al., 2011) in order to correct for \( K_h \) and surface tension effects in both PC and PR estimates of the water relations parameters of Tradescantia virginiana epidermal cells and have determined the relation of \( L_{p,OUT} \) to \( L_{p,IN} \) in these cells.

RESULTS AND DISCUSSION

Comparison of ACPP and Cell Pressure Probe

While the overall processes involved in ACPP and cell pressure probe (CPP) operation are very similar (Fig. 1A) and repeatable values of \( P_{cell} \) can be obtained for sequential measurements of cells in a tissue using either system (Fig. 1B), the operator skill required to accomplish

Figure 2. A, Schematic presentation of a microcapillary tip at equilibrium in a water drop or cell (for abbreviations, see “Materials and Methods”). B, \( \Delta p \) \( (P_{oil} - P_{H2O}) \) as a function of \( r_{m}^{-1} \) for a tip in a water drop \( (P_{H2O} = 0) \) at equilibrium. The linear regression line obtained from measured values of \( r_{m}^{-1} \) \((x)\) and \( \Delta p \) \((y)\) was \( y = 0.0524x + 0.0001 \) \((r^2 = 0.92)\), resulting in an experimentally determined value of slope \((a)\) of 52.4 mN m\(^{-1}\). Dashed lines indicate the 95% confidence intervals as calculated from Student’s percentage \( t \) distribution as obtained from 36 to 115 frames. The dotted line indicates a theoretical value based on \( u = 50.4 \) \( \mu \)N m\(^{-1}\) for dimethyl silicone oil, similar in composition to the silicone oil used. C, Linear relation between \( K_p \) \((x)\) and \( K_h \) \((y)\) was \( y = 0.0056x + 0.1753 \) \((r^2 = 0.95)\). The inset shows the relationship between tip resistance \((y)\) \( (K_h) \) and tip i.d. \((x)\) of \( y = 0.507\exp^{-1.308x} \) \((r^2 = 0.72)\).
these measurements is much less for the ACPP. Because $P_{\text{cell}}$ in the ACPP is automatically controlled by a proportional integral derivative algorithm to maintain either the meniscus position or the pressure at a set value, there is a tradeoff among the speed of response, overshoot or undershoot, and noise in the controlled or controlling parameters (Wong et al., 2009). However, the initial disturbance to $P_{\text{cell}}$ when moving through a series of cells in a tissue is generally less with the ACPP (Fig. 1B) than with the CPP (Fig. 1C). An example of a tissue is generally less with the ACPP (Fig. 1B) than with turbulence to parameters (Wong et al., 2009). However, the initial disturbance to $P_{\text{cell}}$ when moving through a series of cells in a tissue is generally less with the ACPP (Fig. 1B) than with the CPP (Fig. 1C). An example of a tissue

One advantage of the ACPP is that synchronous data on the location of the meniscus within the capillary, the capillary diameter at that location, and $P_{\text{cell}}$ are routinely collected (Wong et al., 2009; Wada et al., 2011), and short-duration video recording is also possible. Video recordings have been useful in documenting the relatively small volume errors (less than 1%) resulting from changes either in the meniscus size or shape (Wong et al., 2009), and for this study, video recordings were used to determine features such as the wetting angle within the capillary (Fig. 2). Data on the location and size of the meniscus allowed the routine correction for differences between $P_{\text{oil}}$ and $P_{\text{cell}}$ due to both static (surface tension) and dynamic (tip conductance) effects, and while the corrections were typically not large for the microcapillaries used to measure $T.\ virginiana$ cells, both corrections increase with decreasing capillary size and, hence, may be important when using smaller microcapillaries for smaller cells. For static (surface tension) effects, the estimated theoretical relation was well within the 95% confidence intervals of the empirically determined relation (Fig. 2B); hence, the empirically determined relation was used to routinely correct for surface tension effects in all subsequent data. For the tips and conditions used in this study, the correction for surface tension effects was generally less than 0.01 MPa. Routine correction for dynamic ($K_{\text{c}}$) effects required measurement of either tip pneumatic conductance ($K_{\text{p}}$) or tip size (Fig. 2C), but because the data for $K_{\text{p}}$ was routinely available as part of microcapillary manufacturing (Wada et al., 2011), this was the method used. The measured $K_{\text{p}}$ values for the tips used in this study were 0.66 to 1.07 standard cubic centimeters per minute (SCCM) MPa$^{-1}$ giving a calculated $K_{\text{c}}$ of 75 to 160 nL s$^{-1}$ MPa$^{-1}$, very similar to the hydraulic resistance of tips of a similar size reported by Zhang and Tyerman (1991). From Equation 2 ($P_{\text{cell}} - P_{\text{oil}} = a/r_m + dV/dt \times 1/K_{\text{c}}$), the differences between measured $P_{\text{cell}}$ and $P_{\text{cell}}$ due to $K_{\text{c}}$ were generally less than 0.0001 MPa; hence, for the tips and cells used in this study, the effects of $K_{\text{c}}$ and surface tension were very small, but the magnitude of both of these effects will increase if smaller tips are used.

Synchronous data of meniscus position and $P_{\text{cell}}$ also allow the quantification of irregular meniscus movement by classifying meniscus data into occurrences of movement (the rate of the volume change $dV/dt \neq 0$) or temporary nonmovement ($dV/dt = 0$) under conditions when $P$ was changing (the rate of the pressure change $dP/dt \neq 0$) and meniscus movement would be expected. Storage of tips at high relative humidity substantially reduced the fraction of time this phenomenon was observed (from 0.44 to 0.05; Table I) when tips were tested in a water drop, although the basis for the nonmovement itself is not clear. In the literature, similar meniscus behavior has often been attributed to tip plugging by cell debris (Zimmermann et al., 1980), but for these tests there was no cell. Since the apparent plugging was reversible, and we observed small pockets of oil adhering to the inner surface of water-filled glass capillaries, we hypothesize that temporary plugging may be due to small quantities of oil temporarily bridging across the interior of the capillary tip, establishing an inner and outer oil/water meniscus of sufficiently small radius that comparatively large pressure differentials are required to break the bridge. The influence of humidity may be to condition the glass surface (possibly an electrostatic effect) and reduce the chance of oil bridges forming.

**Table I. Effects of microcapillary storage at low and high humidity on irregular meniscus movement during ACPP experiments**

| Treatment         | Total No. of Frames per Tip Analyzed for Which $dP_{\text{cell}}/dt \neq 0$ | No. of Frames for Which $dV/dt = 0$ | Fraction of $dV/dt = 0$ Frames |
|-------------------|-----------------------------------------------------------------------------|-----------------------------------|--------------------------------|
| Low RH (45%-55%)  | 141.2 ± 26.7                                                                | 63.5 ± 30.7                       | 0.44 ± 0.19                   |
| High RH (100%)    | 120.5 ± 23.3                                                                | 5.2 ± 5.2                         | 0.05 ± 0.05                   |

**Cell Water Relations Parameters of T. virginiana Leaf Epidermal Cells**

The ACPP was used to make a series of relatively small PCs (±0.02 MPa), PRs (±28 pL), and $P$ pulses (maximum ±0.07 MPa) in the same cell over a period of about 30 min (Fig. 3). These changes are well below the levels of the $P$ difference at which plasmodesmata closure was observed (e.g. 0.2 MPa in *Nicotiana clevelandii*; Oparka and Prior, 1992) or $P$ disturbance (0.1–0.2 MPa) reported to give a significant decline in $L_p$ in maize
and Durachko (1986) and expected from theory, as the pulse were used (Fig. 4). As reported by Cosgrove only end-point values or all values collected during the express the same of cell fluid in the capillary for repeated PC and PR experiments on the same plant. Also shown are the water relations parameters calculated from each experiment. Following the PR experiments, a series of pulses were manually applied in order to expand the range for measurement of for this cell. Asterisks indicate the manual switch between PC and PR modes.

![Figure 3](image1.png)

**Figure 3.** Example ACPP recording showing turgor \(P_{\text{cell}}\) and \(V\) of cell fluid in the capillary for repeated PC and PR experiments on the same \(T. virginiana\) epidermal cell. Also shown are the water relations parameters calculated from each experiment. Following the PR experiments, a series of pulses were manually applied in order to expand the range for measurement of \(dV/dP\) for this cell. Asterisks indicate the manual switch between PC and PR modes.

cortical cells after 1 min (Wan et al., 2004). For the latter hydraulic disturbance, we have similarly noted increased \(T_{1/2}\) in \(T. virginiana\) epidermal cells when applying \(P\) steps of greater than 0.15 MPa (data not shown). Thus, it appears important to limit the size of \(P\) and \(V\) steps using the pressure probe in order to obtain an accurate estimate of membrane hydraulic properties in situ. \(P\) pulses are only used to establish an empirical relation of the applied step change in pressure \((\Delta P)\) to the corresponding change in volume \((\Delta V; dV/dP)\) for each measured cell at its current \(P\), and the most common approach to establishing this relationship is to measure the maximum (end-point) \(V\) that occurs for each of a series of imposed \(\Delta P\) pulses. For a cell at constant \(P\), however, all instantaneous values of \(P\) and \(V\) for any short-term changes in either value should express the same \(dV/dP\), and this was the case whether only end-point values or all values collected during the \(P\) pulse were used (Fig. 4). As reported by Cosgrove and Durachko (1986) and expected from theory, as the size of the \(\Delta P\) pulses increase and more time is required to complete the pulse, the opportunity for water flow across the cell membrane increases and the observed relation between \(\Delta P\) and \(\Delta V\) becomes nonlinear. For large \(\Delta P\) pulses, Equation 3a of Tomos et al. (1981) can be used to estimate \(dV/dP\) near the origin, but for the small control oscillations (outside of PC, PR, and \(P\) pulse tests in Fig. 3), a simple linear regression through the oscillation data was equivalent to the sigmoidal fit (Fig. 4, inset). Also, in many cases, a sigmoidal fit could not be obtained due to the limited range of \(P\) and \(V\) values during the oscillation (data not shown). The best estimate for \(dV/dP\) will be from the smallest \(\Delta P\) values available, and in our case, the control oscillations \((\Delta P) of \pm 0.01\) MPa, which presumably cause a minimal disturbance to the cell water relations, were sufficient to obtain this relation (Fig. 4). All reported estimates of \(dV/dP\) for each cell were obtained using the control oscillations.

For PCs, the size of the step in \(P\) is set and the measured response is the change in the \(V\) of fluid in the capillary over time, whereas for PRs, the change in \(V\) is set and the change in \(P\) is measured. In both cases, the measured response is statistically fit to an exponential decay (Eq. 3 or 8) to obtain the parameters needed to

![Figure 4](image2.png)

**Figure 4.** Relationship between \(dV\) and \(dP\) for the cell shown in Figure 3, either during the period of \(P\) pulses (white symbols) or during periods when \(P\) and \(V\) were stable but oscillating due to feedback control (black circles). The \(dV\) and \(dP\) relationship is typically obtained from conventional \(P\) pulses using only the end-point values for each pulse (white triangles), but this relation is consistent with all the \(P\) pulse data (all white symbols) and consistent with the sigmoidal relation (dashed line) expected from theory (see text). The inset shows the \(dV/dP\) relation from control oscillations around the meniscus set point at each equilibrium \(P_{\text{cell}}\) (Fig. 3). Each data point is the mean \(\pm 95\%\) confidence interval of \(n = 6\) to 504 frames collected at each meniscus location as calculated from Student’s percentage \(t\) distribution (most error bars are hidden by the symbol). In the inset, the long dash line is the sigmoidal relation fit to the mean oscillation points, with the short dash lines indicating the 95\% confidence interval for this relation. The solid line within this confidence interval is a simple linear regression \((y = 0.0044x - 0.0002 [r^2 = 0.99]) fit to all oscillation data points.
Table II. Water relations parameters of individual leaf epidermal cells of *T. virginiana* measured using the ACPP to make repeated PC and PR experiments in the same intact cell

Data for each cell are means (coefficient of variation [CV]) of *n* = 3 to 4 PCs or PRs as in Figure 3, pooling both inflow and outflow results. The range for *P* <sub>ref</sub> for these cells was 0.41 to 0.83 MPa, and that for osmotic potential, determined on nearby cells, was from −1.08 to −0.70 MPa. *s*<sub>i</sub> is the initial volume flow rate observed during a PC.

| Cell | *T*<sub>1/2</sub> | *V*<sub>o</sub> | *e* | *L*<sub>p</sub> |
|------|----------------|-------------|-----|--------------|
|      | PC            | PR          | PC  | PR           | PC                           | PR                      |
|      | s  | nl | MPa | × 10<sup>−7</sup> m<sup>−1</sup> s<sup>−1</sup> MPa<sup>−1</sup> | *s*<sub>i</sub> (10<sup>−16</sup> × m<sup>−3</sup> s<sup>−1</sup>) | × 10<sup>−7</sup> m<sup>−1</sup> s<sup>−1</sup> MPa<sup>−1</sup> |
| 1    | 16.4 (13.4)   | 1.3 (6.3)   | 2.0 (18.3) | 2.0 (7.3) | 8.5 (7.3)     | 10.4 (13.3) | 18.9 | 12.9 (11.0) |
| 2    | 40.9 (13.0)   | 2.7 (36.1)  | 1.5 (16.4) | 1.6 (7.6) | 9.8 (7.6)     | 4.10 (6.9)  | 6.2  | 5.44 (32.6) |
| 3    | 43.2 (10.7)   | 1.2 (42.5)  | 2.0 (13.2) | 2.5 (10.5) | 17.0 (10.5)  | 4.54 (14.5) | 8.3  | 10.1 (69.2) |
| 4    | 37.1 (32.2)   | 1.7 (13.9)  | 1.8 (29.3) | 1.9 (4.0) | 12.8 (4.0)   | 5.34 (27.6) | 8.9  | 6.53 (16.1) |
| 5    | 17.5 (29.3)   | 1.1 (8.8)   | 1.5 (25.8) | 2.0 (5.3) | 11.1 (5.3)   | 10.4 (35.1) | 15.3 | 12.2 (11.2) |
| 6    | 29.6 (37.1)   | 1.3 (19.2)  | 1.2 (27.1) | 1.2 (1.3) | 11.0 (1.3)   | 5.80 (35.9) | 7.1  | 8.81 (21.1) |
| 7    | 42.8 (29.6)   | 1.5 (13.3)  | 1.1 (23.0) | 1.3 (2.7) | 13.7 (2.7)   | 3.85 (26.6) | 4.6  | 5.96 (13.4) |
| 8    | 32.4 (32.6)   | 1.5 (4.2)   | 2.1 (21.3) | 2.7 (11.3) | 17.3 (11.3)  | 6.65 (30.8) | 12.4 | 6.53 (15.1) |
| 9    | 33.8 (28.3)   | 2.3 (17.3)  | 1.4 (33.6) | 1.5 (7.1) | 11.2 (7.1)   | 5.20 (24.1) | 7.5  | 5.05 (20.1) |
| 10   | 17.9 (13.5)   | 1.0 (11.3)  | 1.1 (17.7) | 1.3 (2.3) | 12.7 (2.3)   | 9.08 (11.1) | 11.4 | 9.95 (9.8)  |
| 11   | 56.2 (26.4)   | 2.0 (22.1)  | 1.2 (19.4) | 1.4 (6.1) | 9.3 (6.1)    | 3.21 (24.5) | 4.1  | 6.77 (15.8) |
| 12   | 16.3 (23.0)   | 1.3 (6.6)   | 1.3 (16.3) | 1.7 (5.4) | 6.6 (5.4)    | 10.2 (21.4) | 3.5  | 15.7 (11.3) |
| 13   | 39.5 (27.3)   | 1.9 (3.3)   | 2.0 (20.0) | 2.7 (8.4) | 7.2 (8.4)    | 5.51 (33.9) | 2.4  | 11.1 (13.4) |
| 14   | 31.7 (5.8)    | 1.7 (5.1)   | 1.4 (20.1) | 1.8 (6.4) | 10.9 (6.4)   | 4.85 (12.9) | 5.3  | 7.35 (7.1)  |
| 15   | 25.4 (58.9)   | 1.1 (18.3)  | 1.4 (27.4) | 1.5 (1.6) | 7.9 (1.6)    | 7.48 (44.3) | 4.2  | 14.4 (17.4) |
| Mean ± SD | 32.1 ± 11.8 | 1.6 ± 0.5  | 1.5 ± 0.2 | 1.8 ± 0.5 | 11.1 ± 3.2   | 6.41 ± 2.48 | 8.0 ± 4.7 | 9.25 ± 3.40 |

<sup>a</sup>Optically determined *V*<sub>o</sub> was on average 2.56 nl, ranging from 1.50 to 4.93 nl.  <sup>b</sup>Average for each cell was determined from *dP*/<sup>dV</sup> during pressure oscillations and *V*<sub>o</sub> as determined from PR.  <sup>c</sup>Average of the CV values for each cell.

calculate *L*<sub>p</sub> and *V*<sub>o</sub>, as described previously (Steudle, 1993). In some cases (e.g. PR replicate 3 in Fig. 3), the variation (noise) in the response made it impossible to obtain a reliable fit (nonconvergence condition in SAS PROC NLIN), but for most cases, the fits were very good, with narrow confidence limits on the fit line (Supplemental Figs. S1 and S2) as well as relatively small se for 1/<sup>b</sup> (Supplemental Table S1), which is directly proportional to *T*<sub>1/2</sub> (Eq. 4). The average and se estimates, respectively, for 1/<sup>b</sup> for all cells were 46.0 and 0.22 s for PC and 1.95 and 0.07 s for PR. The estimates obtained for *T*<sub>1/2</sub>, ∆*V*<sub>o</sub> and *P*<sub>o</sub>−*P*<sub>e</sub> (see Eq. 5) using the same method repeatedly on the same cell were reasonably reproducible (Fig. 3). For the 15 cells of this study, within-cell repeatability was generally better for PR tests than for PC tests, with the PR method giving average coefficients of variation of 15.2%, 5.8%, and 19% for *T*<sub>1/2</sub>, *V*<sub>o</sub> and *L*<sub>p</sub>, respectively, and the PC method giving average coefficients of variation of 25.4%, 22%, and 24.2% for the same parameters (Table II). Cell volumes as determined from either method were between 1 and 2.7 nl, which was in the range of the optically estimated *V*<sub>o</sub> values of 1.5 to 4.9 nl for these cells (data not shown), and *L*<sub>p</sub> ranged from about 3 to 13 × 10<sup>−7</sup> m<sup>−1</sup> s<sup>−1</sup> MPa<sup>−1</sup>.

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**Figure 5.** Relation of *V*<sub>o</sub> (A) and *L*<sub>p</sub> (B) as determined by PR (x axis) or PC (y axis) experiments for the cells of Table II. Each data point is the mean ± SD of *n* = 3 to 4 PCs or PRs performed in the same cell as in Figure 3, pooling both inflow and outflow results. The solid line is the linear regression of PC and PR data. The linear regression statistics shown are for mean PR (x) and PC (y) points and for *V*<sub>o</sub> is *y* = 0.65*x* + 0.36 and for *L*<sub>p</sub> is *y* = 0.55*x* + 1.32 × 10<sup>−7</sup>. The dotted line indicates a 1:1 relation.
which extends to the upper range of values reported for
T. virginiana (Tomos et al., 1981) and pea (Pisum sativum)
epicotyl (Cosgrove and Steudle, 1981). There was a clear
relationship between the two methods for \( V_o \) and \( L_p \) (Fig. 5),
but in both cases, the differences increased with in-
creasing magnitude, particularly for \( V_o \) (Fig. 5). In both
cases, the slope of the linear regression was significantly
less than 1:1, and for \( V_o \), the intercept was significantly
greater than 0 (\( P = 0.03 \), but for \( L_p \), the intercept was not
different from 0 (data not shown). The only critical as-
sumptions for the calculation of \( V_o \) using either the PC
or PR are that cell total water potential does not change
from the initial to the final state and that all solutes are
retained by the cell (i.e. that the cell can be regarded as
an ideal osmometer; Wendler and Zimmermann, 1982).
For the same cell, we assumed the same value of osmotic
potential for both methods, so the systematic difference
between methods in calculated \( V_o \) indicates that there
was a systematic difference in the overall \( P \) change for a
given change in \( v \) (see Eq. 5) or \( \Delta V \) (Eq. 9). The reason
for this discrepancy is not known but currently under
investigation. Compared with the PC method, the PR
method is completed in a shorter time and does not require
an extrapolation over time to obtain \( v \), but \( V_o \)
from the PR method also includes elastic effects (\( dv/dP \);
Eq. 5) that are not included in the PC method because \( P \)
is maintained constant (Wendler and Zimmermann,
1982). Hence, it is difficult to suggest which method
should be regarded as the most accurate.

In this study, both PC and PR methods were used on
the same cell, and both methods used inflow and outflow
conditions (Fig. 3). Hence, a very powerful statistical test,
equivalent to pooling many paired comparisons, for the
effect of method and flow direction on cell water relations
parameters was possible. For all parameters, these tests
showed significant to very highly significant effects, with
the PC method giving generally lower estimates of \( V_o \)
and \( L_p \) and higher estimates for \( T_{1/2} \) than the PR
method and inflow conditions giving generally longer
\( T_{1/2} \) and lower \( L_p \) than outflow conditions (Table III).
For the same cell, \( L_p \) and \( T_{1/2} \) are closely related (Eq. 7),
but they are calculated differently for the PC and PR
methods, and in addition, the values of \( T_{1/2} \) do not
depend on an assumed cell membrane area. Hence, the
fact that there was a 12% to 27% reduction in \( L_p \) and a
corresponding increase in \( T_{1/2} \) for inflow compared with
outflow, regardless of method (Table III), is strong evi-
dence that these cell membranes and/or the membrane
complex exhibit polarity in water transport. \( T_{1/2} \) for PC will
always be longer than \( T_{1/2} \) for PR in the same cell because
cell wall elastic effects are not present in PC (Wendler and
Zimmermann, 1982), and the significant interaction term
between method and direction for \( T_{1/2} \) in Table III simply
reflects the larger difference for the larger value.

As reported by Dainty (1963), the effects of USLs in our
experiments were found to be negligibly small. For inflow
and outflow during PRs, which have the highest potential
for USL effects, the maximum USL thickness was only 0.2
to 1.39 \( \mu \)m (Ye et al., 2006; Eq. 3), compared with cell
diameters of 70 to 140 \( \mu \)m. Using our measured cell osmotic
pressure (\( \pi_c \)) values as the bulk solute concentra-
tion, maximum fluxes of water across the cell membrane
of \( 4.5 \times 10^{-7} \) m s\(^{-1} \) (from our \( L_p \) times the maximum
applied \( P \) pulse, \( dP/dV \times AV \)), and a conservative diffu-
sion coefficient for solutes in aqueous solution of \( 5 \times 10^{-10} \)
m\(^2\) s\(^{-1} \) (Dainty, 1963), the difference in solute concentra-
tion between the bulk solution and the membrane was
0.11% (Ye et al., 2006; Eq. 1). Even assuming a much lower
diffusion coefficient for the cell wall space of \( 3 \times 10^{-11} \)
m\(^2\) s\(^{-1} \) (Kramer et al., 2007), this difference is only 1.9%.
Hence, the potential effect of USLs on our estimates of \( L_p \)
is negligible.

A similar degree of \( L_p \) polarity, in the same direction,
has been reported for stem parenchyma in Saccharum
species (Moore and Cosgrove, 1991), although in their case
\( L_p \) \( \text{cm}^2\text{s}^{-1} \) \( \text{M}^{-1} \) was greater for PR (1.65) than for PC (1.16)
methods, and we found the opposite (1.11 for PR and
1.27 for PC methods). Moore and Cosgrove (1991) sug-
gested that the greater polarity found for the PR method
may have been due to the fact that this method imposed
larger changes in \( P_{\text{cell}} \) than the PC method, but in our
case, the \( P_{\text{cell}} \) changes for both methods were small and
similar (Fig. 3). Based on the loss of polarity that oc-
curred when cells were treated with mercury (Tazawa
et al., 1996), it is reasonable to propose that aquaporins
are responsible for polarity, either intrinsically, by pore
asymmetry, or by active regulation, as suggested by
Tyerman et al. (2002). If active regulation is responsible
for the asymmetry that we observed, then, particularly
for the PR method (\( T_{1/2} \) of about 1.5 s), this regulation

| Parameter       | Method | Inflow | Outflow | Method P | Direction P |
|-----------------|--------|--------|---------|----------|-------------|
| \( V_o \) (nL)   | PC     | 1.78 ± 0.49 | 1.55 ± 0.60 | 0.0014   | 0.0358      |
|                 | PR     | 1.96 ± 0.72 | 1.97 ± 0.65 |          |             |
| \( L_p \) (\( \times 10^{-7} \) m s\(^{-1} \) MPa\(^{-1} \)) | PC     | 5.28 ± 2.39 | 6.71 ± 2.83 | 0.0001   | 0.0143      |
|                 | PR     | 8.18 ± 3.62 | 9.14 ± 3.10 |          |             |
| \( T_{1/2} \) (s) | PC     | 36.7 ± 14.5 | 28.6 ± 12.7 | 0.0001   | 0.0052      |
|                 | PR     | 1.73 ± 0.71 | 1.45 ± 0.37 |          |             |
must be relatively rapid and highly reversible. The PR steps in this study were made at a time interval of about 90 s, but the ACPP (Wong et al., 2009) can make more frequent steps and, hence, will be instrumental in testing this hypothesis. Tazawa et al. (1996) have suggested that aquaporins increase both \( L_p \) as well as polarity, but if this were the case, then for the relatively wide range of \( L_p \) values that we observed in the 15 cells of this study (Table II; Fig. 5B), it would be anticipated that cells with higher \( L_p \) might be expected to exhibit higher values of \( L^{\text{OUT}}_p / L^{\text{IN}}_p \), but no such correlation was observed (Supplemental Fig. S3). Hence, these data are suggestive that the observed polarity may be an intrinsic biophysical property of the intact membrane/protein complex. Polarity may play an important role in directed solute/water transport processes, such as localized refilling of embolized xylem (Brodersen et al., 2010). A higher \( L^{\text{OUT}}_p \) than \( L^{\text{IN}}_p \) would mean that, at steady state, a smaller area of membrane would be required to support water outflow into the xylem than the area of membrane supporting inflow to the cell, possibly allowing the refilling process to be restricted to pit areas, as suggested by Brodersen et al. (2010). The same geometric principle and advantage of a higher \( L^{\text{OUT}}_p \) than \( L^{\text{IN}}_p \) may also apply generally to water uptake by roots, since the transport path is radial, and hence, the membrane area for transport decreases along the path, particularly for the cells close to the xylem.

**MATERIALS AND METHODS**

**Plant Material and Cell Turgor Measurements**

Experiments were conducted with greenhouse-grown *Tradescantia virginiana* plants. Plants were grown under greenhouse conditions in 2-L pots filled with a mixture of GrowCoir (Greenclay), clay pellets, and perlite (4:1:1 by volume) in a mixture of GrowCoir (Greenclay), clay pellets, and perlite (4:1:1 by volume) in a temperature-controlled greenhouse (day/night cycle of 30°C/20°C ± 3°C; 40%/70%; ± 10% relative humidity [RH]; and natural light with a daily maximum of 1,200 μmol photons m⁻² s⁻¹ photosynthetically active radiation). Plants were fully watered daily with a modified Hoagland nutrient solution (in mM: N O₃⁻, 6.85; NH₄⁺, 0.43; PO₄³⁻, 0.84; K⁺, 3.171; Ca²⁺, 2.25; Mg²⁺, 0.99; SO₄²⁻, 0.50; and in μmol: Fe³⁺, 28.65; Mn²⁺, 4.91; BO₃⁻, 24.05; Zn²⁺, 1.83; MoO₄²⁻, 0.17; Cu²⁺, 2.52) with electrical conductivity of 1.00 dS m⁻¹ at pH 5.75 and an osmotic potential of −0.04 ± 0.01 MPa. Plants were typically kept for several days under laboratory conditions (diffuse fluorescent light and 25°C air temperature) for measurement. \( P_{\text{cell}} \) was measured using the ACPP described by Wong et al. (2009), typically collecting \( P \) and \( V \) data at 7.5 Hz for epidermal cells in fully expanded leaves of 361 ± 85 mm in length and 20 ± 4 mm in width (mean ± SD of \( n = 9 \) leaves). In most cases, the plants were exposed to laboratory environmental conditions, but in some cases, all but the test leaf was enclosed in a clear plastic bag to minimize plant transpiration and any changes in overall plant water status over time.

The performance and sensitivity of the ACPP system were also compared with the observed value of \( \Delta P \), but if this were the case, then for the relatively wide range of \( L_p \) values that we observed in the 15 cells of this study (Table II; Fig. 5B), it would be anticipated that cells with higher \( L_p \) might be expected to exhibit higher values of \( L^{\text{OUT}}_p / L^{\text{IN}}_p \), but no such correlation was observed (Supplemental Fig. S3). Hence, these data are suggestive that the observed polarity may be an intrinsic biophysical property of the intact membrane/protein complex. Polarity may play an important role in directed solute/water transport processes, such as localized refilling of embolized xylem (Brodersen et al., 2010). A higher \( L^{\text{OUT}}_p \) than \( L^{\text{IN}}_p \) would mean that, at steady state, a smaller area of membrane would be required to support water outflow into the xylem than the area of membrane supporting inflow to the cell, possibly allowing the refilling process to be restricted to pit areas, as suggested by Brodersen et al. (2010). The same geometric principle and advantage of a higher \( L^{\text{OUT}}_p \) than \( L^{\text{IN}}_p \) may also apply generally to water uptake by roots, since the transport path is radial, and hence, the membrane area for transport decreases along the path, particularly for the cells close to the xylem.

**Microcapillary Manufacturing**

Microcapillary tips were prepared from borosilicate microcapillary glass (o.d./i.d. of 1.00/0.75 mm; Stoelting) using a micropipette puller (Kopf 750; David Kopf Instruments). Tips were beveled in a jet stream of beveling solution (Ogden et al., 1978) at an angle of approximately 45° using a microscope (100×). Tip size was controlled by adjusting beveling time (30–120 s), speed of the jet stream (2.0–2.3 m s⁻¹), and depth of the tip within the stream (25–50 μm). During beveling, pressurized air (approximately 0.37 MPa) was applied to the basal capillary end to prevent the entry of grinding compound through the open tip, and \( K_p \) of each capillary was calculated as described by Wada et al. (2011). After beveling, microcapillary tips, still under pressure, were dipped in glass cleaner (S.C. Johnson and Son) and rinsed with a jet of distilled water. Tips used in ACPP experiments had an i.d. of 2 to 3.5 μm and were typically stored for 2 d at 100% RH before usage (see below).

**Microcapillary Storage**

In preliminary experiments, microcapillaries were typically stored under laboratory conditions (45%–55% RH) before being filled with oil (dimethyl silicone fluid; Thomas Scientific) and assembled onto the ACPP. These microcapillaries could be inserted into a droplet of water and a stable oil/water meniscus established at any chosen point within the capillary by adjusting \( P_{\text{out}} \). However, it was observed that when \( P_{\text{out}} \) was changing (when \( \Delta P/dt \) was not 0), the meniscus did not move smoothly from one location to another but rather moved in jumps, appearing to be periodically “stuck” at different locations. This was not commonly observed for freshly pulled tips. The effect of tip storage at low (45%–55%) and high (100%) humidity for 14 d on this phenomenon was quantified by adjusting the feedback control parameters of the ACPP (Wong et al., 2009) to achieve approximately sinusoidal oscillations in \( P_{\text{out}} \) with a frequency of about 0.5 Hz and a variable amplitude (0.0001–0.0088 MPa) and observing the rate of change (μm s⁻¹) in meniscus location when \( dP_{\text{out}}/dt \) was not 0. This test was performed while collecting \( P_{\text{cell}} \) and meniscus location data at 30 Hz and at locations corresponding to approximately 30 and 100 μm of inner capillary diameter for each tip tested (\( n = 6 \)).

**Correcting for Surface Tension and \( K_h \)**

The surface tension (\( \gamma \) in mN m⁻¹) at a silicon oil/water interface (meniscus) will cause a pressure difference (\( \Delta P \)) across the meniscus according to the Young-Laplace equation (Ghosh, 2009):

\[
\Delta P = \frac{2 \gamma \cos \theta}{r_m} - \frac{\gamma}{r_m}
\]

where \( r_m \) is the meniscus radius (assumed equal to half the capillary i.d.) and \( \theta \) is the contact angle between the water and the capillary wall. This equation predicts a reciprocal relation between \( \Delta P \) and \( r_m \) with a slope, \( a/(2\cos\theta) \). To empirically determine \( a \), the meniscus was moved to a series of positions of different \( r \) (10–100 μm, corresponding to a distance of 65–595 μm from the tip) by changing \( P_{\text{out}} \). At each stationary meniscus location, \( P_{\text{out}} \) was measured together with \( r_m \). For values of \( 10 \) to 100 μm, \( \Delta P \) was relatively constant at 50° ± 2.9° (mean ± SD, as obtained from \( n = 8 \) images of the static meniscus). A value of \( t = 40 \text{ mN m}^{-1} \) for dimethyl silicone (Xue et al., 2006) was used together with the observed value of \( \theta \) to calculate an expected value for \( a \).

\( K_h \) was determined as the slope of the linear relationship between the rate of the volume change of water (\( dV/dt \)) in the capillary and the pressure difference between the water in the capillary (\( P_{\text{out}} \), Eq. 1) and the water in a water drop with \( r \) = 1 mm (0.00015 MPa, assumed equivalent to atmospheric pressure). Water volume in the capillary was calculated as described by Wong et al. (2009) and measured at 7.5 Hz. All data reported for \( P_{\text{cell}} \) in this work include corrections to \( P_{\text{cell}} \) for the static effects of surface tension (\( \gamma \) and \( \theta \); Eq. 1) as well as dynamic effects (\( K_h \)), according to Equation 2:

\[
P_{\text{cell}} = P_{\text{oil}} + \frac{2 \gamma \cos \theta}{r_m} \frac{1}{K_h} \frac{dV}{dt}
\]

Values of \( dV/dt \) were defined as negative for movement of the meniscus toward the tip and positive for movement away from the tip.

**PR Experiments**

PR experiments were conducted using the “meniscus-position step” function described by Wong et al. (2009), recording the change in \( P_{\text{cell}} \) over time [\( P_{\text{cell}}(t) \)] after the meniscus had reached its new set point and the volume step was complete. The change (relaxation) in \( P_{\text{cell}} \) over time is described by a monophasic-exponential function (Zhu and Steudle, 1991; Steudle, 1993):
\[ P_{\text{ad}}(t) = a \cdot e^{r \cdot t} + \epsilon \]  
(3)

where \( a = P_{\text{eq}} - P_{o} \), \( b \) is the rate constant of cell water exchange, and \( \epsilon = P_{r} - P_{o} \) is the final equilibrium \( P_{\text{eq}} \) at the end of the relaxation. The \( T_{1/2} \) of cell water exchange during a PR (\( T_{1/2}' \)) can be calculated as follows:

\[ T_{1/2}' = \frac{\ln 2}{r} \]  
(4)

In this study, data for \( P_{\text{ad}}(t) \) were only analyzed after the meniscus had reached the set point in order to exclude the elastic effects that occur during the step. An alternative approach to correct \( P_{\text{ad}}(t) \) for transient elastic effects during a PR is presented by Steudle et al. (1980). Cell volume was also determined from PRs \( (V_{o}) \) according to the theory of Malone and Tomos (1990; see their Eq. 5):

\[ V_{o} = \frac{\pi_{0}(P_{o} - P_{o})}{P_{o} - P_{o}} + \frac{(P_{o} - P_{o})}{dV/DP} \]  
(5)

where \( \pi_{0} \) and \( dV/DP \) are as already defined, \( P_{o} \) is the original equilibrium cell pressure, and \( v \) is the volume of liquid removed from or introduced to the cell. Because the ACP records \( V \) and \( P \) at a relatively high frequency (10–30 Hz), a number of alternative approaches were used to measure \( dV/DP \) (see “Results and Discussion”).

The \( \epsilon \) was calculated as follows:

\[ \epsilon = V_{c} \cdot \frac{dP}{dV} \]  
(6)

The cell hydraulic conductivity \( (L_{p}) \) was determined based on values of \( T_{1/2}' \), \( V_{o} \), \( \epsilon \), and \( \pi_{0} \), according to:

\[ L_{p}' = \frac{V_{c} \cdot h_{2}}{A \cdot T_{1/2}' (\epsilon + \pi_{0})} \]  
(7)

where \( A \) was determined from \( V_{c} \) by assuming that cells were column shaped with radius \( (r) \) and length \( (l) \). Cell dimensions were measured microscopically on 88 cells using an image-analysis software (NIH Image version 1.61; National Institutes of Health). Since the ratio of \( l \) to \( r \) was 3.8 ± 1.65 (mean ± SD, \( n = 88 \)), the mean \( l/r \) value was regarded as the representative ratio to calculate \( A \) from \( V_{o} \) with the conversion \( A = 5.77V_{o}^{1/3} \).

PC Experiments

PC experiments were conducted using the “pressure-step” function described by Wong et al. (2009), recording the change in volume over time \( (\Delta V(t)) \) after the final clamped pressure had been reached (i.e. the pressure step was complete). The change (relaxation) in \( V \) over time is described by a monophasic-exponential function (Wendler and Zimmermann, 1982):

\[ \Delta V(t) = a \cdot e^{r \cdot t} \]  
(8)

where \( a \) is total volume change in the microcapillary and \( b \) is the rate constant for cell water exchange. As for the PR, \( \Delta V(t) \) data were only used after the pressure had reached the set point in order to exclude transient elastic effects. The corresponding \( T_{1/2} \) of the volume relaxation \( (T_{1/2}' \)) can be calculated according to Equation 4, substituting \( T_{1/2}' \) for \( T_{1/2} \). Cell volume from a PC \( (V_{c}) \) was determined based on the theory of Wendler and Zimmermann (1982; their Eq. 5):

\[ V_{c} = \frac{\Delta V}{A} (\pi_{0} + \Delta P) \]  
(9)

where \( \Delta V \) is the overall change in liquid volume in the microcapillary, from the initial equilibrium state to the final equilibrium state (i.e. \( \Delta V \) at infinite time from Eq. 8). Note that Wendler and Zimmermann (1982) refer to \( \Delta V \) as “the change in cell volume,” but once \( P \) is clamped, there should be no change in cell volume. \( \Delta P \) is the applied step change in pressure. The reflection coefficient \( (\sigma) \) was assumed to be unity, which is considered a reasonable approximation when the capillary tip is located in the vacuole (Steudle, 1995; Murphy and Smith, 1998).

The cell hydraulic conductivity from PC experiments \( (L_{p}) \) was calculated according to Equation 10 (Wendler and Zimmermann, 1982). Equation 10 is independent of \( e \) but relies on \( A \) as derived from the measured \( V_{c} \):

\[ L_{p}' = \frac{s_{p}}{A \cdot \Delta P} \]  
(10)

where \( s_{p} \) is the initial slope of the volume relaxation \( (s_{p} = \Delta V/\Delta t \text{ for } t=0) \), which was determined from the fitted curve of Equation 8. As for the PR experiments, only the data after the pressure had reached its new set point were used to determine the fitted curve. \( A \) was determined from \( V_{c} \) as described for PR experiments (see above).

\( \pi_{0} \)

The cell \( \pi_{0} \) in the vicinity of the cells used for \( P \) measurements was measured directly in some cases using a nanoliter osmometer (Clifton Technical Physics) as described by Shackel (1997). The same relation between epidermal cell \( \pi_{0} \) and bulk tissue \( \pi_{0} \) was found as that reported by Nonami and Schulze (1989), so for the remaining cells, \( \pi_{0} \) was either estimated from a measurement of bulk tissue \( \pi_{0} \) or simply taken as a mean value \((-0.73 \text{ MPa})\), since all measurements exhibited a relatively narrow range \((-0.67 \text{ to } -0.83 \text{ MPa})\) and since this estimate would not affect any comparisons conducted within the same cell.

Microscope

Epidermal cells of \( T. \) virginiana leaves were viewed at \( 600 \times \) through a microscope equipped with a vertical illuminator (BHIM system; Olympus) and a \( \times 20 \) objective (L-M546; Olympus) linked to a monochrome digital camera (model CV-50, JAI Technologies), as described by Wong et al. (2009). The length, width, and thickness of the cells were measured using image-analysis software (NIH Image version 1.61).

Data Analysis

All statistical data analyses were performed using SAS (version 9.2; SAS Institute). Nonlinear regression (PROC NLIN) was used to fit the measured changes of \( P \) or \( V \) to the expected exponential decay over time following a step change in \( P \) or \( V \), respectively (Eq. 3 or 8), using only data collected after the targeted step change had been reached. Typically, data for two replicate PCs and PRs for both inflow and outflow directions were collected from each cell, as shown in Figure 3. Traditional measures of goodness of fit \((r^{2})\) are not available in PROC NLIN, but approximate \( SE \) values for the fit parameters are given. Cell parameters (e.g. \( V_{c} \), \( L_{p} \), and \( T_{1/2}' \)) were determined separately for each replicate PC or PR, and these estimates were considered as subsamples within a cell. Statistical tests (SAS PROC GLM) for the significance of flow direction, clamping method, or their interaction on cell parameters were based on considering individual cells as a factor, and hence, these tests are equivalent in power to that of a pooled pairwise comparison within the same cell.

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Figure S1. An example of PC data showing an exponential fit to the raw data of the observed change in volume within the capillary over time.

Supplemental Figure S2. An example of PR data showing an exponential fit to the raw data of the observed change in pressure over time.

Supplemental Figure S3. Lack of relation between the \( L_{p}^{\text{OC/IN}} / L_{p}^{\text{IN}} \) ratio and the average \( L_{p} \).

Supplemental Table S1. Statistical results for the parameters of the individual fit curves shown in the supplemental Figures S1 and S2.

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LITERATURE CITED

Brodersen CR, McElrone AJ, Chaot B, Matthews MA, Shackel KA (2010). The dynamics of embolism repair in xylem: in vivo visualizations using high-resolution computed tomography. Plant Physiol 154: 1088–1095
Cosgrove D, Steudle E (1981) Water relations of growing pea epicotyl segments. Planta 153: 343–350

Cosgrove DJ, Durachko DM (1986) Automated pressure probe for measurement of water transport properties of higher-plant cells. Rev Sci Instrum 57: 2614–2619

Cosgrove DJ, Ortega JK, Shrophshire W Jr (1987) Pressure probe study of the water relations of Phycomyces blakesleeanus sporangiothepores. Biophys J 51: 413–423

Dainty J (1963) The polar permeability of plant cell membranes to water. Protoplasma 57: 220–228

Dainty, Hope AB (1959) Ionic relations of cells of Chara australis. I. Ion exchange in the cell wall. Aust J Biol Sci 12: 395–411

Fricke W (2000) Water movement between epidermal cells of barley leaves: a symplastic connection? Plant Cell Environ 23: 991–997

Ghosh E (2009) Colloide and Interface Science. AK Ghosh, New Delhi, India

Hüskens D, Steudle E, Zimmermann U (1978) Pressure probe technique for measuring water relations of cells in higher plants. Plant Physiol 61: 158–163

Kramer EM, Frazer NL, Baskin TI (2007) Measurement of diffusion within the cell wall in living roots of Arabidopsis thaliana. J Exp Bot 58: 3005–3015

Malone M, Tomos AD (1990) A simple pressure-probe method for the determination of water in higher-plant cells. Planta 182: 199–203

Moore PH, Cosgrove DJ (1991) Developmental changes in cell and tissue water relations parameters in storage parenchyma of sugarcane. Plant Physiol 96: 794–801

Murphy R, Smith JA (1998) Determination of cell water-relatio parameters using the pressure probe: extended theory and practice of the pressure-clamp technique. Plant Cell Environ 21: 637–657

Nonami H, Schulze ED (1989) Cell water potential, osmotic potential, and turgor in the epidermis and mesophyll of transpiring leaves: combined pressure-clamp technique. Plant Cell Environ 3: 129–137

Ogden TE, Citron MC, Pierantoni R (1978) The jet stream microbeveler: an inexpensive way to bevel ultratine glass micropipettes. Science 201: 469–470

Oparka KJ, Prior DA (1992) Direct evidence for pressure-generated closure of plasmodesmata. Plant J 2: 741–750

Shackel KA (1987) Direct measurement of turgor and osmotic potential in individual epidermal cells: independent confirmation of leaf water potential as determined by in situ psychrometry. Plant Physiol 83: 719–722

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 J Smith, H Griffiths, eds, Water Deficits: Plant Responses from Cell to Community. Bios Scientific Publishers, Oxford, pp 5–36

Steudle E, Oren R, Schulze ED (1987) Water transport in maize roots: measurement of hydraulic conductivity, solute permeability, and of reflection coefficients of excised roots using the root pressure probe. Plant Physiol 84: 1220–1232

Steudle E, Smith JA, Lütge U (1980) Water-relatio parameters of individual mesophyll cells of the Crassulacean acid metabolism plant Kalanchoë daigremontiana. Plant Physiol 66: 1153–1163

Steudle E, Tyerman SD (1983) Determination of permeability coefficients, reflection coefficients, and hydraulic conductivity of Chara corallina using the pressure probe: effects of solute concentrations. J Membr Biol 78: 85–96

Steudle E, Zimmermann U (1974) Determination of the hydraulic conductivity and of reflection coefficients in Nitella flexilis by means of direct cell-turgor pressure measurements. Biochim Biophys Acta 332: 399–412

Steudle E, Zimmermann U, Zillikens J (1982) Effect of cell turgor on hydraulic conductivity and elastic modulus of Eclipta leaf cells. Planta 154: 371–380

Tazawa M, Asai K, Iwasaki N (1996) Characteristics of Hg- and Znsensitive water chanels in the plasma membrane of Chara cells. Bot Acta 109: 388–396

Tazawa M, Shimmie T (2001) How characean cells have contributed to the progress of plant membrane biophysics. Aust J Plant Physiol 28: 523–539

Thomas TR, Matthews MA, Shackel KA (2006) Direct in situ measurement of cell turgor in grape (Vitis vinifera L.) berries during development and in response to water deficits. Plant Cell Environ 29: 993–1001

Tamos AD, Leigh RA (1999) The pressure probe: a versatile tool in plant cell physiology. Annu Rev Plant Physiol Plant Mol Biol 50: 447–472

Tamos AD, Steudle E, Zimmermann U, Schulze ED (1981) Water relations of leaf epidermal cells of Tradescantia virginiana. Plant Physiol 68: 1135–1143

Tyerman SD, Niemietz CM, Bramley H (2002) Plant aquaporins: multifunctional water and solute channels with expanding roles. Plant Cell Environ 25: 173–194

Wada H, Matthews MA, Choat B, Shackel KA (2011) In situ turgor stability in grape mesocarp cells and its relation to cell dimensions and microcapillary tip size and geometry. Environ Control Biol 49: 61–73

Wada H, Shackel KA, Matthews MA (2008) Fruit ripening in Vitis vinifera: apoplastic solute accumulation accounts for pre-veraison turgor loss in berries. Planta 227: 1351–1361

Wan XC, Steudle E, Hartung W (2004) Gating of water channels (aquaporins) in cortical cells of young corn roots by mechanical stimuli (pressure pulses): effects of ABA and of HgCl2. J Exp Bot 55: 411–422

Wendler S, Zimmermann U (1982) A new method for the determination of hydraulic conductivity and cell volume of plant cells by pressure clamp.

Wendler S, Zimmermann U (1985) Determination of the hydraulic conductivity of Lamprothamnium by use of the pressure clamp. Planta 164: 241–245

Wong ES, Slaughter DC, Wada H, Matthews MA, Shackel KA (2009) Computer vision system for automated cell pressure probe operation. BioSystems Engineering 103: 129–136

Xue HT, Fang ZN, Yang Y, Huang JP, Zhou LW (2006) Contact angle determined by spontaneous dynamic capillary rises with hydrostatic effects: experiment and theory. Chem Phys Lett 432: 326–330

Ye Q, Kim YM, Steudle E (2006) A re-examination of the minor role of unstirred layers during the measurement of transport coefficients of Chara corallina internodes with the cell pressure probe. Plant Cell Environ 29: 964–980

Zhang WH, Tyerman SD (1991) Effect of low O2 concentration and azide on hydraulic conductivity and osmotic volume of the cortical cells of wheat roots. Aust J Plant Physiol 18: 603–613

Zhu GL, Steudle E (1991) Water transport across maize roots: simultaneous measurement of flows at the cell and root level by double pressure probe technique. Plant Physiol 95: 305–315

Zimmermann U, Hüskens D, Schulze ED (1989) Direct turgor pressure measurements in individual leaf cells of Tradescantia virginiana. Planta 149: 445–453