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Water transport across roots

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Abstract

Usually, roots are looked at as rather perfect osmometers with the endodermis being the 'root membrane' which is equivalent to the plasma membrane of cells. However, this 'single-equivalent-membrane model' of the root does not explain the findings of a variable hydraulic resistance of roots as well as of differences between hydraulic and osmotic water flow and of low reflection coefficients of roots. Recent work with the root pressure probe is reviewed and discussed which indicates that the simple osmometer model of the root has to be extended by incorporating its composite structure, i.e. the fact that there are different parallel pathways for water in the root, namely, the cell-to-cell and apoplasmic path. The new 'composite transport model of the root' readily explains the experimental findings mentioned above. Pressure probe work with roots in which the endodermis was punctured to create an additional parallel path as well as anatomical studies support the model.

Introduction

Roots are very efficient in taking up water from the soil and meet the requirements of the transpiring shoot. This means that the hydraulic conductivity of roots and its components (cell membranes, cell walls, etc.) has to be rather high. On the other hand, roots have to be sufficiently 'tight' to prevent a substantial leakage of nutrients once accumulated in the xylem. Thus, the 'passive' permeability of roots to solutes has to be low compared with that of water. The 'optimization' of the problem has led to the 'invention' of the root endodermis. This structure is thought to have rather perfect semipermeable properties in that water passes the membranes much faster than nutrient ions (usually the permeability of cell membranes to water is larger by 3 to 8 orders of magnitude than that for solutes). In addition, the Casparian band nearly completely interrupts the passage of water and hydrophilic solutes across the walls. In principle, this picture may be true. However, there are some problems with it because, in some instances, water and solute relations of roots deviate from those predicted from the simple model. The deviations include:

(i) Water flow is not linearly related to the driving force (water potential difference across root) over the entire range of forces (cf. Fiscus, 1975; Kramer, 1983; Steudle, 1989, 1992, 1993, 1994; Weatherley, 1982).

(ii) There is an offset of pressure (water potential) which has to be overcome before water is taken up into the root (Passioura, 1988).

(iii) There is some variability in the hydraulic conductivity of roots which is related to the nature of the driving force used. Osmotic water flow can be much smaller than hydrostatic. (Hallgren et al., 1994; Steudle et al., 1987; Steudle, 1989, 1992, 1994).

(iv) Root reflection coefficients are substantially lower than unity for solutes present in the xylem sap (σ_{sr} = 1 refers to the perfect osmometer and σ_{sr} = 0 to an osmotic barrier which does not distinguish between water and solute) (Steudle and Frensch 1989; Steudle and Brinckmann, 1989; Steudle, 1989, 1992, 1994).

These points are important since they either refer directly to the root hydraulics (Lp_r) or to the forces driving water across roots (σ_{sr}). Evidence for deviations comes from work of different laboratories which use quite different techniques such as the pressure-chamber technique (Fiscus, 1975), stop-flow techniques (Miller, 1985; Pitman et al., 1981), or the balancing pressure technique (Passioura, 1988). A lot of evidence concerning items (iii) and (iv) has been collected in the past few years using the root pressure probe (Azaizeh and Steudle, 1991; Birner and Steudle, 1993; Heydt and Steudle, 1991; Melchior and Steudle, 1993; Peterson et al. ,1993; Steudle, 1989, 1992, 1993, 1994; Steudle et al., 1993). In the following, this work is reviewed and discussed in relation to transport models of roots. The 'composite transport model of the root' is presented as a new concept which explains the deviations. The root pressure probe technique has been described in detail in many original contributions (see above and Hallgren et al., 1994; Melchior and Steudle, 1994).

Transport models of roots

In the traditional view, the endodermis is thought to be the main hydraulic and osmotic barrier in roots. Since root cell protoplasts are considered to represent a hydraulic resistance much larger than that of the root apoplast, the preferred path for water in the cortex is the wall path, i.e. water moves around the protoplasts. At the endodermis, the Casparian band blocks the apoplasmic passage and a transport across two membranes is required. In the stele, transport is again mainly apoplasmic up to the vessels which (when mature) represent a path of high conductance. In this picture, the endodermis with the fully developed Casparian band provides a rather perfect semipermeable barrier. Roots, like cells, have been regarded to be nearly ideal osmometers (Weatherley, 1982). Since the endodermis is considered to be 'the root membrane', the osmometer model may also be called a 'single-equivalent-membrane model' (Dainty, 1985). This implies that 'the root membrane' behaves like a 'homogenous membrane', an assumption which may be questioned.

Although the term may be not used directly, many textbooks refer to the root as a rather perfect (semipermeable) osmometer. This is so because this model fulfills two requirements for a proper functioning of roots: (i) Water uptake (or loss) may be rate-limited or controlled at the endodermis. (ii) The loss (leakage) of nutrients accumulated in the xylem by active processes is effectively prevented. The first requirement has been thought to be important to protect the root cortex against rapid changes in xylem water potential as they occur in the transpiring plant, or to dampen the stelar tissue against changes in soil water potential. At least in young, non-suberized roots, the first requirement is not fulfilled since these roots have high radial hydraulic conductivities and quickly equilibrate with their immediate surroundings. The second requirement (roots impervious to nutrients and other solutes) seems to be fulfilled. Here, most of the evidence comes from observations of the radial movement of apoplasmic dyes or other tracers which do show that the endodermis is an effective barrier for solutes (Clarkson, 1991; Marschner, 1986).

Single-equivalent-membrane model of root: quantitative formulation of transport

The analogy between the cell and root osmometer has led to the application of basic transport equations of irreversible thermodynamics to the root system which have been successfully used at the cell level (Fiscus, 1975; Steudle et al., 1987). The root has been treated as a two-compartment system with an internal compartment (xylem tracheary elements) and an external (soil solution or root medium) separated by a membrane-like barrier. The transport equations for roots describe the flow of water (or of volume in the more precise notation of the theory) per unit surface area of a root and the forces (hydrostatic or osmotic pressure gradients) driving it, as well as the solute (nutrient) flow (again per unit root surface area). In the description, interactions between flows, i.e. between the water (volume) (J_{Vr}) and the solute (J_{sr}) flow are taken into account: The latter play an important role in roots and are well-known from guttation, exudation and other root pressure phenomena. If we accept the two-compartment, root membrane model, and, for the sake of simplicity, consider the flow of only one solute (subscript's') besides the water flow, we get (Steudle, 1989, 1992, 1993, 1994):

$$J_{Vr} = -\frac{1}{A_r}\frac{dV_x}{dt} = Lp_r \cdot P_r - Lp_r \cdot \sigma_{sr} \cdot RT \cdot (C_s^x - C_s^o), \tag{1}$$

and

$$J_{sr} = -\frac{1}{A_r}\frac{dn_s^x}{dt} = P_{sr} \cdot (C_s^x - C_s^o) + (1 - \sigma_{sr}) \cdot \overline{C}_s \cdot J_{Vr} + J_{sr}^*. \tag{2}$$

Here, A_r and V_x are the root surface area and the volume of mature xylem, respectively. P_r = root pressure or tension in root xylem (atmospheric pressure is taken as the reference); σ_{sr} = root reflection coefficient; $C_s^{o,x}$ = concentration of solute 's' in the medium (superscript 'o') or in the xylem (superscript 'x'); n_s^x = amount of solute 's' in the xylem; P_{sr} = permeability coefficient of root; $\overline{C}_s$ = mean concentration of 's' in the root ($\approx (C_s^o + C_s^x)/2$). J_{sr}^* = active solute flow.

It should be noted that, according to Eq. (1), water flow has a hydrostatic and osmotic component. Osmotic and hydrostatic pressure gradients are related to water flow by different coefficients (Lp_r and $\sigma_{sr} \cdot Lp_r$, respectively). This expresses the fact that there are interactions between water and solutes as they pass the membrane which cause the differences. It is seen from Eq.(1) that hydrostatic and osmotic gradients yield the same flow (i.e. they show the same apparent conductivity) only for a semipermeable membrane or barrier ($\sigma_{sr} = 1$; $P_{sr} = 0$). If the selectivity of the membrane for the given solute 's' is reduced ($\sigma_{sr} < 1$), the osmotic flow is smaller by the factor of σ_{sr} for the same gradient applied. At an extreme, $\sigma_{sr} = 0$. This means that there is no selectivity and osmotic gradients cause no water flow. Conditions for the latter situation are found in the apoplast (such as in the root; see below).

According to Eq. (2), the solute flow (J_{sr}) has three different components. The diffusional component ($= P_{sr} \cdot (C_s^x - C_s^o)$) represents the passive permeation of solute across the root cylinder according to Fick's first law. The second term on the right side is the solvent drag which quantifies the effect of water flow on solute movement. In roots, this component will usually be negligible. The third component (J_{sr}^*) represents the active transport of 's', i.e., it relates solute flow to metabolism, e.g. to the splitting of ATP by an ATPase. J_{sr}^* provides a possibility of a regulation of solutes in roots. However, since J_{sr}^* affects concentrations (C_s^x), it will also affect water flow. The relative contribution of solute flow should be most important at low or zero transpiration. It is easily verified from Eqs. (1) and (2) that, at zero water and solute flow (J_{sr}, $J_{Vr} = 0$), the steady-state root pressure (P_{ro}) will be (Birner and Steudle, 1993; Steudle, 1994):

$$P_{ro} = -\sigma_{sr} \cdot RT \frac{J_{sr}^*}{P_{sr}}. \qquad (3)$$

It is plausible that P_{ro} increases with increasing solute pumping (J_{sr}^* being more negative) and decreases with increasing P_{sr} (increasing leak of solutes across the root cylinder). As a borderline case, Eqs. (1) and (2) incorporate the perfect osmometer ($\sigma_{sr} = 1$; $P_{sr} = 0$). However, they extend the simple model in that they allow for more realistic properties of roots, namely, some leakage of solutes ($P_{sr} > 0$) and some deviation from properties of the ideal osmometer ($\sigma_{sr} < 1$). Despite this extension, the idea of a homogeneous 'root membrane' and the simple two-compartment model still forms the basis of the concept given by Eqs. (1) and (2)

The model has been successfully used to explain experimental data. Most importantly, the linear relationships between flows and forces have been basically verified in experiments. However, the two-compartment, single-equivalent-membrane model has been criticized and more complex models have been proposed such as models consisting of three or more compartments in series (Newman, 1976). In fact, one could think of multi-compartment models in which each cell layer represents a compartment. In other models, the compartmentation of the root into apoplast and symplast has been considered to be important: solutes are transferred between compartments and water follows changes in osmotic pressure ('canal model of root', Katou and Taura, 1989).

Multi-compartment models require detailed information about the transport across borders of different compartments, their sizes, internal concentrations etc. These data are usually not available. In addition, all complex series compartment models are not easily handled. Therefore, it has been claimed that the root-membrane model should persist for practical reasons and deviations would have to be interpreted in terms of variable transport coefficients (Lp_r, σ_{sr}, P_{sr}) or active components (J_{sr}^*) (Dainty, 1985). However, this point of view does not seem to be convincing. An alternative would be to use the simple approach as a starting point and to extend it, if necessary, which in turn requires techniques to measure relevant transport parameters. Such concepts are briefly reviewed in the following. First, some evidence is presented in favor of the simple model. Then, extensions are given to correct for non-linear force/flow relations. This is followed by the presentation of a new root model which also accounts for deviations, but, for the first time considers different parallel transport pathways in the root. The concept of an osmotic barrier exhibiting transport properties of a homogenous membrane is given up and a 'composite barrier model' is used to describe water and solute transport across roots still using the basic concepts of irreversible thermodynamics.

Evidence for the single-equivalent-membrane model of the root

Most of the evidence for the model comes from experiments in which excised roots freely exude xylem sap.

In early studies, the differences between the osmotic concentrations of xylem sap and root medium was found to be proportional to the (osmotic) water flow in these experiments (for a review, see Anderson, 1976). Also in the absence of a concentration difference, there was a 'non-osmotic' water flow which is understandable if there is an active uptake of solutes which drags some water along with it so that, at the steady state, $J_{sr}^{*}/J_{Vr} = C_s^x$. In other experiments, the rate of exudation was increased by pressurizing the root system or by applying a vacuum to the cut root surface (Cruz et al., 1992; Fiscus, 1975; Mees and Weatherley, 1957). It was found that, in general, pressure-volume curves were curvilinear and the apparent Lp_r increased with increasing flow. Rather than rejecting the simple model, this was interpreted as being due to a dilution effect in the root xylem, i.e. part of the force driving the flow did vary with J_{Vr} (Fiscus, 1975).

In other exudation experiments, 'stop flow techniques' were used. A stationary root exudation (constant J_{Vr}) was first established which was then stopped (i.e. J_{Vr} set zero) by either increasing the osmotic pressure of the medium or by applying a hydrostatic pressure pulse to the cut surface of the root (Miller, 1985; Pitman et al., 1981). These experiments yielded values similar to those obtained from the earlier measurements of steady-state flows. There were also indications of a $\sigma_{sr} < 1$ (Miller, 1985). Values of Lp_r obtained by different techniques were similar, although the comparison of the data obtained by using either osmotic or hydrostatic pressure gradients yielded a fairly broad spectrum of Lp_r values. There were clear indications of a lower Lp_r in the presence of osmotic forces than in the presence of hydrostatic (Cruz et al., 1992; Hallgren et al., 1994; Steudle, 1989; Steudle et al., 1987).

Deviations from the simple model: root pressure probe measurements

The trend of Lp_r(osmotic) < Lp_r(hydrostatic) continued when the root pressure probe was introduced. The technique has been explained in detail in earlier publications (see, for example, Hallgren et al., 1994; Melchior and Steudle, 1994). It is based on the measurement of root pressure in excised roots using a little manometer adapted from the conventional cell pressure probe. Root pressure is measured by tightly fixing the excised root to the equipment without interrupting xylem tracheary elements. This yields the steady-state root pressure (P_{ro} in Eq.(3)) which is a measure of the active pumping of a root. Root pressures and transport properties of roots can be measured either with root tips, segments, or whole root systems. In a single root tip, measurements are usually possible for one to two days. In root systems, measurements have been performed for up to 10 days (see below).

Hydraulic data (hydraulic conductivity of root, half time of water exchange between root and medium) are obtained by either changing the root pressure with the aid of the equipment or by changing the osmotic pressure of the medium to induce an osmotic water flow. In a variation of the technique, J_{Vr} and Lp_r are obtained by pressure clamp (Steudle and Frensch, 1989). Thus, there are different techniques to evaluate Lp_r. As already mentioned, the Lp_r obtained with the technique using hydrostatic gradients was larger and in some cases much larger (one to three orders of magnitude) than that obtained by osmotic gradients, although this did not hold for all species investigated. Thus, the trend was similar to that known from the literature (see above), but could be worked out much more precisely.

In contrast to the simple exudation technique, root pressure probe techniques also provided data of solute parameters (σ_{sr} and P_{sr}). Reflection coefficients were determined by changing the osmotic concentration of the root medium and recording the maximum change in root pressure at $J_{Vr} = 0$. For roots of herbaceous species, reflection coefficients were substantially smaller than unity and ranged between 0.5 and 0.8 even for solutes for which cell membranes exhibit a $\sigma_s \approx 1$ (salts, sugars, PEG, mannitol, urea, etc.). Careful investigation showed that the result could not be explained by unstirred layers (Steudle and Frensch, 1989). Hence, the simple single-equivalent-membrane model of the root in which the root membrane represented a nearly semipermeable barrier (see above) was questioned (Steudle et al., 1987; Steudle, 1989, 1992, 1993, 1994). Despite the low reflection coefficient, permeability coefficients of roots were low which demonstrated that the roots used were not unusually leaky. Absolute values of P_{sr} for salts, sugars and mannitol were of an order of magnitude similar to those of root cells. In studies with fluorescent dyes it was determined that solutes could not flow across the cortical apoplast in the area where the root was sealed to the probe (Peterson and Steudle, 1993). If there were such a flow, this would have artifactually increased the permeability of the system.

Longitudinal vs. radial transport

Roots were also checked for a substantial contribution of the longitudinal (axial) hydraulic resistance (Frensch and Steudle, 1989; Melchior and Steudle, 1993). It was found that the longitudinal component was usually negligible at distances from the root tip where xylem had already matured (about 20 mm in young corn roots and 30 mm in onion roots grown in hydroponics). Using the root pressure probe, both longitudinal and radial hydraulic resistances have been worked out along the developing root to determine how both components would influence the efficiency of roots for water uptake. The results showed that, except for the tip regions of roots, the simple two-compartment model which neglects the axial component provides a good basis for the evaluation of data. If the axial component were co-limiting transport along with the radial, this would complicate the situation and more sophisticated models would have to be used (Frensch and Steudle, 1989).

Axial resistances obtained with the root pressure probe for maize and onion have been compared with those calculated from Poiseuille's law. In maize, calculated values were smaller (conductances higher) by a factor of 2 to 5 than the measured. Near the base and the tip of onion roots grown in hydroponics, measured and calculated axial resistances were similar. However, at distances between 200 and 300 mm from the apex measured values were smaller by more than an order of magnitude than the calculated, probably, because of remaining cross walls between vessels members. During root development, the axial resistance in onion was reduced by three orders of magnitude. The differences between measured and calculated values of axial hydraulic resistances are known from the literature. They may be referred to the fact that the assumption of ideal cylindrical capillaries does not hold or to difficulties in identifying all vessels in stained cross sections (Frensch and Steudle, 1989; Melchior and Steudle, 1993).

In maize, the radial hydraulic conductivity did not change at distances between 20 and 120 mm from the tip where the Casparian band of the endodermis was already developed but no suberin lamellae were present. In onion roots, the radial hydraulic conductivity decreased at distances larger than 150 mm from the tip which was correlated with suberin lamellae in the endodermis and/or with the maturation of the exodermis (Melchior and Steudle, 1993).

Negative root pressure

In order to test whether or not the transport parameters measured with the root pressure probe in the range of positive pressures would also apply to the range of negative pressures (tensions in the xylem), the technique was also used in the range of negative pressures using young maize roots as the objects (Heydt and Steudle, 1991). This test is important because it may be argued that parameters (Lp_r, P_{sr}, σ_{sr}) could change when switching to tensions in the xylem (the condition usually occuring in the transpiring plant). Tensions (negative pressures) are not easily created in the measuring system because of the danger of cavitations. The system had to be kept free of any air bubbles prior to the experiments. This means that air also had to be removed from the root tissue by vacuum infiltration. Under these conditions, tensions of as large as - 0.2 MPa (- 2 bar) could be created by applying hypertonic solutions to the root. Osmotic experiments yielded similar Lp_r, P_{sr}, and σ_{sr} values in the presence of tensions as were obtained at positive root pressures. It was also established that infiltration did not change the transport properties of roots. The experiments indicated that, at least for maize, there was no change in transport properties when switching from an overpressure to tensions in the root xylem.

Combination of root and cell pressure probes

In order to test current models of preferred pathways for water in the root cylinder, hydraulic measurements have also been performed at the level of root cells by combining the cell and root pressure probes (Steudle and Jeschke 1983; Steudle et al., 1987; Steudle and Brinckmann, 1989; Zhu and Steudle, 1991). Neglecting the apoplasmic component, the data allowed an estimation of root Lp_r using the measured cell Lp. The calculated root Lp_r was then compared with the measured. Provided that water flow was mainly from cell to cell, the calculated and measured Lp_r should be similar. However, if there were a substantial apoplasmic component, the measured Lp_r should be larger than the calculated. For some species (barley, bean), the data indicated a preference for a cell-to-cell transport, regardless of whether hydrostatic or osmotic gradients were used to drive water flows across the roots. In others (maize, onion), the measured Lp_r was much larger than expected from the cell Lp when hydrostatic gradients were applied. When osmotic gradients were used, transport occurred mainly from cell to cell. These findings indicated that, at least in some species, the transport pathways changed with the driving force used. In hydrostatic measurements, flow was mainly around protoplasts, whereas in osmotic it was from cell to cell. It has been concluded that the differences between species may be related to the developmental state of the endodermis. If the Casparian band effectively interrupts water flow, this would result in a preferred cell-to-cell transport across the root cylinder regardless of the nature of the driving force. If there were differences, this should point to either an apoplasmic water flow in the endodermis or to flow in regions in the endodermis lacking an intact Casparian band (e.g., root primordia; see below).

Simultaneous measurements at the cell and root level have been also performed (Zhu and Steudle, 1991). These experiments did show how changes in the water potential (osmotic pressure) of the root medium propagated across the root cortex and eventually reached the xylem. From the stationary changes in cell and root pressure, reflection coefficients were evaluated for both cortical cells and the root during the experiment. The data demonstrated that reflection coefficients decreased for cells with increasing distance from the root surface which was in agreement with the composite transport model. However, when the cell σ_s was measured with the roots not attached to the root pressure probe, the σ_s was close to unity as expected from the composite transport model (Azaizeh and Steudle, unpublished). In the presence of an osmotic gradient between xylem and medium, there will be a counterflow of water in the root which should effect not only the overall root reflection coefficient but also that of living tissue cells. Thus, the experiments in which the cell σ_s has been measured in the presence and in the absence of osmotic and hydrostatic gradients between xylem and medium are a further indication for the validity of the model.

By measuring the initial water flow, the simultaneous measurement of hydraulic properties at the cell and root levels also allowed an estimation of the hydraulic conductivity of the apoplasmic path (Lp_{cw}; Zhu and Steudle, 1991). This was done by comparing the cell-to-cell component with the overall flow. Data of Lp_{cw} are not easy to obtain for technical reasons. However, they are badly needed for modelling of water relations of plant tissue.

Effects of external factors on root hydraulics: high salinity and anoxia

The root pressure probe technique may be employed to evaluate the effects of external factors on the ability of roots to take up water and solutes (nutrients). The most important factors would be: low temperature, high salinity, drought, mycorrhiza, and anoxia. To date, effects of salinity and anoxia have been studied in detail. The precise mechanism(s) of the adverse effects of high salinity on the root are still not fully understood. They may include osmotic effects as well as toxicity caused by certain ions (Cheeseman, 1988; Epstein, 1985). Reductions of growth could be caused by an inhibition of water uptake due to a reduced Lp of root cells. This, in turn, may result in a reduced overall hydraulic conductivity of the roots (Lp_r). It is known that effects of salinity could, in part, be reversed by calcium (Cramer et al., 1986; Lynch et al., 1987). It is thought that at high salinity, Na^+ competes for Ca^{2+} at the cell membranes and this has deleterious effects on the proper function and integrity of the membranes. In order to determine the effects of high salinity on the water relations of roots, Azaizeh and Steudle (1991) performed root pressure probe experiments using young maize roots. The hydraulic conductivity of the cell membranes of cortex cells was measured as well (Azaizeh et al., 1992). Maize seedlings grown in a nutrient solution plus 100 m*M* NaCl showed an Lp_r which was reduced by 30 to 60% compared to the control (1/5 Hoagland solution; 0.5 m*M* Ca). Increased levels of Ca (10 m*M*) had an ameliorative effect on the Lp_r of salinized roots so that the water permeability increased again, thus, increasing the availability of water for the plant. It was also shown that the effects were much larger at the cell than at the root level. High salinity (100 m*M* NaCl) caused a reduction of the cell Lp by a factor of 3 to 6 which was reversed by a factor of 2 to 3 in the presence of calcium. The differences in the effects of NaCl on either the root Lp_r or the cell Lp were due to the fact that in the roots the water flow was mainly around cells under hydrostatic conditions (see above). The findings of a reduced root Lp_r are in line with those of Munns and Passioura (1984) and O'Leary (1969) for bean and lupin. Conversely, salinity had no effect on Lp_r in barley, tomato, and sunflower (Munns and Passioura, 1984; Shalhevet et al., 1976). The findings are at variance with those of Tyerman et al. (1989) who found no change of the root cell Lp of *Nicotiana tabacum* at high salinity, i.e. for a plant which can grow reasonably well at high salinity (Flowers et al., 1986)

Similar to increased salinity, anoxia also has a negative effect on root Lp_r as shown in root pressure probe experiments with maize roots (Birner and Steudle, 1993). It is likely that the reason for the decrease of Lp_r was also due to a decrease in the hydraulic conductivity at the level of cell membranes (Lp). In addition to the changes in water transport reported in the literature (e.g. Everard and Drew, 1987; Zhang and Tyerman, 1991), the results obtained with the root pressure probe allowed quantification of changes in root pressure, the permeability of roots to nutrients (P_{sr}), and of the selectivity of roots (σ_{sr}). The stationary root pressure of maize roots strongly decreased during anoxia to reach a low value after several hours. Since ion leakage from the roots (permeability coefficient, P_{sr}) was also reduced at a constant σ_{sr}, this indicated that the active uptake of solutes (nutrient salts) was inhibited (Eq.(3)). Remarkably, roots treated in anoxia for several hours did not recover as quickly as they changed their transport properties. In the experiments, great care was taken to characterize the physiological status of the roots by performing osmotic experiments and other tests which proved that, despite anoxic conditions, the roots were still properly functioning as osmometers.

The two examples presented above demonstrate that the root pressure probe may be used to determine changes in both water and solute uptake of roots in response to environmental stresses such as pollutants, low pH, heavy metals etc. Combining the approach with measurements at the cell level using the cell pressure probe makes the method even more powerful, since this combination reveals deeper insight into mechanisms.

Modified roots: root steaming, dissecting, and puncturing

Work with modified roots provides another tool for getting a deeper insight into the mechanisms of water and solute flow in roots. Root properties may be modified by removing part of the cortex by scraping or by microdissection (Peterson et al., 1993). Parts of roots may be also killed by steaming, thus, removing the entire barrier for radial water flow except for that of the lateral walls of mature vessels (Peterson and Steudle, 1993). Another possibility of modifying roots is the puncturing of the endodermis with needles (e.g. with the tip of a cell pressure probe) to create small holes of some ten μm in diameter (Steudle et al., 1993).

The puncturing of the endodermis offers the possibility to create additional 'apoplasmic by-passes' which, by area, are only a fraction of 10^{-2} to $10^{-3}\%$ of the entire surface area of the endodermis. It may be expected that this would affect the hydraulic conductivity, solute permeability, and reflection coefficients of roots and should allow one to extrapolate to the possible size of by-passes in the intact system. The experiments indicated that only small by-passes (leaks) were required to considerably reduce root pressure and to substantially lower σ_{sr} at a fairly constant hydraulic conductivity of the root. It should be noted that usually puncturing did not result in a root pressure of zero. This means that, according to the pump/leak model of the root (Eq.(3)), the passive leak of ions could be compensated for by active pumping. Thus, the by-passes present in the intact system such as those across the root primordia, across the root tip, or some leakiness of Casparian bands could be sufficient to cause a $\sigma_{sr} < 1$ as found. On the other hand, the fact that the root hydraulic conductivity (half-time of water exchange) did not increase significantly upon puncturing, would be simply explained by the high overall Lp_r of the intact system. Thus, at least for young maize roots, the radial hydraulic resistance of roots is more evenly distributed across the living tissue. The result contradicts the general view that the endodermis represents the main hydraulic resistance in roots. By contrast, the puncturing experiments did show that the endodermis was the main barrier for solutes, a conclusion which would be in line with the traditional view of the function of the endodermis. The puncturing experiments demonstrated that only small apoplasmic by-passes would substantially reduce σ_{sr} and substantially increase P_{sr}. In maize, a careful quantitative evaluation of apoplasmic pathways in the root endodermis lacking a Casparian band (root primordia, root tip) showed that these would be sufficient to explain the low reflection coefficients (Steudle et al., 1993). Thus, the puncturing experiments did support the composite transport model.

Results from experiments in which roots were scraped or dissected were in line with the findings in the puncturing experiments. As long as the endodermis was not damaged by the manipulations, root pressure remained high or recovered after healing of the endodermis. The hydraulic conductivity of the modified roots only increased substantially when considerable amounts of cortical tissue were removed.

In the steaming experiments, the manipulation was most severe. Killing of a few millimeters of a young maize root of a total length of about 100 mm resulted in an immediate decrease in root pressure to nearly zero and in an increase of the overall Lp_r from which the hydraulic conductivity of the steamed zone could be evaluated. The Lp_r of the steamed zone was larger than that of the intact root by a factor of 3 to 13. In the steamed zone, the remaining radial hydraulic resistance could be attributed to the walls of mature xylem vessels. This allowed measurement of the lateral hydraulic conductivity of the walls of the vessels (Lp_x). It turned out that, although Lp_x was much higher than Lp_r, the contribution of the lateral hydraulic resistance to the overall radial resistance was not negligible.

Tree roots

At a first glance, tree roots or better tree root systems grown in soil may be considered to be objects which, because of their size and heterogeneity, are much more difficult to handle with the root pressure probe than roots of herbaceous species which can be easily grown in hydroponics. Fortunately, this is not the case. In some respects, roots of woody species are even easier to handle. For example, when tree root systems are fixed to the root pressure probe, there is practically no danger of crushing the woody xylem in the sealing area (as sometimes occurs with herbs). In addition, excised root systems of tree seedlings may be used for longer periods of time. For example, with oak seedlings, root pressure could be measured up to ten days.

To date, root systems of saplings of oak (*Quercus robur* and *Q. petraea*), walnut (*Juglans regia*), and Norway spruce (*Picea abies*) have been investigated with the pressure probe (Steudle and Meshcheryakov, in preparation; Hallgren et al., 1994). Trees were grown in sand culture and watered regularly with nutrient solution. This provided a defined osmotic pressure in the soil solution and made possible a rapid exchange of the regular soil solution for solutions to which osmotica were added (osmotic experiments). Prior to the experiments, the trees were well watered and shoots enclosed in plastic bags to prevent transpiration and to fully hydrate the plants so that upon excision no or little air entered the xylem. Root systems were allowed to exude for some time before they were attached to the root pressure probe to build up a steady root pressure which took several hours. Then experiments were performed (for a detailed description, see Hallgren et al., 1994) which involved typical root pressure relaxations (osmotic and hydrostatic), but also pressure clamp experiments and experiments in which the pressure chamber was combined with the root pressure probe. The pressure chamber was also used to perform classical root exudation under pressure (Fiscus, 1975). The different approaches allowed us to cross check the validity of root pressure probe data. In order to express water flows and root hydraulic conductance on the basis of root surface area, the root systems were removed from the sand after the experiments and stained with toluidine blue for better contrast. Surface areas were then determined using an image analyzing system.

Root pressures of the two ring-porous trees were 0.05 to 0.15 MPa (0.5 to 1.5 bar) and much larger than those of the conifer (-0.001 to +0.004 MPa; -10 to + 40 cm of water column) which is in agreement with literature data. Although the exchange rates for water between xylem and soil solution were fast in hydrostatic experiments, the hydraulic conductivity was, on average, smaller by an order of magnitude than that usually observed in herbaceous species. The difference may be explained by differences in the root anatomy and may be mainly due to the larger degree of suberization of the tree roots. For oak, the contribution of fine roots (diam.: < 1.0 mm) to the overall surface area was 80% of the total and thus the absolute value of Lp_r did not change very much assuming water flow occurred only in fine roots. Thus, per unit surface area, tree roots exhibited a hydraulic resistance higher than that of roots of non-woody species. Nevertheless, half-times of water flow equilibration were short because of the large surface area of roots (a few seconds or even smaller). This means that the root systems would tend to have water potentials close to those of the soil. However, this is only true in the presence of hydrostatic gradients. In osmotic experiments, half-times of tree roots were larger than in hydrostatic by one to three (!) orders of magnitude and the Lp_r was smaller by the same factor. Thus, the large differences found in some herbaceous species between osmotic and hydrostatic water flow was even more pronounced in trees.

As already discussed, the large differences between hydrostatic and osmotic hydraulic conductivities suggest differences in the transport pathways. In terms of the composite transport model they are understandable. If the model applies, the large differences in Lp_r would then also suggest smaller reflection coefficients. These were found. Reflection coefficients for salts, sugars, mannitol etc. were as small as $\sigma_{sr} = 0.1$ to 0.5, i.e. they were, on average, smaller by a factor of two than those measured for herbs (see above). Low reflection coefficients were found in trees in spite of very low permeability coefficients which were usually not measurable with the root pressure probe. The apparent contradiction is also solved by the model.

Low reflection coefficients and low osmotic Lp_r should be important during osmotic or water stress (drought) which may require some osmoregulation of roots. We may conclude from the data that, under these conditions, osmotic processes in tree roots may be slow. On the other hand, the data indicate that tree roots represent an efficient hydraulic system under well-watered conditions and in the presence hydrostatic gradients driving water flow. It would be interesting to see if hydraulic properties (Lp_r) would change in roots during water stress. This could be checked with the root pressure probe. Other interesting areas of application would be effects of low pH or toxic metals, effects of mycorrhiza, or of low temperature on both hydraulic properties and active and passive solute (nutrient) relations.

Composite transport model of root

The root pressure probe data briefly reviewed above as well as other evidence strongly indicate deviations from the single-membrane-equivalent model. Structurally, there is a basis for deviations from the simple model because of the existence of different parallel pathways for water and solutes such as the apoplasmic and cell-to-cell path, or root zones of different permeability patterns. This would enhance the composite character of roots due to different tissues arranged in series (see above). There is considerable experimental and theoretical evidence which indicates that the model has to be extended. Mainly, the evidence is based on (i) the finding of low root reflection coefficients (σ_{sr}) and of apoplasmic by-passes of solutes, (ii) apparent differences between osmotic and hydrostatic root Lp_r, and (iii) the effect of water flow on the absolute value of root Lp_r.

These findings are directly related to basic functions of roots. They are important for the limitation and control of water and solute flows across them. Changes of root Lp_r and σ_{sr} will directly affect the efficiency of roots for taking up water. They may be readily explained in terms of a new root model which has been termed a 'composite transport model of the root'. The model integrates the different experimental findings listed above as well as known anatomical features of roots. It focuses on the fact that there are parallel transport elements in the root. Obvious parallel transport pathways are the apoplasmic and cell-to-cell path, but also root zones of different states of development. The theory of the transport properties of composite membranes have been investigated in detail by Kedem and Katchalsky (1963). Although the root system is a much more complicated structure, basic results of the theory may be applied.

The model is depicted in Figure 1. The root interior (xylem) is separated by the root cylinder from the medium. Due to the active uptake of solutes (nutrients) into the root xylem the internal concentration is increased and a root pressure is built up. Along the cell-to-cell path (superscript 'cc') the uptake of water will be driven by the osmotic pressure difference which is fully effective since the reflection coefficient here is close to unity. Along the parallel apoplasmic path (superscript 'cw'), the reflection coefficient will be close to zero, and there will be very little water flow into the root along this path. However, along the apoplasmic path there will be a backflow of solution caused by the overpressure in the xylem. Hence, the hydrostatic pressure difference developed in the system at zero water flow will be smaller than the osmotic which means that $\sigma_{sr} < 1$ as found experimentally. For a system with two parallel pathways such as in Figure 1, the overall reflection coefficient (σ_{sr}) would be (Steudle, 1989, 1992, 1993, 1994):

$$\sigma_{sr} = \gamma^{cc} \cdot \frac{Lp^{cc}}{Lp_r}\sigma_s^{cc} + \gamma^{cw} \cdot \frac{Lp^{cw}}{Lp_r}\sigma_s^{cw}. \qquad (4)$$

$Lp^{cc,cw}$ = hydraulic conductivity of the cell-to-cell and apoplasmic pathway, respectively. $\gamma^{cc,cw}$ = fractional contributions of cross-sectional areas of pathways to the overall root area. Lp_r = overall hydraulic conductivity ($Lp_r = \gamma^{cc} \cdot Lp^{cc} + \gamma^{cw} \cdot Lp_{cw}$. From Eq. (4) it is evident that, provided that the apoplasmic passage across the root exhibits a fairly low σ_s^{cw} (e.g., $\sigma_s^{cw} \approx 0$) and a fairly high Lp^{cw}, this component could still contribute to the overall σ_s although the cross-sectional area of the apoplast is small. The physical reason for the effect resulting in a low σ_{sr} is that, in the presence of two parallel pathways exhibiting different values of σ and Lp, opposing water flows would be created in the root which would result in a circulation flow of water. Thus, the model explains the low σ_{sr}. According to Eq.(4), the overall reflection coefficient is a weighted mean of the reflection coefficients of individual pathways which contribute according to their

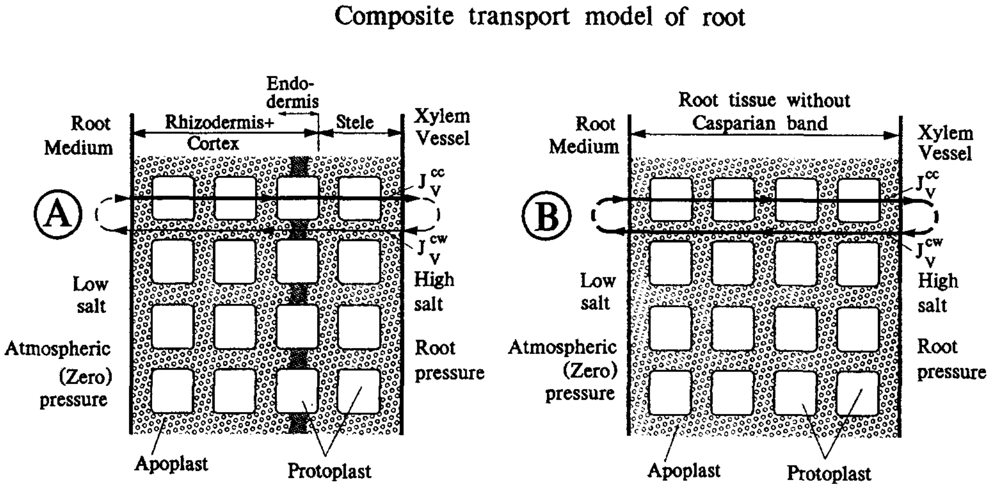

Fig. 1. Diagrammatic scheme of composite transport in a plant root. (A) denotes transport across the root having an endodermis and (B) an area of the root lacking a Casparian band (root primordia or root tip). For the sake of simplicity, rhizodermis and cortex (plus endodermis) are represented by three cell layers and the stelar tissues by a single layer. Different routes of radial flow are denoted, i.e. the flow across the apoplast (superscript 'cw') and the cell-to-cell path (superscript 'cc'). The figure indicates that there will be an osmotic water flow along the cell-to-cell path (J_V^{cc}) directed from the root medium into the xylem (stele) as long as the osmotic concentration in the xylem is larger than that in the medium. The reflection coefficient along this path will be high ($\sigma_s^{cc} \approx 1$). However, as the root pressure builds up in the absence of transpiration, there will be a backflow of water (xylem solution) across the non-selective apoplasmic path ($\sigma_s^{cw} \approx 0$) in (B) and, perhaps, also in (A), if the mature Casparian band is somewhat permeable to water. Opposing water flows or a circulation flow of water across the root result. Opposing flows exactly cancel at a zero net water flow across the root. Under these conditions, the steady-state root pressure will be smaller than expected from the difference in osmotic pressures, i.e. σ_{sr} will be smaller than unity. For further explanation, see text.

hydraulic conductance. The permeability of pathways is not involved and may be still low so that, despite a low σ_{sr}, the overall P_{sr} (leak rate for solutes) is still sufficiently low to provide a high retention of nutrients within the xylem.

With respect to the differences between osmotic and hydraulic Lp_r, the model predicts that in the apoplasmic path the effective osmotic driving force should be low when osmotic gradients are applied to the root. Consequently, the osmotic flow would be low despite a high potential hydraulic conductance of the apoplasmic path. By contrast, the apoplasmic path could be fully used in the presence of hydrostatic gradients. It is clear from the model that a complete interruption of the apoplasmic path (Casparian band) would cause the osmotic and hydrostatic flows to be similar as found for some species. Thus, the variability in root water flow would largely depend on the existence of discrete apoplasmic by-passes lacking Casparian bands and on the developmental state (tightness) of the Caspariarn band itself.

Similarly, a dependence of the root hydraulic conductivity (Lp_r) on the water flow could be understood by the model. At low or zero water flows, the hydraulic resistance of the system would be high because flows would, in part, counterbalance. However, as the hydrostatic gradient across the system is increased by the onset of transpiration, the situation will change and water will flow across the root in the same direction on both pathways.

The model (Eq. (4)) predicts that in roots having a small overall Lp_r (such as the roots of trees; see above), the relative contribution of apoplasmic by-passes to the overall value of σ_{sr} should be larger than in roots having a higher Lp_r (such as the roots of herbs). Thus, the model explains the differences in σ_{sr} values between herbaceous and woody species.

The composite transport model explains important deviations in root transport from that of a nearly perfect osmometer which, in part, have been known for a long time. The model has a sound physical and anatomical basis and predicts that, despite of the deviations, roots will still exhibit basic functions of an osmometer.

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