

OPINION PAPER

# Root pressure and beyond: energetically uphill water transport into xylem vessels?

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## Abstract

**The thermodynamics of root pressure remains an enigma up to the present day. Water is transported radially into xylem vessels, under some conditions even when the xylem sap is more dilute than the ambient medium (soil solution). It is suggested here that water secretion across the plasma membrane of xylem parenchyma cells is driven by a co-transport of water and solutes as previously shown for mammalian epithelia (Zeuthen T. 2010. Water-transporting proteins. *Journal of Membrane Biology* 234, 57–73.). This process could drive volume flow ‘energetically uphill’, against the free energy gradient of water. According to the model, solutes released by xylem parenchyma cells are subsequently retrieved from the sap at the expense of metabolic energy to maintain the concentration gradient that drives the water secretion. Transporters of the CCC type known to mediate water secretion in mammalian cells have also been found in *Arabidopsis* and in rice. The mechanism proposed here for root pressure could also explain refilling of embolized vessels. Moreover, it could contribute to long-distance water transport in trees when the cohesion–tension mechanism of water ascent fails. This is discussed with respect to the old and the more recent literature on these subjects.**

**Key words:** Aquaporin, CCC transporters, cohesion–tension (CT) theory, embolism repair, reflection coefficient, root pressure, water ascent, water co-transport hypothesis, xylem refilling.

## Introduction

The aerial parts of vascular plants are supplied with water and nutrients via the xylem conduit, a network of pipelines extending from roots to leaves. In the absence of transpiration (e.g. when the atmosphere is saturated with water vapour) the root can maintain a positive pressure in the xylem sufficient to push water upwards against the gravitational field of the earth, thus providing the shoot with water required to maintain growth. Droplets form at the leaf margin when xylem sap is secreted via hydathodes, giving rise to guttation. A closely related phenomenon extensively studied by plant physiologists is the continuous

exudation of fluid by a detached root system (‘root pressure exudation’). Xylem sap is secreted at the cut after excision of the shoot. When the cut surface of the root is sealed, a hydrostatic pressure, the ‘root pressure’, is built up in xylem vessels until a steady pressure level of up to ~0.4 MPa (Knipfer and Fricke, 2010) is reached. This pressure can be measured by attaching a miniature pressure sensor (‘root pressure probe’; Steudle and Jeschke, 1983) to the detached root system. These phenomena have puzzled and inspired scientists since Hale’s first report in 1727; in fact the mechanistic and thermodynamic aspects are

Abbreviations: EK, +Nernst potential for K<sup>+</sup>; J<sub>V</sub>, overall net volume flow; J<sub>V,CCC</sub>, volume flow mediated by CCC transporter; J<sub>V,Ψ</sub>, volume flow generated by water potential gradient; J<sub>V,P</sub>, hydrostatic pressure-driven volume flow; J<sub>V,π</sub>, volume flow driven by a gradient in osmotic pressure; k, conductance parameter to decide whether water transport is ‘energetically uphill’; L<sub>P</sub>, hydraulic conductance of the plasma membrane of xylem parenchyma cell; ΔP<sub>xpc</sub>, turgor pressure of xylem parenchyma cell (= P<sub>cytosol</sub>–P<sub>apoplast</sub>); Δπ<sub>xpc</sub>, difference in osmotic pressure between xylem parenchyma cell and stelar apoplast (= π<sub>cytosol</sub>–π<sub>apoplast</sub>); ΔΨ<sub>xpc</sub>, difference in water potential between xylem parenchyma cell and stelar apoplast (= Ψ<sub>cytosol</sub>–Ψ<sub>apoplast</sub>); ΔV<sub>M</sub>=membrane potential of xylem parenchyma cell. σ, reflection coefficient of the plasma membrane of xylem parenchyma cell at a physiological composition of cytosol and xylem sap; σ<sub>r</sub>, reflection coefficient of root tissue for radial water transport.

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not yet fully understood. The elusiveness of root pressure that seemingly collides with the laws of thermodynamics has caused some researchers even to resort to religiously inspired metaphors (or, rather, to indulge in superstition): [Pickard \(2003a\)](#) claimed ‘exorcism of Maxwell’s demon’ by his new model on root pressure, and [Holbrook and Zwieniecki \(1999\)](#), discussing the closely related phenomenon of refilling of embolized vessels, even invoked the necessity of a ‘miracle’.

In this article, I will briefly discuss previous concepts of root pressure and try to elaborate on them in the light of progress in our understanding of water transport in mammalian epithelia that we have witnessed during the last ~10 years. Subsequently, root pressure—and the closely related stem pressure—will be placed into a wider perspective with respect to embolism repair and mechanisms of long-distance water transport in trees.

## Current concepts of root pressure

It is generally agreed upon that water exuding from the cut surface of excised roots flows out of the severed xylem vessels through which it has been transported upwards (but see also [Rowan \*et al.\*, 2000](#)). The question remains of how radial re-supply of water from soil solution to xylem vessels is maintained. In most textbooks (e.g. [Kramer and Boyer, 1995](#)), root pressure is explained by a difference in osmotic pressure between xylem sap and the external medium (i.e. mass flow is driven by a free energy gradient of water). The ‘osmometer model’ considers the tissue separating the xylem sap from the external medium as a semi-permeable transport barrier or ‘membrane’; alternatively, the root symplast is accepted as a third, transitory compartment. While this provides an adequate description in some cases, there are numerous reports in the literature on isotonic water flow, or even radial water flow against an osmotic pressure gradient between the external medium and the guttation fluid or the exudate secreted by the root stump (e.g. [Oertli, 1966](#); [Zholkevich, 1991](#); [Schwenke and Wagner, 1992](#); [Enns \*et al.\*, 2000](#); [Pickard, 2003b](#)). In order to defend the ‘osmotic hypothesis’, it was inferred by several authors that the osmotic pressure in the vicinity of the semi-permeable membrane that controls water release into the vessels might be considerably higher than that of guttation fluid or the bulk xylem sap harvested at the cut surface. [Anderson \*et al.\* \(1970\)](#), inspired by the standing osmotic gradient model proposed by [Diamond and Bossert \(1967\)](#) for epithelia in the animal field, postulated a longitudinal osmotic gradient in the (dead) xylem vessels, with water entering vessels close to the root tip. This gradient was thought to be maintained at the expense of metabolic energy. A similar, more elaborate model was suggested by [Taura \*et al.\* \(1988\)](#); ‘canal model’; instead of longitudinal osmotic gradients in the vessels, they postulated a steep radial osmotic gradient in the apoplast of the stele extending from the vessels to the endodermis. However, no experimental evidence for such osmotic gradients was obtained when the distribution of osmotica in the vessels and adjacent tissues was measured with high spatial resolution ([Enns \*et al.\*, 1998, 2000](#)).

Therefore, [Zholkevich \(1991\)](#) introduced a distinction between an osmotic and a non-osmotic, ‘metabolic’ component of root pressure. The latter is susceptible to various blockers, including those of respiration. The fact that cytochalasin B and other drugs that interfere with the cytoskeleton also had a strong impact on the metabolic component of root pressure was taken as evidence in favour of a peristaltic mechanism involving the xylem parenchyma cells.

Other approaches focused on the radial reflection coefficient of the root (in the case of two-compartment models) or, if xylem sap and soil solution were thought to be separated by two membranes with the symplast as an intermediate compartment, on the reflection coefficient of the inner boundary, namely the plasma membrane of xylem parenchyma cells. The latter approach was introduced by [Ginsburg \(1971\)](#), who transferred the general model of [Patlak \*et al.\* \(1963\)](#) to the problem of radial water transport in roots. Their three-compartment model featured two membranes in series, with the inner membrane having a significantly lower reflection coefficient than the outer one. If osmotica accumulate in the intermediate compartment (the root symplast) due to metabolic energy-driven uptake of solutes from the outer compartment (the soil solution), a hydrostatic pressure (the turgor pressure of root cells) is maintained in the intermediate compartment. Under these conditions, a permanent, directed water flow is generated from the outer into the inner compartment (the root xylem). A variation of this model was suggested by [Schwenke and Wagner \(1992\)](#), who hypothesized the reflection coefficient of the plasma membrane of xylem parenchyma cells to oscillate due to dynamic changes in the activity of stretch-activated channels (thus explaining intrinsic oscillations in the root exudation rate). Later, [Pickard \(2003a\)](#) proposed the co-existence of two different pathways for water secretion into xylem vessels, ‘orthodox’ aquaporins that utilize the free energy gradient of water (with a reflection coefficient of ~1) and larger pores with water flow being driven by the hydrostatic pressure gradient alone (the reflection coefficient of this pathway equalling zero). [Pickard](#) pointed out that the second pathway would be associated with a massive loss of solutes from the symplast to the xylem sap that would require re-absorption by membrane transporters at the expense of metabolic energy. The major problem with these models is that even though they are sound from a thermodynamic point of view, they could not be verified experimentally. Thermodynamics is purely phenomenological and does not provide any clue with respect to the structural (e.g. anatomical) basis of the phenomenon, nor does it imply a (molecular) mechanism.

The most widespread and popular model describing radial water transport in roots has been, however, the composite transport model introduced by [Steudle and co-workers \(Steudle and Peterson, 1998; Steudle, 2000, 2001\)](#), that was built on earlier two-compartment models of [Fiscus \(1975\)](#) and of [Miller \(1985\)](#). The composite transport model focuses on two parallel pathways of radial water transport: Besides the symplastic pathway that involves water and ion transport across at least two membranes and is associated with a reflection coefficient close to one, there is an apoplastic pathway

across the continuum of the cell walls. The significance of this 'bypass' to the symplast is under debate, since cortex and stelar apoplast are separated by suberin depositions in the cell walls of the endodermis, known as the Casparian band (Steudle and Peterson 1998; in many cases, there is an additional barrier of similar properties in the cortex, the exodermis). A  $\sigma_r$  value of zero was ascribed to this pathway that was supposed to support Navier–Stokes flow in the pores of the cell wall matrix. When the xylem hydrostatic pressure response  $\Delta P$  upon addition of various osmotica to the bath medium (resulting in an increase in osmotic pressure,  $\Delta\pi$ ) was measured with a root pressure probe, radial reflection coefficients (calculated from the ratio  $\sigma_r = \Delta P / \Delta\pi$ ) were frequently significantly lower than one. According to Steudle and co-workers, this was a 'mixed' value that allowed quantification of the relative contribution of apoplastic and symplastic pathways to overall volume flow upon an osmotic challenge. Hydrostatic pressure pulses applied to the xylem via the root pressure probe, on the other hand, were supposed to be dissipated predominantly via an apoplastic pathway. More recently, the composite transport model and the experimental approach on which it was based were criticized for basic misconceptions (Bramley *et al.*, 2007; Wegner and Zimmermann, 2009; Knipfer and Fricke, 2010), and the interpretation of  $\sigma$  values according to the model of Steudle no longer appears feasible. Most importantly, the experimental approach of Steudle apparently tends to overestimate the purely apoplastic pathway of volume flow. With the exception of a few species, for example rice, this extracellular pathway appears to play a minor role only.

### **A new hypothesis: energetically uphill water transport across the plasma membrane of xylem parenchyma cells, taking advantage of the free energy gradients of ions and sugars**

First of all, it is important to note that it is not the goal of this opinion paper to present 'just another' hypothesis on root pressure that is meant to replace the previous ones. Rather, it is the author's intention to open up a new perspective on root pressure (and beyond) that is not mutually exclusive to previous concepts discussed above.

The idea that water during passage through the roots gains free energy, and hence is transported 'energetically uphill', is not novel; it was already put forward by Oertli as early as 1966. However, at that time, no molecular mechanism was conceivable, and no adequate tools for rigorous experimental testing of any mechanistic hypothesis would have been available. However, the field of water transport has experienced tremendous progress, especially in the last 15 years, that came—not for the first time—from research on mammalian epithelia. T. Zeuthen and his co-workers provided multiple evidence that co-transporters exist in various mammalian epithelia (such as the small intestine or the choroid plexus epithelium) that transport ions together with water at a fixed stoichiometry of 160 to 500 water molecules per ion or pair of ions, respectively (Zeuthen, 2010). Therefore,

water can be secreted across the serosal, or 'exit' membrane against its chemical potential, if the energy is provided by the coupled ion transport process (and, therefore, each turnover of the protein is still associated with a net dissipation of free energy). In order to maintain the ionic gradient across the membrane that is dissipated by this transport step, ions have to be retrieved again from the extracellular compartment (in plant roots: the stelar apoplast) at the expense of metabolic energy.

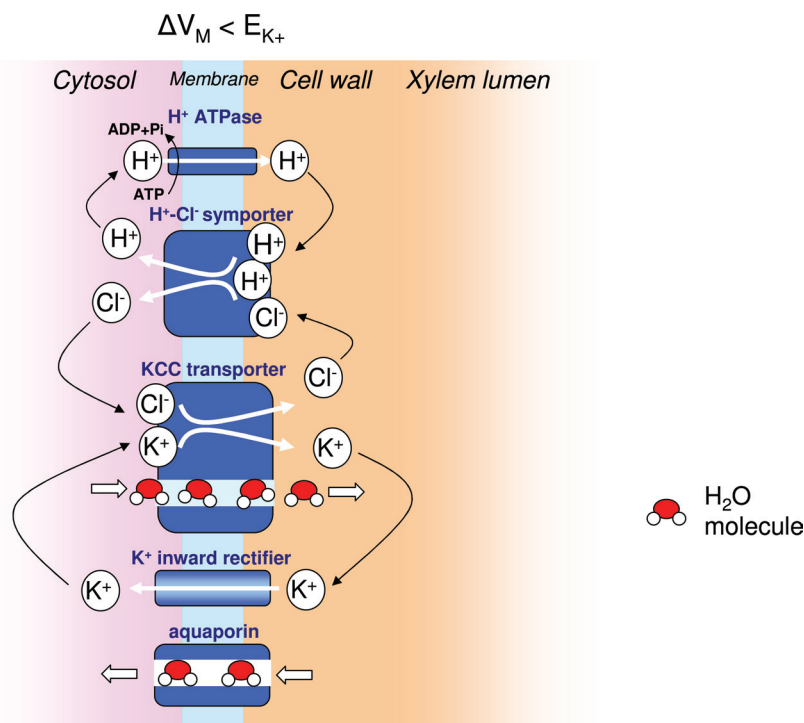
Much experimental work has focused on members of the CCC superfamily of cation/ $\text{Cl}^-$  co-transporters, recently renamed SLC12 (for a review, see Gamba, 2005). According to the cation which they translocate together with  $\text{Cl}^-$ , these membrane proteins are divided into three subfamilies. The KCCs and NCCs exclusively use  $\text{K}^+$  and  $\text{Na}^+$  as a co-substrate, respectively, whereas the NKCCs perform  $\text{Na}^+ - \text{K}^+ - 2\text{Cl}^-$  co-transport. Interestingly, these transporters apparently also mediate water flow tightly coupled to salt translocation. The most intensively studied transporter, the KCC of the choroid plexus epithelium, was calculated to shift 500  $\text{H}_2\text{O}$  molecules per protein turnover (Zeuthen, 2010). As a consequence, epithelial cells started to swell upon exposure to 50 mM KCl even though the osmotic pressure inside the cells was twice as low. However, when 50 mM NaCl was added instead, cells shrunk, as expected from the gradient in chemical potential of water. At a concentration of 150 mM [300 mOsmol, corresponding to 0.75 MPa (!)], water uptake by co-transport matched efflux driven by the osmotic gradient, and cell size remained constant. When KCl was added in the presence of the KCC blocker furosemide, only the osmotic response was retained, and cells responded in the same way as observed with NaCl. The 'water co-transport hypothesis', based on experiments of this type and repeated on various other proteins expressed heterologously in oocytes, was vigorously criticized and challenged (Lapointe *et al.*, 2002; Lapointe, 2007; Mollajew *et al.*, 2010). The main concern was that salt transport across the membrane would give rise to the formation of unstirred layers in the vicinity of the membrane (particularly since diffusion in the cytosol is somewhat retarded with respect to a dilute aqueous solution) providing an osmotic driving force for water transport, without any need to assume a flux-coupling mechanism located within the protein. However, in a more recent paper, Zeuthen and McAulay (2012) could provide compelling evidence for a true co-transport mechanism: when the NKCC1 transporter was reconstituted in oocytes, isoosmotic addition of 15 mM KCl to the bath (by lowering the NaCl concentration accordingly, or by replacing the same concentration of cholineCl) elicited continuous water influx, again measured by cell expansion, within a second (!). It is hard to envisage how unstirred layers sufficient to drive water flux against the bulk osmotic gradient could have evolved within this short period of time. Immediate cell swelling was even induced when KCl addition coincided with an increase in bath osmolarity. Crystallization and determination of the 3D structure of several co-transporters also provided indirect evidence in favour of a direct coupling of water and salt transport (Abramson and Wright, 2009), although the precise mechanism of co-translocation is still not fully understood.

Irrespective of the current status of the debate on the precise coupling mechanism (either directly by a conformational change, or indirectly by solute accumulation at the protein surface), this is of little relevance for the putative physiological role of these and other transporters.

Interestingly, homologues of the CCC family have also been discovered in plants. Kong *et al.* (2011) cloned a cation–chloride co-transporter in rice that seems to belong to the KCC subfamily since it apparently transported  $K^+$ , but not  $Na^+$ . These authors tested the subcellular localization of a CCC–GFP (green fluorescent protein) fusion protein transiently overexpressed in onion epidermis and could demonstrate that the protein was predominantly allocated to the plasma membrane. This was also confirmed for rice root cells. The only CCC-type transporter found in *Arabidopsis* also showed the highest homology with the KCCs, but reconstitution in oocytes showed that  $Rb^+$  (as a tracer for  $K^+$ ) was only translocated in the presence of  $Na^+$ , indicating that this transporter functions like an NKCC (Colmenero-Flores *et al.*, 2007). Interestingly, the co-transporter found in *Arabidopsis* turned out to be prominently expressed in vascular tissue. It is conceivable that these transporters are involved in the directed, radial transport of water into xylem vessels by which root pressure is built up, playing the same part as in mammalian epithelia (Fig. 1). Unfortunately, no information on water permeability of the plant transporters is available

as yet. Re-absorption of  $K^+$  and  $Na^+$  (required for ‘keeping the battery charged’) would be brought about by inward-rectifying  $K^+$  channels in xylem parenchyma cells (Wegner and Raschke, 1994; Wegner *et al.*, 1994). For  $Cl^-$ , this role could be played by  $Cl^-/2H^+$  symporters. Note that salt release by co-transporters is an electroneutral process (Zeuthen and McAulay, 2012) and would not interfere with  $K^+$  re-uptake by ion channels that requires a membrane potential more negative than the Nernst potential of  $K^+$ , which is maintained by proton pump activity (Fig. 1). Evidence for ‘simultaneous’ uptake and release of  $K^+$  has indeed been obtained for root tissue, using refined radioactive tracer techniques (Britto and Kronzucker, 2006). Rapid, seemingly ‘futile cycling’ of ions is apparently a common phenomenon at root membranes that was found for  $K^+$ ,  $Na^+$ , and  $Cl^-$ , and becomes more prominent at elevated concentrations of these ions. Futile cycling consumes metabolic energy, but its benefit for the plant seemed to be elusive and so far has remained an open question. Water secretion may be part of the answer.

In his quantitative biophysical analysis of coupled ion and water transport by CCC transporters, Zeuthen (2010) did not make use of the framework of the thermodynamics of irreversible processes. However, this formalism can conveniently be applied to arrive at a quantitative expression for the hypothesis on root pressure developed in this communication. Overall volume flow across the plasma membrane of xylem parenchyma



**Fig. 1.** Hypothetical interplay of membrane transporters in the plasma membrane of xylem parenchyma cells for water secretion. Coupling between water and ion transport occurs in a KCC-type co-transporter that translocates  $K^+$  and  $Cl^-$  together with a fixed number of water molecules. Note that this transport is electrically silent. The ions are at least partly recycled via a  $K^+$  inward-rectifying channel and a  $Cl^-/2H^+$  symporter, respectively. These processes are energized by the activity of a  $H^+$  ATPase that maintains the  $H^+$  gradient and hyperpolarizes the membrane to values more negative than  $E_{K^+}$ . Aquaporins may to some extent short-circuit co-transport-driven water flow if their activity is not down-regulated. Note that all transporters have been demonstrated to co-exist in the plasma membrane of root stelar cells.  $\Delta V_M$ =membrane potential of xylem parenchyma cell.  $E_{K^+}$ =Nernst potential for  $K^+$ . For more details, see the text. (This figure is available in colour at JXB online.)



cells can be described as the sum of two separate components, namely volume flow driven by the chemical potential of water (or, rather, in the nomenclature generally preferred by plant scientists, the water potential  $\Delta\Psi_{xpc} = \Delta P_{xpc} - \Delta\pi_{xpc}$ ) and volume flow driven by the CCC transporter (assuming that, apart from the activity of the CCC transporter, the membrane is ideally semi-permeable, i.e. other solute transport processes that may interact with water transport, including frictional interaction, are neglected). For this simplified model, we can write:

$$J_V = J_{V,CCC} + J_{V,\Psi} \quad (1)$$

with

$$J_{V,\Psi} = J_{V,p} + J_{V,\pi} \quad (2)$$

Moreover,

$$J_V = L_P \times (\Delta P_{xpc} - \sigma \Delta \pi_{xpc}) + J_{V,CCC} \quad (3)$$

Equation 3 is equivalent to the general water flow equation:

$$J_V = L_P \times (\Delta P_{xpc} - \sigma \Delta \pi_{xpc}) \quad (4)$$

defining  $\sigma$  as a generalized reflection coefficient of the membrane under physiological conditions. Hence

$$L_P \sigma \Delta \pi_{xpc} = L_P \Delta \pi_{xpc} - J_{V,CCC} \quad (5)$$

and

$$\sigma = 1 + \frac{J_{V,CCC}}{J_{V,\pi}} = 1 - \frac{J_{V,CCC}}{L_P \Delta \pi_{xpc}} \quad (6)$$

It is instructive to have a closer look at this simple equation. Usually, osmotica (including  $K^+$  and  $Cl^-$ ) tend to be more concentrated in the cytosol than in the xylem sap (but not necessarily; see calculations on the direction of CCC-driven transport depending on ionic gradients across the membrane of xylem parenchyma cells by Teakle and Tyerman, 2010); hence  $L_P \Delta \pi_{xpc}$  and  $J_{V,CCC}$  will both carry a positive sign. Inhibition of the CCC transporter (e.g. by the blocker furosemide) leads to a reflection coefficient equal to one, and the membrane becomes ideally semi-permeable. Deviation of the  $\sigma$  value from one depends on the ratio of CCC-driven flow and the osmotic flow (which is not specific to any osmoticum). If  $J_{V,CCC} < L_P \Delta \pi_{xpc}$ ,  $\sigma$  attains values ranging between zero and one, i.e. the effective osmotic pressure gradient (and the net water flow driven by it) will be reduced. If  $J_{V,CCC} > L_P \Delta \pi_{xpc}$ ,  $\sigma$  attains a negative value and co-transport of  $K^+$  ( $Na^+$ ),  $Cl^-$ , and  $H_2O$  (usually directed towards the xylem vessels) dominates the osmolyte-driven flow component. Theoretically,  $\sigma$  could even be larger than one if osmotic flow and  $J_{V,CCC}$  happen to operate in the same direction.

A formal criterion for energetically uphill water transport is given by the following relationship:

$$k = \frac{J_V}{\Delta\Psi_{xpc}} < 0 \quad (7)$$

$k$  is a kind of conductance and attains negative values if net volume flow is directed against the water potential gradient,  $\Delta\Psi$ . This parameter can be expressed in terms of the parameters well known from thermodynamics of irreversible processes by comparison with Equation 4:

$$k = L_P \frac{\Delta P_{xpc} - \sigma \Delta \pi_{xpc}}{\Delta P_{xpc} - \Delta \pi_{xpc}} \quad (8)$$

It has to be kept in mind that the volume excreted into the xylem via CCC co-transporters has a defined salt concentration. For the KCC in choroid plexus epithelium, the KCl concentration is 0.11 M, corresponding to an osmolarity of 0.22 Osmol. Given a cytosolic  $K^+$  concentration of ~75 mM (Walker *et al.*, 1996), this would not allow self-sustained water secretion, since the  $K^+$  gradient provides the main driving force for KCl/water efflux. Hence, re-absorption of  $K^+$  and  $Cl^-$  from the sap (that is supposed not to interact directly with volume flow across the membrane) would be an indispensable element of the apparatus for water secretion (see also calculations by Teakle and Tyerman, 2010).

A critical aspect of the putative role of cation-chloride co-transporters in water secretion into the xylem is their dependence on the availability of  $Cl^-$  as a substrate. In glycophytes growing in natural habitats, tissue chloride concentration is said to range from 7 mM to 70 mM (White and Broadley, 2001), but cytosolic concentration may be lower to evade toxic effects. This may limit the activity of CCC transporters in glycophytes. It is as yet unknown whether in plant co-transporters  $Cl^-$  could be replaced by other monovalent anions that do not play a role in animal tissues (e.g. by nitrate). For NKCC transporters serving as a secretory water pathway,  $Na^+$  is additionally required as a substrate. This subtype would be expected in halophytes that take up  $Na^+$  as an osmoticum (Shabala and Mackay, 2011). However, the *Arabidopsis* CCC transporter when expressed in oocytes translocated  $Rb^{86}$  only in the presence of  $Na^+$ , suggesting that it would be primarily active under saline conditions (Colmenero-Flores *et al.*, 2007). On the other hand, AtCCC-deficient mutants had a clear phenotype even under low salt conditions, and salt stress did not affect the expression in the wild type (Colmenero-Flores *et al.*, 2007), providing circumstantial evidence that the transporter was not silent even under low salt conditions. Possibly, small amounts of  $Na^+$  permanently cycling between the cytosol and apoplast could be sufficient to sustain at least basic activity of this transporter.

Co-transport of water together with one or more substrates appears not to be a unique property of the CCC transporters. The Na-glucose co-transporter SGLT1 and the glucose transporter GLUT1 also translocate water at a fixed stoichiometry (Loo *et al.*, 1999; Zeuthen, 2010), and evidence was obtained that the same is true for a range of amino acid transporters. Sugar transporters play an important role in plants in the context of assimilate allocation in sinks, such as roots. Notably they are involved in phloem unloading and in the retrieval of monosaccharides, for example by root cells after sucrose splitting by apoplastic acid invertases. No information is available regarding whether these transporters possess

the ability to co-transport water, but it is tempting to speculate that release of Münch water by phloem is mediated, at least partly, by a co-transport mechanism with sucrose. This water, being secreted into the stelar apoplast, could contribute significantly to root pressure, at least in intact plants.

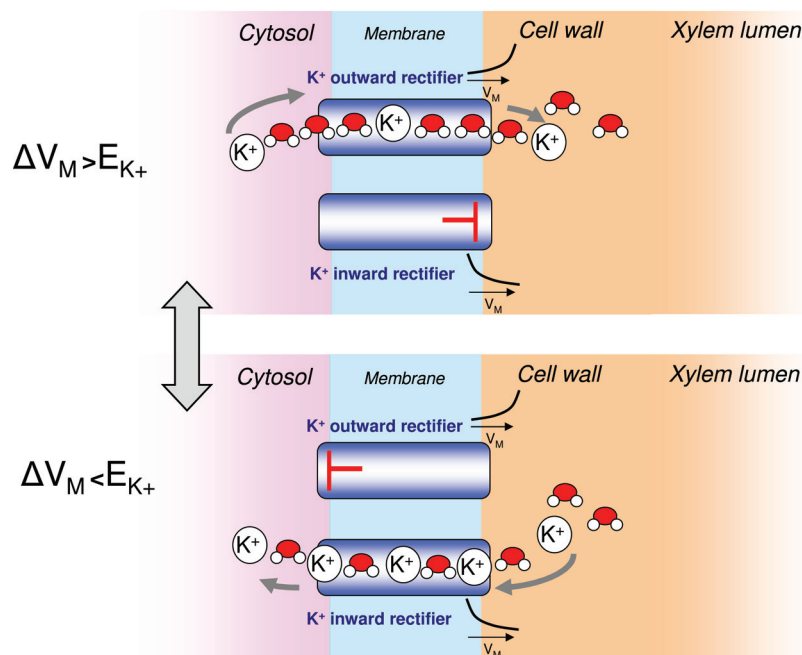
Note that the energetically uphill transport of water is not restricted to the transporters discussed so far (although they seem to be most effective with respect to the number of water molecules transported per substrate). Ion channels also conduct water as a 'by-product', with one ion being accompanied by 4–12 water molecules. Few studies have been undertaken to study this phenomenon quantitatively, but in one case data on an ion channel of plant origin are available. [Hornblom and Vetter \(1992\)](#) investigated coupling of water and ion transport in the maxi  $K^+$  channels from *Chara* droplets. These authors provided evidence that water could drive  $K^+$  flow through these channels (and vice versa). Uphill water transport could potentially be brought about by the cooperation of outward- and inward-rectifying  $K^+$  channels (as described for xylem parenchyma cells previously; [Wegner and Raschke, 1994](#); [Wegner et al., 1994](#); [Wegner and de Boer, 1997](#)), with the important prerequisite that outward rectifiers mediate water transport more efficiently than inward rectifiers ([Fig. 2](#)). At present, this is purely based on speculation since no experimental evidence is currently available showing that channels differ in that respect. A channel-mediated futile  $K^+$  cycle ([Scsnerba et al., 2006](#)) would imply that both  $K^+$  channels operate alternately, co-ordinated by oscillations of

the membrane potential, as previously shown for guard cells ([Gradmann et al., 1993](#)). Indeed, short-term oscillations in root exudation ([Zholkevich et al., 2005](#)), electrical potentials ([Toko et al., 1990](#)), and ion fluxes ([Shabala et al., 2006](#)) have been demonstrated. Another putative candidate for ion-coupled water flow across the membrane of xylem parenchyma cells is the NORC, a channel that is outwardly rectifying and poorly selective among cations and anions ([Wegner and De Boer, 1997](#)). Conspicuously, this channel type has also been found in other secretory tissue. Its role in water translocation across the membrane remains to be tested. This is still hampered by our lack of information on the genetic basis of this ion channel.

Transporters of neurotransmitters were also found to be involved in water translocation in brain cells ([McAulay and Zeuthen, 2010](#)). Interestingly, Zholkevich and co-workers have repeatedly demonstrated that neurotransmitters such as acetylcholine, adrenaline, and serotonin stimulate root pressure exudation (e.g. [Zholkevich et al., 2003](#)). It is worth testing whether water secretion coupled to the transport of these substances is involved in the generation of root pressure.

Possibly, several pathways for co-transport of water and ions (solutes) may co-exist in the membrane to achieve the required rate of water secretion under various conditions, as previously also described for epithelia ([Zeuthen, 2010](#)).

It should be noted that the hypothesis developed here refers to isotonic radial water flow (i.e. in the absence of an osmotic gradient between the xylem sap and the ambient



**Fig. 2.** Alternative model for water secretion that makes use of different water–ion coupling ratios in outward- and inward-rectifying  $K^+$  channels. Arbitrarily, the  $K^+$  outward rectifier is thought to carry three water molecules together with one  $K^+$  ion, whereas the inward rectifier transports water and  $K^+$  on a 1:1 basis. Both rectifiers operate alternately, co-ordinated by membrane potential oscillations. In this way, futile  $K^+$  cycling is organized that drives a net water flow from the cytosol into the apoplast. Note that this  $K^+$  cycling consumes metabolic energy when the membrane potential is hyperpolarized by proton pump activity (for simplicity, other transport processes are omitted in the figure).  $\Delta V_M$  = membrane potential of xylem parenchyma cell.  $E_{K^+}$  = Nernst potential for  $K^+$ . (This figure is available in colour at JXB online.)

medium) or when radial inflow is sustained even though the xylem sap is more dilute than the external medium (Oertli, 1966; Anderson *et al.*, 1970; Zholkevich, 1991; Schwenke and Wagner, 1992; McCully, 1999; Enns *et al.*, 2000; Pickard, 2003b). In other cases, however, the osmotic pressure of the xylem sap is higher than that of the ambient medium (e.g. Miller, 1985); under these conditions, the osmometer model holds water, and aquaporins come into play.

## How do aquaporins fit into this scenario?

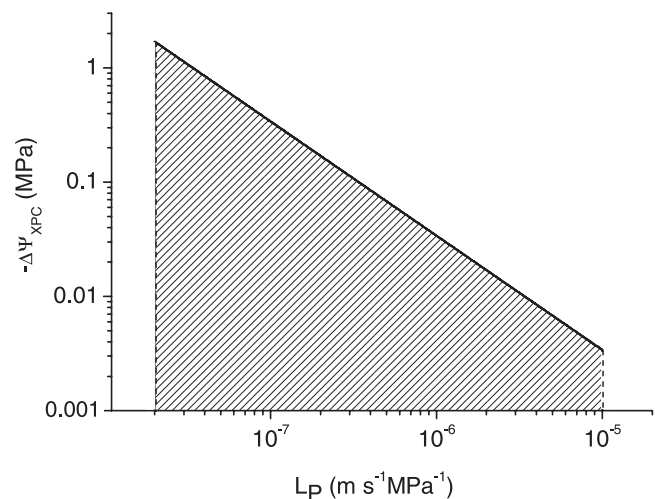
The most obvious and serious objection against the hypothesis described in the previous paragraph is the presence of aquaporins in the plasma membrane of xylem parenchyma cells (e.g. Postaire *et al.*, 2010). Aquaporins conduct  $H_2O$  molecules passively along with the driving force. Aquaporin activity contributes strongly to the hydraulic conductance of the membrane and, together with the natural water conductivity of the bilayer, tends to dissipate existing water potential gradients. Hence these transmembrane pathways for water would short-circuit the activity of solute-driven water transport and potentially establish 'futile water cycles' across the membrane. This appears to be 'a waste of energy' and seems to be at variance with the hypothesis advocated here.

To bring this aspect into perspective, it has to be remembered here that under daylight conditions—even at a low light intensity—in most cases the hydrostatic pressure gradient across the membrane (i.e. the difference between the turgor of xylem parenchyma cells and the xylem pressure) plays a major role for radial water transport in the root (Wegner and Zimmermann, 2009). Under these conditions, water transport will become more efficient at an increased hydraulic conductance due to aquaporin activity, and water secretion into the vessels (that is indirectly coupled to the consumption of metabolic energy) is likely to be down-regulated. But what about those processes of water secretion into xylem vessels that are the focus of this study? Is net water flow against a transmembrane gradient in water potential compatible with the existence of aquaporins?

In the case of plants, a quantitative treatment of this issue is hampered by our lack of information on the rate of water secretion, for example by CCC transporters, that depends on both their density in the plasma membrane and the ion gradients. For a model calculation, a value of  $3.4 \times 10^{-8} \text{ m s}^{-1}$  was adopted from the literature on mammalian epithelia (Zeuthen, 2010). Moreover, in plant cells, the hydraulic membrane conductance can vary greatly. Depending on species, cell type, time of the day, nutrient availability, etc.,  $L_p$  can range from  $\sim 2 \times 10^{-8} \text{ m s}^{-1} \text{ MPa}^{-1}$  to  $10^{-5} \text{ m s}^{-1} \text{ MPa}^{-1}$  (Maurel, 1997). Based on these data, the water potential gradient across the membrane can be calculated at which water secretion, for example by co-transport, would be counter-balanced by a passive  $J_{V\Psi}$  (i.e. net volume flow across the membrane,  $J_V$ , is 0; Fig. 3). At lower water potential gradients, indicated by the shaded area in Fig. 3, water secretion would dominate over the passive flow component, and 'energetically uphill' water transport would be feasible. As expected, the range of

water potential gradients allowing water secretion depends strongly on  $L_p$ . At the lowest value (that may be close to the background conductance of the bilayer), water efflux could be sustained against a water potential gradient of  $>1 \text{ MPa}$  (!), and, even at a higher conductance of  $10^{-6} \text{ m s}^{-1} \text{ MPa}^{-1}$ , a disequilibrium of up to  $\sim 0.04 \text{ MPa}$  could be maintained. At maximum aquaporin activity, however, the water potential gradient would be kept close to the equilibrium. Nevertheless, isotonic water efflux would still be possible if water secretion and resorption of the transported ions were orchestrated in such a way that gradients in water potential across the membrane remain very small (because the secreted volume is isoosmotic to the xylem sap) and passive backflow of water is diminished by the lack of a driving force! After all, conclusive data on mammalian epithelia that also have aquaporins co-located with CCC transporters (e.g. in kidney tubules) prove the feasibility of the concept.

From an energetic point of view, it is favourable to keep futile water cycling across the membrane at a minimum. Hence, it is most likely that water secretion and passive transport via aquaporins are inversely regulated and that aquaporin activity is low when water secretion by a co-transport mechanism is active, for example under conditions of isotonic radial water transport. Aquaporin activity has been demonstrated to be kept under tight physiological control (e.g. by phosphorylation, cytosolic pH, and divalent cations; Maurel, 1997). In agreement with this line of evidence, Dustmamatov and Zholkevich (2008) showed that  $HgCl_2$ , an inhibitor of aquaporins, enhanced root pressure rather than inhibiting it



**Fig. 3.** Double-logarithmic plot of the water potential gradient against which water secretion could be maintained (shaded area) as a function of the hydraulic conductance of the plasma membrane (range of values following Maurel, 1997). The upper limit (continuous line) was calculated according to  $-\Delta\Psi_{XPC} = J_{V,CCC}/L_p$ . At these water potential gradients, water efflux by co-transport would be counter-balanced by water potential-driven influx (note that  $\Psi$  is assumed to be higher in the xylem than in the adjacent cell). For simplicity,  $J_{V,CCC}$  was assumed to be independent of  $\Delta\Psi_{XPC}$ . A value of  $3.4 \times 10^{-8} \text{ m s}^{-1}$  for  $J_{V,CCC}$  was adopted from the literature on mammalian epithelia (Zeuthen, 2010).



when it was applied at concentrations supposed to be most effective (80–250  $\mu\text{M}$ ), before unspecific toxic effects prevailed. This finding is at variance with water flow being driven by osmotic gradients. Clearly, there are several studies reporting a close correlation between root pressure exudation, expression of aquaporins, and hydraulic conductivity of the root (e.g. Lopez *et al.*, 2003; Postaire *et al.*, 2010), but in these cases the osmotic pressure of the xylem sap was high, and volume flow was predominantly driven by an osmotic gradient between the bath and xylem lumen, which is in line with a dominant role for aquaporins facilitating passive movement of water from the ambient medium into the xylem.

## Root pressure and embolism refilling—two sides of the same coin?

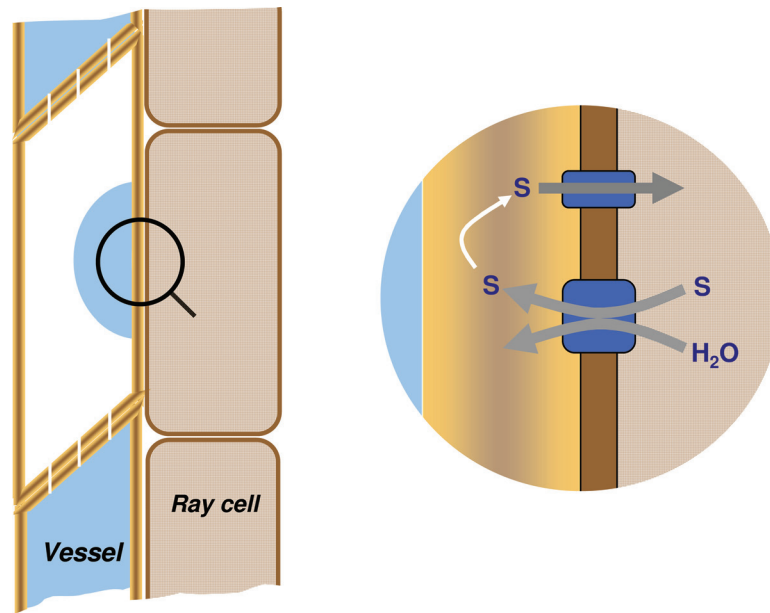
Energetically uphill water transport is most probably not restricted to the root, but can occur everywhere along the vascular system. Stem pressure (analogous to root pressure) is a well-known phenomenon, and AtCCC expression in *Arabidopsis* was not restricted to the root but was also found in the vascular tissues of the shoot (Colmenero-Flores *et al.*, 2007). The ability of plants to refill void xylem vessels that underwent cavitation and, as a consequence, became embolized has frequently been documented even for individual xylem vessels, for example by magnetic resonance imaging (Holbrook *et al.*, 2001; Kaufmann *et al.*, 2009), by computed tomography (Brodersen *et al.*, 2010; Lee *et al.*, 2013), and, indirectly, by cryo-microscopy (McCully *et al.*, 1998; McCully, 1999). Embolism repair requires vessels to be filled with xylem sap secreted by adjacent cells. It is generally believed that some overpressure has to be built up in a vessel during the refilling process in order to dissolve residual inclusions of gas completely; removal of cavitation nuclei appeared to be a prerequisite for a vessel to regain functionality (Holbrook and Zwieniecki, 1999; Zwieniecki and Holbrook, 2009; Nardini *et al.*, 2011; Brodersen and McElrone, 2013). Usually refilling occurs overnight when transpiration is low and little or no tension prevails in adjacent, functional vessels. However, even during the day, repair of embolized vessels has been observed when pressures below vacuum were apparently established in those parts of the xylem that were still conductive (Nardini *et al.*, 2011). The puzzling aspect of this phenomenon even bordering on a miracle (Holbrook and Zwieniecki, 1999) is how a steep pressure gradient between vessels undergoing repair and others under tension is maintained; water being secreted into the void vessels should immediately be swept into the functional vessels unless those vessels during refilling are hydraulically isolated from their environment. The problem becomes somewhat less dramatic if one takes into account that tensions in the xylem of transpiring plants have been grossly overestimated and are likely not to increase beyond a few tenths of a megaPascal (Zimmermann *et al.*, 2004; but see, for example, Tyree, 2003, for an opposing opinion). Clearly, pits connecting the vessels have to be occluded, and the hydraulic conductivity of the walls separating the vessels has to be extremely low to ensure that water influx into vessels

under repair coming from adjacent cells exceeds water loss to adjacent, conductive vessels (Zwieniecki and Holbrook, 2009).

In search of a mechanism that could explain the movement of water from cells into void xylem vessels, osmotic forces have most frequently been considered. Release of sugars or salts into the apoplast by vascular tissue could provide the driving force for water movement into the vessels, and indeed this mechanism was confirmed for refilling of embolized vessels during springtime in deciduous trees. In birch, for example, osmotically active sugars are mobilized by starch breakdown and released into the vessels providing the driving force for passive water movement (Westhoff *et al.*, 2008). Moreover, longitudinal gradients are established in the stem that could induce water ascent (provided that the xylem is sectioned by solute-reflecting barriers). However, springtime refilling is a special case, and previous attempts to establish a similar mechanism for embolism repair in transpiring plants have failed (Tyree *et al.*, 1999). Recently, the involvement of sugars as osmotica in the refilling process was reconsidered (Zwieniecki and Holbrook, 2009; Nardini *et al.*, 2011; Secchi and Zwieniecki, 2011, 2012). Indeed, residual water in embolized vessels contained measurable amounts of sugars, in contrast to non-embolized ones; however, under many conditions, the overall osmotic pressure of this vessel content was much too low to provide a sufficient driving force for attracting water from adjacent cells by a passive mechanism (Secchi and Zwieniecki, 2012). Alternatively, some authors have suggested a refilling mechanism that involves tissue pressure (e.g. Enns *et al.*, 2000; Bucci *et al.*, 2003), but no conclusive evidence for such a mechanism has been presented either.

It is suggested here that water may be secreted into gas-filled vessels by solute–water co-transport (Fig. 4) in a very similar way to that suggested for root pressure above. In fact, a common mechanism for both phenomena has been suspected before (Holbrook and Zwieniecki, 1999; McCully, 1999; Enns *et al.*, 2000). Ions and/or sugars being released into the apoplast are immediately taken up again by the cells to maintain the concentration gradient that serves as a driving force for water release—hence the bulk concentration of osmotica in the secreted volume remains low. High-resolution computed tomography imaging has demonstrated that droplets form at certain sites along the vessel walls, preferentially where xylem walls border on xylem parenchyma cells (Brodersen *et al.*, 2010; Lee *et al.*, 2013), indicating that water was secreted by these cells, and that transport proteins involved in that process are not distributed uniformly over the membrane but are functionally clustered. Water secreted into embolized vessels may pressurize and dissolve the gas phase so that those vessels become refilled with fluid again (Brodersen and McElrone, 2013). Moreover, if water is simultaneously ‘pumped’ into embolized and adjacent conductive vessels, the pressure gradient between these vessels (that was probably overestimated anyway, see above) may be relieved at least partially, and futile transfer of water from vessels undergoing repair to neighbouring, functional vessels may be less severe. Circumstantial evidence points to proton–sucrose antiporters being involved in this process





**Fig. 4.** Schematic representation of embolism refilling according to the water secretion mechanism introduced in this communication. Water is transported into the central, void vessel. Processes at the membrane level are shown under the 'magnifying glass': water is transported together with a substrate 's' (possibly a sugar) that is retrieved again after the co-transport process to maintain the concentration gradient. This simple mechanism drives water transport into the xylem and eventually allows generation of an overpressure in the vessel sufficient to dissolve the vapour phase. (This figure is available in colour at JXB online.)

(Secchi and Zwieniecki, 2012), but detailed information is still lacking.

Again, the role of aquaporins in this process needs some attention. Evidence for the involvement of aquaporins in the refilling process has been obtained (Secchi and Zwieniecki, 2010), but, as previously pointed out by Nardini *et al.* (2011), this does not necessarily imply that aquaporins are directly involved in the secretory process. Xylem parenchyma cells themselves cannot provide the water required for vessel refilling; secreted water has to be retrieved from storage water in fibres, from the phloem, or from below or above the site of refilling. Aquaporins may be required to ensure water supply from these resources to the site of secretion.

### A new perspective for long-distance water transport in plants?

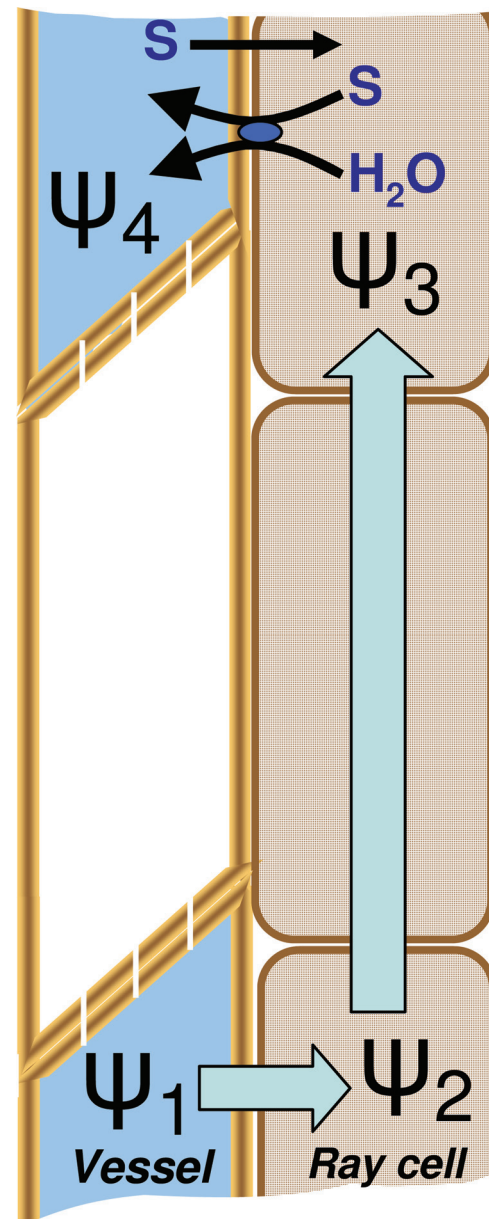
There is general agreement that root pressure can contribute significantly to long-distance water transport in plants, varying strongly with species, environmental conditions, and time of the year (e.g. Fisher *et al.*, 1997; for a review, see Kramer and Boyer, 1995). However, according to most textbooks, ascent of sap is supposed to be driven predominantly by transpiration, as postulated by the cohesion–tension (CT) theory (Dixon and Joly, 1894, 1895; Tyree, 2003). Water loss to the atmosphere is thought to induce a hydrostatic pressure gradient in the continuum of the network of xylem conduits that extends down to the roots. The main problem of this theory is that pressure has to decrease with tree height by some 0.02–0.03 MPa m<sup>-1</sup> to induce continuous transpirational flow, thus overcoming the gravitational force

(~0.01 MPa m<sup>-1</sup>) and additional frictional resistances of the xylem conduit. As a consequence, pressures in the vessels are supposed to drop below vacuum in the canopy of trees exceeding a height of 3–5 m. This is feasible from a thermodynamic point of view, but water in the fluid phase at below-vacuum (negative) pressure is jeopardized by spontaneous gas formation, known as cavitation. Cavitation is associated with a pressure jump to the equilibrium value for co-existence of gas and fluid phase, slightly above vacuum at 20 °C. Since water at a negative pressure is in a metastable state, and the existence of hydrostatic pressure gradients in the vessels of tall trees, as postulated by the CT theory, could not be demonstrated convincingly by experimental methods, the validity of this theory has repeatedly been questioned, and alternative hypotheses have been proposed (Canny, 1998; Laschimke *et al.*, 2006). To overcome the ambiguities and contradictions of the 'orthodox' CT theory, a multiforce theory was introduced by Zimmermann *et al.* (2004). In contrast to the CT theory, that postulates continuous water columns extending from the top of the canopy to the root tips even in tall trees, Zimmermann *et al.* suggested segmentation of the transport pathway. The CT mechanism itself is not altogether dismissed since it satisfactorily explains water ascent e.g. in most transpiring herbaceous plants (Wegner and Zimmermann, 1998). However, in stems of tall trees, the CT mechanism fails to provide a comprehensive explanation of water supply to the canopy (Zimmermann *et al.*, 2007; Westhoff *et al.*, 2009). Therefore, Zimmermann *et al.* (2004) suggested that cohesive water ascent in the conduits driven by a pressure gradient (flanked by other mechanisms, see the original review article for details) only operates within a segment (i.e. along a distance of a few metres at most). Connection between segments

is thought to be brought about by intermittent ‘watergates’; passage through a watergate is associated with an increase of free energy of the water (which is equivalent to a pressure jump in the conduit, since the osmotic pressure of the sap in transpiring plants is usually low and does not change with height; Westhoff *et al.*, 2008). Upon passing a watergate, water is dragged upwards in the subsequent segment by tension forces. Zimmermann *et al.* (2004) did not propose a clear (molecular) mechanism for the mode of operation of these watergates, which compromised the acceptance of the theory. An obvious candidate, in the light of the above discussion, would again be co-transport-driven water secretion into vessels against the chemical potential of water.

Most probably, putative watergates are closely related to the process of refilling of embolized vessels as discussed in the previous paragraph. From an energetic point of view, it would be most efficient if uphill water secretion was initiated only once cavitation had occurred in a group of vessels in a way that affects water supply to the canopy of a tree. This implies that water ascent would function according to the ‘orthodox’ CT mechanism as long as no bottleneck in long-distance transport is formed. However, when local cavitation in a few vessels has occurred, a feedforward process is initiated, since the resistance of the stem increases and more tension is built up in those vessels that are still conductive, thus increasing their cavitation probability. When most vessels are embolized at a particular site, water tends to bypass the cavitated xylem area, moving through adjacent cells. Re-supply to the vessels could be brought about by water secretion at a site some distance above the ‘cavitation hot spot’, and the secreted water will be sucked upwards into the subsequent segment (Fig. 5). Note that water flow through the distal xylem segment is no longer driven by transpiration, but by water–ion or water–sugar co-transport involved in the secretion process (and the metabolic energy required to keep this mechanism going). A particular role for the phloem in this process is likely (Nardini *et al.*, 2011). Local water storage sites provided by non-conducting vessels (e.g. after formation of tyloses) and fibres may also be involved in this process and function as a buffer. In these non-functional vessels, pressure is at above-atmospheric values. When vessels are substantially embolized at a particular site, water will be retrieved from these stores and be swept upwards. The buffer is refilled by secretion of water originating from vessels under tension; however, this refilling is not necessarily a simultaneous process but may start with some delay (e.g. at night, when transpirational water loss is low and, in turn, only mild tensions prevail in xylem vessels).

Although a conclusive network of evidence is not available yet, some information in support of this model can be obtained from the literature. In a very recent study, Melcher and Zwieniecki (2013) have shown that water can easily bypass embolized xylem vessels in leaf petioles, obviously flowing through cells. Moreover, Westhoff *et al.* (2009) completely sectioned boles of birch trees to determine the filling status of xylem vessels in terms of dependence on height, using various methods. Interestingly, most of the vessels at the stem base, just above the insertion of roots, were embolized



**Fig. 5.** Same molecular process as in Fig. 4 to bypass embolized vessels through ray cells. Flat water potential gradients prevail up to the ray cell at position 3 (i.e.  $\Psi_1 \approx \Psi_2 \approx \Psi_3$ ). Re-supply into the xylem, however, can occur against a water potential gradient even of a few tenths of a megaPascal due to the water secretion mechanism, namely  $\Psi_4 > \Psi_3$ . For more details, see the text. (This figure is available in colour at JXB online.)

(even under pre-dawn conditions), as expected for a ‘watergate’. This may have been due to a higher susceptibility to air seeding via the pits. Irrespective of the mechanism, their finding is definitely hard to explain on the basis of the ‘orthodox’ CT theory. Another relative minimum of ‘cohesive water’ was found at a height of ~13 m in most of the trees. Also at the base of many branches, most xylem vessels appeared to be cavitated. Apart from these general features, distribution of xylem water appeared to be highly variable, which is in line with a highly dynamic pattern of segmentation. Evidence for the involvement of water secretion by xylem parenchyma in

long-distance water transport was also obtained for grapevine, using high-resolution computed tomography imaging (Brodersen *et al.*, 2010). The importance of water storage sites functioning as a ‘capacitance’ in long-distance water transport in trees has repeatedly been highlighted (Meinzer *et al.*, 2003). Clearly, we are just beginning to understand how long-distance water transport in plants is organized.

## An agenda for further research

The hypothesis put forward here to explain root pressure (with implications for refilling of embolized vessels and long-distance transport in trees) needs further rigorous experimental testing. Proteins of the CCC family are the most likely candidates for mediating water secretion by water–salt co-transport in plants. More information is urgently required on members of this family in the plant genomes, and their expression patterns in vascular tissue, particularly in roots and in the stem of trees. Their biophysical properties have to be tested by heterologous expression, for example in oocytes, to establish that these proteins can mediate water transport against the chemical potential gradient for water in plants, much as was done for similar transporters in animals. Moreover, the ion selectivity and stoichiometry of these transporters need further testing. If these transporters play the role ascribed to them here, knockout or antisense mutants of *Arabidopsis* and rice that lack the CCC transporter(s) should lose the ability to generate root pressure and to repair embolized vessels. In parallel, the ability of outward-rectifying ion channels to participate in water secretion should be quantified, for example those of the outward-rectifying channel, SKOR, and of the NORC. Experiments of this kind will hopefully contribute to overcome the current deadlock in our understanding of long-distance water transport that we are facing despite tremendous experimental effort being invested in this field of research.

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