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**Review of the Composite Transport Model of Solute
and Water Uptake Into Roots**

S. Koh

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Water Uptake Into Roots**

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Abstract

The mechanism by which water and solutes are taken up into the root is an unresolved area in plant physiology. A few models have been developed which are capable of explaining observed root transport behaviour, but as yet no widely accepted model exists. Further verification of these models is required before conclusions about general mechanisms of root transport can be made. An understanding of these mechanisms is important for advancing our basic knowledge of plant biology. Reliable models of transport through roots have important applications in the management of plant health and productivity.

This report reviews one of these models, the 'Composite transport model' (CTM). The CTM model makes an interesting prediction of the roles of apoplastic and non-apoplastic pathways in the root, but has not been translated into a numerical formulation which can be tested quantitatively. A proposal to develop a numerical model based on the CTM is outlined here. One advantage in developing this model is that the data from which the CTM's conclusions have been drawn are clearly identifiable and abundant in the literature. Experimental techniques which have been used to collect this data, and the mathematical theory and equations which have been used to describe root transport, will also be reviewed.

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1 INTRODUCTION

1.1 Role of the root

Roots are the point of solute and water uptake into the plant. They must simultaneously (Peterson *et al.*, 1993; Steudle, 1994; Hose *et al.*, 2001):

- a) exclude harmful substances
- b) enhance water uptake
- c) reduce the loss of water and nutrients.

Roots are the region of greatest resistance to solute and water transport, and play a major role in plant survival and adaptation. Therefore it is of great interest to understand how roots regulate the transport of solutes and water, as well as how the above functions are performed and how these are affected by environmental conditions. This is important not only for physiological understanding, but also for applications in conservation (e.g. enhancement of plant survival under harsh conditions, and understanding the effects of toxins, salinity, or waterlogging) and in plant productivity (e.g. enhancement of yield through increased nutrient uptake under given growth conditions) (Peterson *et al.*, 1993; Steudle and Peterson, 1998). Surprisingly relatively little is understood about the mechanisms of root function.

Most of the uptake by roots occurs radially, and the main resistance to transport is radial not axial (Peterson *et al.*, 1993; Steudle and Peterson, 1998). Radial transport is the focus of this research. Water and solute uptake is thought to be controlled by the following major factors (Steudle, 1994; Steudle and Peterson, 1998):

- transpiration rates
- osmotic pressure due to solute gradients between the inside and outside of the root
- demand from other parts of the plant
- environmental factors such as water or nutrient stress.

1.2 Root anatomy

The roots considered here are from maize, for most of the data for root transport is available for maize. Maize roots exhibit primary growth only (*cf.* secondary, woody growth), and the main tissue regions are the epidermis, exodermis, cortex, endodermis, and stele which is the central region containing the tracheary elements (Figure 1) (Barber, 1995; Steudle and Peterson, 1998). The number of layers of cortical cells between the endodermis and exodermis is usually between 5 and 10 (Barber, 1995). In maize the endo- and exodermis are one cell-layer thick and uniform (*cf.* dimorphic) (Hose *et al.*, 2001).

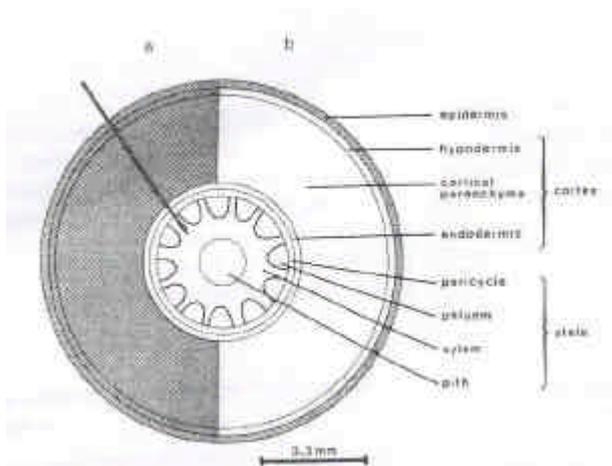


Figure 1. Schematic diagram of a root cross-section (in primary growth): (a) Hypodermis is not an exodermis; stippled area shows that cell walls of the epidermis and cortex are permeable to the soil solution. Arrow indicates the direction of soil solution passage to the xylem. (b) Hypodermis is an exodermis; only the cell walls of the epidermis and outer tangential walls of the exodermis are accessible to the soil solution (Peterson, 1988).

For most species (including maize), the root has two sites of major resistance – the endodermis and exodermis (Peterson, 1988). The former surrounds the whole root and the latter surrounds the stele (or vascular cylinder). The hypodermis is the cell layer just beneath the epidermis and may or may not contain a Casparian Band (CB); where it does so it is an exodermis. CB are waxy, hydrophobic deposits in the cell wall which inhibit solute and water movement, and characterize the endo- and exodermis (*ibid.*). These regions may also contain suberin lamellae (SL) which are also thought to inhibit the passage of solutes and water (*ibid.*). Where CB and/ or SL occur those regions are said to be suberized. The extent of suberization (and resistance) of these regions varies with age and stage of development. In the young areas (near the tip) the main radial resistance is in the cortex and the resistance is evenly distributed across the root layers, but in older regions the main resistance is in the endodermis (Tyree, 2003). The axial hydraulic conductivity increases from the tip to the base (i.e. as roots age), due to an increase in the number and diameter of xylem conduits with age. The ratio of axial to radial resistance depends on the diameter and number of xylem conduits (*ibid.*). See sections 2.4.1 – 2.4.3 for further discussion on the effects of suberization.

Transport across the stele is probably similar to that in the cortex (Steudle, 2000a). The three major (radial) regions to be considered in exploring the mechanisms of root transport are then the exodermis, cortex, and endodermis.

It is generally accepted, and has been experimentally verified, that there are several pathways water can take through a plant tissue (Molz and Ferrier, 1982). The three possible pathways for water and solute transport are (Steudle and Peterson, 1998) (Figure 2):

a) Apoplastic path

Where flow is through the apoplast, which are the non-living parts of the cell outside the plasma membrane. This includes cell walls, intercellular spaces, and the lumen of tracheary elements. This has also been termed the 'free space' (Barber, 1995).

b) Symplastic path

Where flow is through the symplast, which are the living parts of the cell. This includes the continuum of cytoplasm interconnected by plasmodesmata, and excludes vacuoles.

c) Transcellular path

Where the flow crosses the plasma membrane and cell wall, and involves crossing two plasma membranes per cell layer.

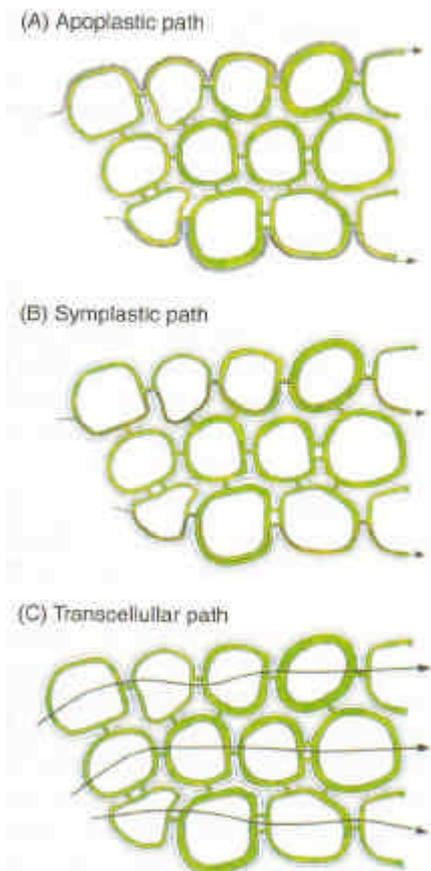


Figure 2. Three parallel pathways for radial root transport of solutes and water (Steudle and Peterson, 1998).

All these paths have a high permeability to water. However, membranes strongly impede the passage of solutes and are the major barrier for ion and solute uptake (Barber, 1995). The transcellular path is the only one which crosses multiple membranes, and is an important path for solute selectivity (Steudle and Peterson, 1998).

Root anatomy is complex and highly variable – between habitats, species, roots of a plant, and within a single root. It also changes with age and stage of development. Therefore it is difficult to generalize experimental results (Steudle and Peterson, 1998; Tyree, 2003), and transport properties of the root system are unlikely to be uniform (Hose *et al.*, 2001). However, it is reasonable to believe that there are uniform general mechanisms governing transport.

1.3 Brief history of root models

1.3.1 Series compartment models

Roots should have efficient water uptake and low nutrient leakage. This means that hydraulic conductivity should be high and solute permeability lower than water permeability. The first models of root transport considered the root endodermis to be the primary osmotic barrier, analogous to the cell membrane and largely impermeable to solutes. This osmometer, or ‘single-equivalent-membrane’ model, is a one-membrane two-compartment model where a single homogenous membrane (the endodermis) separates the xylem and root cortex compartments. Two-compartment models allow the application of basic transport equations for cells to roots (Steudle, 1994).

The osmometer model is simple and useful to an extent, but has been inadequate in explaining certain aspects of root behaviour. One ongoing major subject of interest has been the nonlinear relationship between pressure gradient and water flux rate, typically observed at low flux rates in excised roots. Two of the first one-membrane two-compartment models which provided plausible explanations for this were by Dalton *et al.* (1975) and Fiscus (1975). However, some predictions of these models were contrary to observation, even with solute-buildup at the endodermis taken into account (Murphy, 2000). These models have persisted, however, because information for multi-compartment models are often unavailable (Steudle, 1994). With more complex models, adequate techniques to measure the relevant parameters must also be developed (*ibid.*).

Since the 1970s a few two-membrane three-compartment models have arisen. These have been reviewed by Murphy (2000). The three compartments are usually the xylem, cortical symplast, and external medium, with the endodermis separating the xylem and symplast, and the exodermis separating the symplast and external medium. These models typically include solute buildup at the endodermis, and essentially differ from each other in how solute dynamics between membranes are dealt with, and what factors are included which lead to particular solute distributions across the membranes. These models all failed to completely explain observations (Murphy, 2000).

Murphy (2000) proposed a two-membrane four-compartment model, with external medium, cortical symplast, stelar symplast, and xylem compartments. This model included an outward flux of photoassimilates from the stele to the cortex, in addition to an inward flux of solutes from the external medium. Thus, unlike previous models it include supply from the shoot to the root. It also examined the impact of plasmodesmata behaviour in the endodermis on solute and water fluxes across the endodermis. This model seems to explain many root transport observations and parameter behaviour. However, with this model and other three-compartment models reviewed by Murphy (2000), only the symplasm was modelled, with the apoplasm ignored. Murphy (2000) suggests that the symplastic compartment is the major component in transport because many observations can be explained without the inclusion of an apoplastic component.

Some detailed root models have been made specifically for studying root pressure, which is the process by which plants are thought to push water up the xylem during low or zero transpiration rates, through positive pressure generated in the root. Pickard (2003) has recently developed such a model. Transport processes considered were diffusion, water channel activity, active transport and ionic flow, with the apoplastic pathway excluded. His model is a two-membrane three-compartment model, with the compartments the same as the three-compartment models listed above.

1.3.2 Parallel compartment models

The pathways by which water is transported in the root is a continuing subject of contention. Theoretical analysis by Tyree (1970) concluded that symplastic transport based on irreversible thermodynamics is feasible, while Murphy (2000) has also concluded, using a modelling approach, that most transport occurs symplastically. Ginsburg and Ginzburg (1970), from experiments on Maize, concluded that ion transport into the root must be through rather than between the cells (including the cell walls).

Experiments using the root pressure probe by Steudle *et al.* (1987) suggest that transport is primarily apoplastic except at low transpiration rates, where it is symplastic or transcellular. Molz and co-workers (Molz *et al.* 1979) have looked at the problem of different pathways in the root, and built on the classical mathematical theory of water movement through plant cells and tissues produced by Philip (1958a,b,c). Molz and Hornberger (1973) extended Philip's theory to include diffusible solutes, while Molz and Ikenberry (1974) extended it to include the cell wall pathway, without a diffusible solute. Their conclusion was that a significant portion of water flow occurred through the cell walls. Molz (1979) developed the theory further to specifically include the symplasm (not just transcellular flow), with the inclusion of permeability of the plasmodesma membrane, without a diffusible solute.

In the 1980s Ernst Steudle and co-workers developed an alternative model to those above. Looking at the roles of apoplast and non-apoplast flows in the cortex, they put forward the hypothesis that many root observations can be explained by interactions between parallel apoplastic and non-apoplastic pathways. This was termed the composite transport model (CTM) (Steudle and Frensch, 1996), and suggests that transport is mainly apoplastic at high transpiration rates but at lower transpiration rates it is mainly symplastic or transcellular.

Theoretically, the CTM allows for multi-compartments in series, but regards the parallel pathways as more important than the serial compartments (Steudle, 1993). Since it has concentrated on the endodermis rather than the exodermis, it is a one-membrane two-compartment model (Steudle *et al.*, 1987; Pickard, 2003). Unlike the classical osmometer model, it assumes that resistances are more distributed across the root rather than only contained in the endodermis (Steudle and Frensch, 1989).

1.3.3 The continuing debate

Conclusions that the root has a dominant symplastic path (Murphy, 2000) and an equally important apoplastic path (Molz and Ikenberry, 1974; Steudle and Frensch, 1996) are quite contrary, but both plausible. The issue of relative contributions of the symplastic, transcellular, and apoplastic pathways for water movement in plant tissues are an ongoing subject of contention and have not been resolved (Murphy, 2000). Currently, there seems to be a wrestle between the model proposed by Murphy (2000), and that of Steudle (2000a). Complicating this has been the lack of accurate anatomical root data to test and differentiate the models, and predictions for both fall within the ranges of transport parameters observed from experiments.

One reason for this is that a proper quantitative and numerical formulation has not been developed for the CTM. It remains highly conceptual in nature (Steudle, 1997), and has not been cast into “a computation-convenient form...[with] extensive numerical results (Pickard, 2003). Many papers about the CTM have been written, and are continuing to be written. A lot of data has been generated to ‘validate’ the model, and parameter behaviour has been found to be in accordance with the model. However, the model is ambiguous, and it is suggested here that the development of a quantitative model based on the CTM is necessary in order to clarify the CTM, assess the validity of the data used to support the CTM, and resolve some of these contentions. Various models of root transport can not be properly compared without an adequate mathematical comparative analysis. Without this, a model will remain ‘plausible’ only (Murphy, 2000; Pickard, 2003).

The CTM model of the root is an interesting theory but requires further investigation. One appeal of this model is that predictions are made directly from measured parameters and coefficients across the root, without the requirement of membrane parameters in particular parts of the root. Membrane parameters are often unavailable and must be estimated (Murphy, 2000). In particular the permeability of the plasmodesma membrane is unknown, and was considered the most uncertain parameter in the models by Molz (1979) and Murphy (2000). A disadvantage of the CTM is that factors affecting these root measurements are unclear.

The CTM is a simple model, but has been viewed as a possible mechanism for nutrient uptake (Steudle and Peterson, 1998) although the movement of nutrients specifically, interactions between nutrients, and active transport has not been included in most papers about the CTM. It can not be considered a nutrient-uptake model. However, if the CTM is validated quantitatively, and more confidence in the model gained thereby, in the future it may be able to be formulated as a nutrient-uptake model which includes the effects of environmental factors on uptake. This is the case for all plausible root transport models. Detailed studies have already been published on the observed effects of high

salinity and low oxygen (anoxia) on transport parameters in young maize roots (Passioura, 1988; Steudle, 1993; Steudle, 1994). These have not yet been incorporated into the CTM.

1.4 Significance of current project

The CTM postulates mechanisms for uptake and how the measured efficiency of solute and water absorption can be achieved (Steudle, 1993). It is suggested that a quantitative model is necessary to test the CTM's predictive ability. By varying inputs and the equations used to represent the transport processes, factors contributing to transport behaviour can be clearly identified in instances where they cannot be done so in the laboratory. One advantage of developing a numerical model for the CTM is that a lot of experimental data which can be used in the model has been generated, and is readily available from the literature.

The proposed project involves:

- A theoretical review of the CTM – assessment of its assumptions and limitations, physical principles and processes incorporated, and equations used to represent these processes;
- Model development – incorporation of equations and processes into a numerical simulation of water and solute transport radially through the root;
- Analysis of results – validation of a quantitative model with experimental data, and testing the extent of its agreements with predictions of the CTM; examination of what this tells us about root transport mechanisms, and if the CTM is a model which appropriately describes these mechanisms.

The best source of data on root transport parameters is from pressure-probe experiments on young maize plants (Tyree, 2003), which will be used to validate the model in this project. The aim is not to develop a model for maize but to understand the physiological processes of root uptake in a general and theoretical sense. A root model developed from this data will encourage and guide future experiments on root uptake and transport in eucalypt seedlings, with a view to numerically modelling these processes in eucalypts.

2 THE COMPOSITE TRANSPORT MODEL

Transport equations essentially describe relationships between forces and flows. Parameters in the equations (e.g. flow conductivity, permeability) modulate this relationship and reflect the physical system. Parameters form the backbone of what we are trying to model – the CTM was developed to explain parameter behaviour which could not be explained by previously existing models.

2.1 General uptake mechanisms

Water transport and uptake in plants is considered to be mostly passive (Steudle and Peterson, 1998). There are three means whereby solutes and ions can be taken up (Barber, 1995; Steudle, 2000a):

- a) Passive transport independent of respiratory energy. This may be via diffusion, facilitated diffusion (using carrier proteins), or through water-filled pores. It may also be via convection with water flow;
- b) Passive transport along an electrochemical gradient dependent on respiratory energy;
- c) Active transport against an electrochemical gradient which requires respiratory energy. The plasma membrane is the main barrier to active uptake.

The CTM model doesn't really consider ion transport, for it models the passive transport of uncharged solutes, ignoring electrochemical gradients and active transport. The CTM is a bulk tissue model, whereas these latter factors operate on a smaller scale, i.e. across membranes. Transport is therefore described by bulk transport parameters rather than kinetics. However, a bulk term for active transport can be introduced into the transport equations, though this has not often been done. At the tissue level it is reasonable to assume that charged solutes are transported like uncharged solutes in the absence of an electrical gradient (see section 6.5).

While the CTM doesn't consider ion uptake specifically, pathways of water transport influence ion transport through changes in water potential and osmotic potential of cells. Most ions move symplastically (such as potassium, phosphorous, and ammonium), however some move apoplastically (such as calcium, and maybe magnesium) (Barber, 1995), and so different transport pathways would be of some relevance to ion uptake. As a starting point to understanding root transport, a simple model is best. Modelling passive transport of uncharged solutes is therefore deemed adequate.

2.2 Transport parameters

2.2.1 Definition of transport parameters

Three important parameters describe the passive transport of uncharged solutes (Katchalsky and Curran, 1967; Steudle and Peterson, 1998):

Reflection coefficient, s

This denotes the passive selectivity of a membrane or barrier. It measures how permeable a membrane is to solutes relative to that of water, and can be considered a measure of the interaction between solutes and water within the membrane. It can refer to the passive transport of charged or uncharged solutes. The value of s is particular to the solute being transported and the membrane travelled through.

- $s = 1$ indicates total selectivity, that is no solutes can pass and the membrane is only permeable to water (a 'semipermeable membrane');
- $s = 0$ indicates zero selectivity, that is all solutes can pass and the membrane is just as permeable to solutes as it is to water. There is no selection of one over the other.

Solute permeability coefficient, p_s

This is simply a measure of how permeable the membrane is to the solute. It is different to s as it doesn't measure selectivity. A low p_s reflects a 'semipermeable' membrane, for membranes are quite permeable to water. The value of p_s depends on the type of solute being transported and the membrane travelled through. The coefficients s and p_s are related: in an individual cell, a low s (low selectivity) is correlated with a high p_s (high passive solute permeability).

Hydraulic conductivity, L

This is the inverse of hydraulic resistance. It shows how conducive the path is to the passage of water, i.e. it is a measure of water permeability (Tyree, 1969). Also called the filtration coefficient. For a given driving force, the greater L is the larger the volume flux of water.

These parameters are related to the physical properties of the membrane, the nature of the transported solutes, and on the external environment (e.g. oxygen levels; salinity and solute concentration levels) (Steudle and Frensch, 1996). Refer to section 6.4.5 for formulaic definitions.

Root parameters (as opposed to membrane parameters) will be denoted by σ_r, p_{sr} , and L_r .

2.2.2 Parameter behaviour in the root tissue

The CTM is able to explain three observations previous models could not (Steudle, 1994; Steudle and Frensch, 1996; Zimmermann *et al.*, 2000):

- Low s_r and low p_{sr} .

A low s_r indicates low solute selectivity and that solutes pass through as easily as water. However, a low p_{sr} indicates that solutes do not pass easily, which is contradictory. The classical view of the endodermis acting as a plasma membrane of cells also implies that roots behave like a near-perfect osmometer with a reflection coefficient near 1, which is not generally observed.

- Differences between osmotic and hydrostatic L_r .

A hydrostatic pressure gradient gave higher L_r values than the equivalent osmotic pressure gradient in pressure-probe experiments. There is no reason from theory

why this should be the case – all components of water potential should have the same effect on the flux.

- Variable L_r

A nonlinear relationship between water flow rate and pressure gradient was found, with L_r increasing with (dependent on) flow rate.

The above differences between theory and experiment may be due to (Steudle, 2000a):

- a) Changes in root morphology and anatomy due to development/ ageing
- b) Changes in transport pattern/ mechanism of water flow
- c) Changing interactions between water and solutes
- d) Changes in the activity of water channels
- e) Internal influencing factors (nutritional and water status, demand from shoot)
- f) External influencing factors (e.g. drought, salinity).

Of these, b) - d) are of central interest since they concern fundamental mechanisms of transport. The other points will not be dealt with in detail in this project.

Quantitative models seek to understand:

- a) Why and how does L_r change?
- b) How does this affect uptake and solute-water interactions (s_r)?
- c) How does root anatomy impact on uptake?
- d) How do changes at the membrane level result in changes at the overall tissue/ organ level under different conditions?
- e) How does the surrounding environment influence uptake?

To test the CTM (a multi-compartment model) ideally requires detailed information for each compartment – e.g. sizes, internal concentrations, pressure gradients – which is unavailable (Steudle, 1994). In the absence of such data, however, a theoretical study can still be carried out by mathematically describing concentration and pressure distributions across the root, varying these, and looking at their effects on transport (Tyree, *pers. comm.*).

2.3 The CTM explanation

2.3.1 Brief outline of the CTM

The CTM postulates that the root switches transport pathways depending on internal (within-plant) or external (environmental) conditions. It is a one-membrane two-compartment model, with the endodermis separating an elastic xylem compartment and an elastic measuring system, fixed symplasm? Cortex an external unstirred layer

The CTM outlines:

- Composite transport comprised of two parallel pathways, and membranes in series
- A hierarchy of coarse and fine regulation
- Changing contribution of pathways to solute and water uptake depending on environmental conditions and driving forces.

It provides an explanation of:

- L_r variability, low s_r , and differences between osmotic and hydrostatic pressure flows
- How roots might adapt to water and nutrient stress, by physical changes in root development, or by switching transport pathways.

The CTM is also able to explain observed parameter differences between woody and herbaceous species (see Steudle, 1994; Steudle and Frensch, 1996; Steudle, 1997).

2.3.2 Transport pathways

Experimentally it is not possible to separate the symplastic and transcellular components, and these have therefore been grouped into the protoplasmic or cell-to-cell pathway in the CTM (Steudle and Frensch, 1996). The former term is more correct than the latter, although the latter is more common (Steudle and Frensch, 1996). We are then left with *two proposed pathways* for water flow:

- Apoplastic path
- Protoplasmic path.

Note that while the apoplastic path and symplastic paths are quite distinct, with the former path exterior to the plasma membrane and the latter interior to it, the above two pathways are not, since water must enter the cell walls to traverse between cells in the protoplasmic path (Molz, 1979). This makes modelling more difficult, and the processes being measured and modelled must be considered carefully.

Most of the time, i.e. under reasonable transpiration rates, water transport occurs through the apoplastic path. Evidence for this includes similar values of L_r and cortical cell L in maize (under hydrostatic gradients), which could only occur if large amounts of water were by-passing the protoplast (lots of membranes in series would decrease L_r) (Steudle, 1993).

The apoplastic and protoplasmic pathways are in parallel and form the basis for the CTM (two-compartment) model. The parallel pathways are made up of resistive components in series (membranes or hydrophobic barriers). These pathways are not mutually exclusive and solutes and water can move between the two for different distances (Steudle and Frensch, 1996; Steudle and Peterson, 1998; Steudle, 2000a). For this pathway interaction, it is assumed that a rapid exchange of water occurs between the two pathways so that there is local water flow equilibrium (zero water potential difference) between them.

This is considered a reasonable assumption since the time scale of water exchange of cells with their surroundings is usually of the order of a few to tens of seconds (Steudle, 1993).

However, papers have not commented on how pathway exchange would affect parameters of the CTM. It has been commented only that “there is considerable exchange of water between pathways”, that the “relative contribution of pathways to overall flow may vary”, and that a more complicated network or system model is needed to properly describe water flow in roots (Steudle and Frensch, 1996). Switching between pathways has been dealt with only in the more mathematically complex papers, by considering differences in water potential across the plasma membrane between two cells in the protoplasmic pathway (i.e. parallel to the pathway), and between the protoplast and the cell wall of a cell in a direction transverse to the pathway, on a cellular level (Molz and Ikenberry, 1974). A switching factor (between 0 and -1) has also been incorporated in some equations, the value of which is dependent on the nature of the driving forces (Steudle and Frensch, 1996). However, switching between pathways needs to be considered in more detail, especially in regards to incorporation into the CTM model.

Solute relations are affected by the nature of the water pathway. Solute and water transport are strongly coupled in the apoplast, but are thought to move largely independently across the protoplasmic path. Interactions between solute and water flows can be important at low rates of transpiration (Steudle and Peterson, 1998).

The properties of the two pathways are as follows (Steudle and Frensch, 1996; Steudle, 1997; Steudle and Peterson, 1998; Steudle, 2000a; Steudle, 2000b):

Apoplastic path:

- Forms about 10% of root cross-sectional area (but its contribution to water flow can be substantial)
- Has a high L_r
- Hydrostatic pressure gradient is the driving force for flow
- Has no membranes
- Has apoplastic barriers - hydrophobic deposits of CB or SL at the endo- and exodermis, which decrease L_r and p_{sr} and increase s_r
- Passive solute transport is via diffusion and bulk flow with water.

Protoplasmic path:

- Forms about 90% of cross-sectional area
- Has a lower L_r
- Hydrostatic and osmotic pressure gradients are the driving forces for flow
- Has membranes, the plasma membrane being the area of major resistance (two membranes are crossed per cell layer)
- Has selective water channels (aquaporins) in the membranes, which contribute to most of the hydraulic conductivity of the membranes
- Solute transport is rate-limited by active or passive permeation across the plasma membrane, and ability to be taken up into the symplasm.

Transport is mostly apoplastic and driven by hydrostatic pressure gradients set up by transpiration, except at the endo- and exodermis where there will be a protoplasmic step (Steudle, 2000a). After passing through the endodermis, solutes may then move back to the apoplast or continue via the symplast (Barber, 1995). However, under stress or low transpirational rates, the osmotic pressure gradient (and thus protoplasmic flow) can be significant, and active transport may also become more important (Steudle, 2000a).

Flow of water in the transcellular path, where for each cell water flows through a membrane, apoplast, then a membrane, is thought to involve water channels (Murphy, 2000). Water channels are thought to be selective mainly because they are narrow (Steudle and Henzler, 1995). The gating of water channels is probably controlled both by metabolic and environmental factors (Steudle and Frensch, 1996; Steudle, 2000a). While water channels are regarded by some as important, the CTM considers that the contribution of water channels would only be important if the protoplasmic pathway is dominating, and the contribution of other components to water conductivity is negligible (Steudle, 1997). Under stress conditions (increased suberization resulting in a dominance of the protoplasmic path) inhibiting water channels can decrease L_r , and may help to reduce water losses (*ibid.*). However, relatively little is known about the control of water channels and their role in water and solute transport (Steudle and Henzler, 1995).

The above can be summarized in two postulates of the CTM.

Postulate 1:

Under different plant or environmental conditions the dominant transport pathway will change, and hence the dynamics of water and solute transport (coarse regulation) will also change.

Postulate 2:

Water channels in membranes may play an important role under severe water or nutrient stress, when roots are heavily suberized (fine regulation) (Steudle and Frensch, 1996; Steudle, 1997; Steudle, 2000a).

2.3.3 CTM explanation of parameter findings

As mentioned previously, the CTM provides a possible explanation of three observations regarding parameter behaviour. Observations in various parts of the root has found the following (Peterson *et al.*, 1993; Steudle and Peterson, 1998; Steudle, 2000a):

- Apoplast: high L_r , low s_r
- Protoplast: lower L_r , high s_r , low p_{sr}
- Radially across the root: variable L_r , low s_r , low p_{sr} .

The different L_r of each pathway would explain why L_r is different under different driving forces, since hydrostatic pressure gradients result in more apoplastic flow, and

osmotic pressure gradients drive flow through the protoplast (Steudle, 1997; Steudle and Peterson, 1998; Steudle, 2000a).

The increase of L_r with flow rate can be explained by an increase in the apoplastic: protoplasmic ratio with flow rate, which is thought to occur by a ‘circulatory’ flow (Figure 3). Under low transpirational rates flow into the root is primarily protoplasmic, which causes a pressure buildup in the xylem and a back-flow of water out of the root through the apoplast, due to the rapid exchange of water between cells. Apart from the dominant protoplasmic flow, the hydraulic resistance would also be high at low flow rates because the direction of flows partly counterbalance each other (Steudle, 1994; Steudle, 2000a; Zimmermann *et al.*, 2000; Hose *et al.*, 2001).

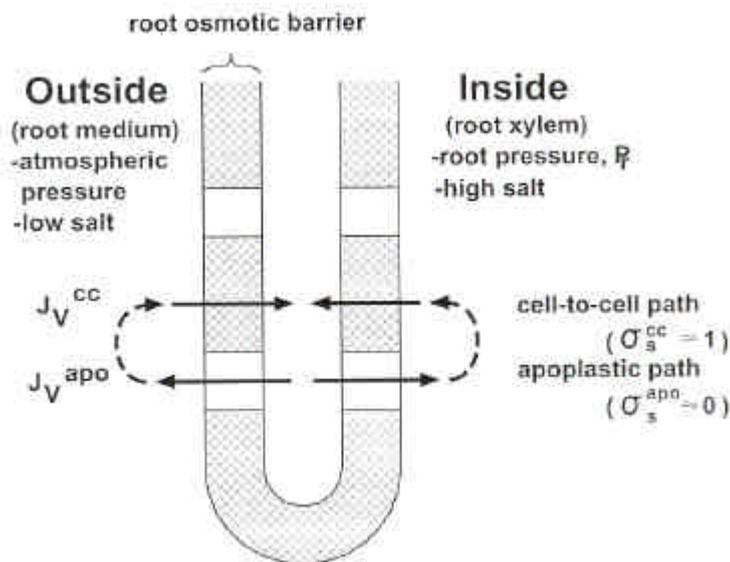


Figure 3. Diagram of circulatory flow. J_v is the volume flow; s_s is the reflection coefficient; ‘cc’ indicates cell-to-cell path; ‘apo’ indicates apoplastic path (Steudle, 2000).

As transpirational rates and the hydraulic gradient increases, the increasing flow of xylem sap will dilute the xylem, lessening the pressure buildup there and causing a cessation of backflow across the apoplast. Water will then flow in the same direction in both pathways (into the root) and L_r will be higher because of increasing apoplastic flow. Under sufficiently large hydrostatic pressure gradients the osmotic driving force will vanish (Steudle, 1994; Steudle, 2000a; Zimmermann *et al.*, 2000; Hose *et al.*, 2001).

Another reason for an increase of L_r with flow rate may be due to increasing water content of cell walls and the filling of intercellular spaces under dominant hydrostatic pressure gradients. This would increase the cross-sectional area available for apoplastic flow (Steudle and Frensch, 1996, Steudle and Peterson, 1998). Katchalsky and Curran (1967) has shown that L is proportional to the water content of the membrane.

This circulatory flow also provides an explanation for low s_r and p_{sr} . The root s_r is a weighted mean of the s_r of each pathway, which contributes according to its L_r (Steudle and Frensch, 1989; Steudle, 1994). Since apoplastic flow is the dominant flow the overall

s_r would be low. Since flows in different pathways are the cause of changes in s_r , the permeability of the pathways does not have to be correlated with s_r as would be the case across the same membrane or pathway (as in a cell). Therefore p_{sr} may still be low in the presence of a low s_r . This is important for the plant, for a low p_{sr} allows nutrients to be retained in the xylem in the presence of rapid/ variable water uptake or release from the roots (Steudle and Frensch, 1989; Steudle, 1994; Steudle, 1997).

A circulation flow has also been postulated to exist within the plasma membrane, with the two parallel pathways being a membrane lipid array, and an array with water channels (Steudle and Henzler, 1995). This has been used to explain low membrane reflection coefficients (*ibid.*). The model also predicts low reflection coefficients for the water channel pathway, which suggests there is some transport of solutes across water channels (*ibid.*).

In some species transport pathways do not seem to change with the driving force. This does not necessarily invalidate the model, for differences between species may be due to different compositions of suberized deposits, and different developmental states of the endo- and exodermis (Steudle, 1994; Steudle and Peterson, 1998). In some roots apoplastic transport appears negligible (Murphy, 2000). However, the effects of anatomy on transport processes requires further research.

2.4 Sites of resistance

Although the CTM only considers parallel pathways in the explanation of parameter behaviour, understanding what happens at the major sites of resistance along the pathways is also fundamental to root transport models. However, the permeability of the endo- and exodermis in intact plants is difficult to measure, and the function of these regions remains unclear (Steudle and Peterson, 1998). There are also no estimates of the reflection coefficient for endodermal cells (Murphy, 2000). The endo- and exodermis follow similar paths of development, implying the roles of each are adapted to each other (Peterson, 1988). However, they would have different functions.

2.4.1 Effects of suberization

Influence of suberization on water flow

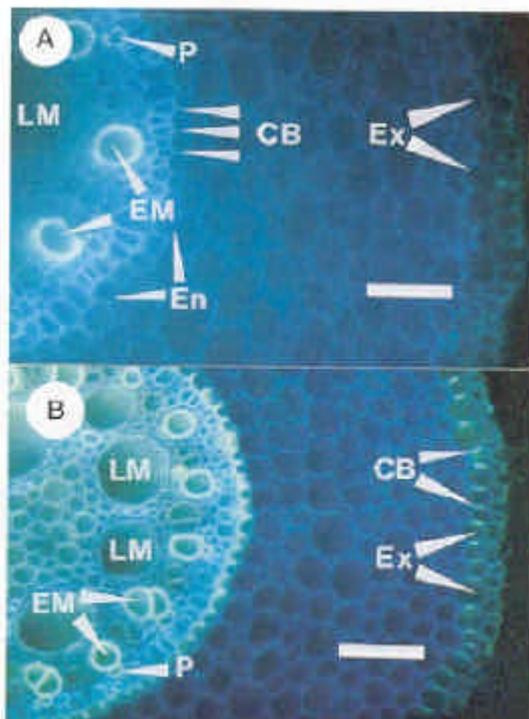
The CB has been assumed to be impermeable to water and solute flow. Recent endodermis puncturing experiments (Steudle and Peterson, 1998), however, have shown that the CB in young maize plants has some permeability to water (Steudle, 2000b). In young plants, the hydraulic resistance is not concentrated in the endodermis but is more evenly distributed across the root tissue (*ibid.*). The cortex also seems to present considerable resistance to radial ion movement (Ginsburg and Ginzburg, 1970).

With age, the endodermis appears to become the major point of resistance in roots without an exodermis (Frensch *et al.*, 1996). The exodermis, where present, also becomes more suberized with age (Clarkson *et al.*, 1987). This could be due to deposition of SL which occurs after the initial CB deposition. Yet the effects of SL are variable. There is evidence that SL decreases the rate of water uptake and some ions, but in some species it

does not seem to appreciably affect permeability (Clarkson *et al.*, 1987; Steudle and Peterson, 1998). It is unclear whether decreased permeability with age is due to SL specifically, or thickening of the walls due to both increased CB and SL deposits (Steudle and Peterson, 1998).

The role of SL is a significant point which needs to be resolved. If SL does decrease permeability it would significantly affect radial flow in the protoplasmic path, since SL is laid down in radial, transverse (end), and tangential walls, while CB is specifically laid down in radial and transverse walls of cells only (Figure 4) (Peterson, 1988; Steudle and Peterson, 1998). This means almost all water passage into the cell would be impeded. Though the processes are not clear, it is generally agreed that a thickening of CB and the formation of SL with age in both the endo- and exodermis would increase localized resistance for water and solute flow in these regions (Frensch *et al.*, 1996; Steudle, 2000b).

Figure 4. Radial cross-section of root of *Zea mays*, showing CB in the endo- and exodermis at different stages of development. Ex: exodermis; En: endodermis; EM: early metaxylem; LM: late metaxylem; P: protoxylem (Steudle and Peterson, 1998).



Influence of suberization on solute flow

There is plenty of evidence that the CB in the endodermis is a barrier to most ions and effectively blocks leakage of nutrient ions from the xylem, leading to an optimization of water supply and nutrient retention in this region (Peterson, 1988; Steudle, 2000b; Hose *et al.*, 2001). Suberization affects all the three transport parameters of hydraulic conductivity, solute selectivity, and solute permeability.

Suberized deposits decrease L_r by reducing the surface area available for water flow. They can increase solute selectivity (and decrease permeability) by acting as filters for some solutes which are too large or hydrophilic, and also through an increased protoplasmic: apoplastic path ratio due to a symplastic path present in these regions. Under suberized conditions a greater proportion of solutes may enter symplastically, and solutes unable to enter the symplasm are impeded (Hose *et al.*, 2001).

If SL blocks the transport of solutes (which is not yet clear), the exodermis in addition to the endodermis could contribute to nutrient regulation, and the cortical apoplast could act as a storage region for nutrient ions leaking out of cortical cells (Steudle and Peterson, 1998). Clearly sites of uptake and nutrient activity could change with SL deposits in this case. But it is not unequivocally clear that the exodermis contains SL (Clarkson *et al.*, 1987).

2.4.2 Role of the exodermis

The role of the exodermis continues to be a subject of contention. Comparisons of aeroponic to hydroponic roots have shown that the former has a greater resistance to water flow, indicating that the exodermis is a substantial barrier (Steudle and Peterson, 1998). However, some consider it still unproved whether the CB in the exodermis is analogous to that in the endodermis (Clarkson *et al.*, 1987).

Experiments on the exodermis of maize suggest that walls with SL remain relatively permeable (lamellae were observed close to the root tip in regions characterized by high rates of water and solute uptake), but become impermeable if roots are exposed to moist air rather than kept completely immersed in culture solution (Clarkson *et al.*, 1987). This indicates that the soil environment has direct and significant effects on suberization in the exodermis. Soil environment may be the most significant determinant of exodermis function – the exodermis has a major impact on sites of uptake from the soil, and stressed roots develop a more suberized endodermis to limit water and nutrient loss to the rhizosphere (Steudle, 2000b; Hose *et al.*, 2001). If soil moisture is important, then in soils which are not uniformly wet one can expect a large heterogeneity with respect to permeability to water along the length of a root (Clarkson *et al.*, 1987). All roots have an endodermis, but not all have an exodermis (Peterson, 1988; Hose *et al.*, 2001).

The presence of an exodermis changes paths for uptake in the outer root (refer to Figure 1), since:

- In roots without an exodermal CB, solutes can move apoplastically as far as the endodermis, allowing all the cortical parenchyma cells to be potential sites of solute uptake from the apoplast (Peterson, 1988);
- With an exodermal CB, uptake is confined to the epidermal walls and outer tangential walls of the exodermis (uptake by cortical parenchyma cells is prevented). If SL is present, uptake across the outer tangential walls is prevented and uptake into the symplast limited to the epidermis (*ibid.*). Uptake confined to the epidermis means that the surface area for uptake of solutes might be greatly decreased. This has led to speculation that the CB in

the apoplast might not block the passage of ions to the same extent as in the endodermis (Hose *et al.*, 2001).

Some exodermal layers have passage cells, which are cortical-like cells thought to have a higher water permeability than exodermal cells, and allow greater uptake into the exodermis (Peterson, 1988). They have CB but lack SL (Hose *et al.*, 2001). Maize, however, has a uniform exodermis (*ibid.*).

2.4.3 Role of the endo- and exodermis together

There is little information in the literature about the effects on solute and water transport of the endo- and exodermis together, for when they are both present their effects on conductivity cannot usually be separated (Frensch *et al.*, 1996). Effects of the endodermis are usually examined in roots where the exodermis is absent (*ibid.*), and the effects of suberization of the exodermis are inconclusive and often looked at separately from the endodermis (Clarkson *et al.*, 1987). In the absence of conclusions about the endo- and exodermis, models can only take into account various possible scenarios based on different assumptions, and apply each of these in turn.

2.4.4 Apoplastic by-passes

Non-selective by-pass flows around apoplastic barriers make it difficult to ascertain the effect of suberized regions. By-passes mean that water and some solutes can avoid apoplastic barriers by taking an alternative path. They complicate the picture because even small by-passes can considerably reduce the osmotic gradient, and substantially lower s_r . Where there are frequent apoplastic by-pass channels, selectivity will be weak (Steudle, 1994; Hose *et al.*, 2001). There is evidence that there are substantial apoplastic bypasses for water in the root, even in the endodermis (Zimmermann and Steudle, 1998). However, this issue requires further research, for Murphy (2000) argues that bypasses in the endodermis are not conclusive, and transport across the endodermis could be entirely symplastic.

2.4.5 Water channels in the plasma membrane

Water channels are an active area of research. Relatively little is known about their role in solute and water transport in plants. Water channels can alter the hydraulic conductivity of cell membranes. They may also modulate membrane selectivity since, although solute permeability across membranes is low, it has been found that there is some passive flow of solutes across water channels (Steudle, 1997).

Water channels may have an important regulatory role in passage cells of the endo- and exodermis. Suberization in the endo- and exodermis may be paralleled by an increased expression of water channels, for there is a higher contribution of the protoplasmic pathway under these conditions. Yet how exactly passage cells and water channels influence water and solute transport is not clear (Maurel, 1997). There is some evidence that nutrient deprivation decreases water channel activity, but this is complicated by the fact that they are under metabolic control (Steudle and Peterson, 1998). Maurel (1997) has made a detailed review of water channel structure and function in membrane transport.

From discussions such as the above, which show that the relative influence of the two transport pathways is not always clear, it has been concluded by Steudle (1997) that changes in apoplastic and protoplasmic resistances should be studied in conjunction. It is difficult to separate the apoplastic component from the action of water channels and plasmodesmata, for a reduced L_r in one may be compensated for by an increased L_r in the other (Steudle, 1997).

2.5 Problems with the CTM

The CTM provides plausible explanations for transport mechanisms and pathways. However, there are many unresolved issues, including:

1. The CTM has not been tested quantitatively and its predictions are still speculative. We cannot say whether it is in fact the mechanism which generates observed transport behaviour in the root, and a means by which plants adapt to environmental conditions.
2. The CTM is based on a simple view of two parallel pathways. This may not be adequate since the serial components, in particular the endo- and exodermis, have not been looked at in depth (Steudle *et al.*, 1987; Tyree, 2003). That is, the serial components have not been disaggregated. Other factors apart from switching of parallel pathways may contribute to a variable L_r , as explained in point 3 below.

The scenario of solute and water exchange between the pathways also adds a tangential dimension to the radial flow, which may only be negligible at the extremes of very low or very high flow, when one pathway dominates. In addition, near the root tip (with immature xylem) the axial hydraulic resistance increases and tends to dominate the overall resistance; to include this region the CTM has to incorporate axial resistance, adding a third axial dimension to the transport equations (Steudle and Frensch, 1996).

3. It has been claimed that the theory of irreversible thermodynamics used in the CTM “exactly fits the experimental results, including the coupling between water and solute transport” (Steudle, 1993). However, Tyree (2003) has stated that measurements of transport parameters have not taken solute-water coupling effects into account. The RPP (see sections 5.1 and 5.3) uses concentration and pressure differences between the medium and the xylem, rather than the effective gradients driving water flow across the resistive components. Equations (4) and (5) which have been used to adjust for unstirred layer effects do not reflect these effective gradients. This may seriously alter measurements since solutes may accumulate in front or within apoplastic barriers, forming an unstirred layer and altering the effective concentration gradient (Freundl *et al.* 1998). Unstirred layers cause many ‘pseudo’ effects in measurements of membrane parameters (Barry and Diamond, 1984).

Unstirred layers and solute/water drag (friction) effects influence the rate of flow, and consequently measurements of s_r , p_{sr} , and L_r . For example, increasing solute concentration causes a reduction in s_r since solute flow: volume flow increases

(Steudle and Tyerman, 1983). L_r (osmotic) is calculated using half-times, but diffusion times of solutes at the barriers may be longer due to solute/ water drag, and back flow of water at the endodermis which reflects from the barrier and sweeps solutes away, delaying their arrival (Tyree, 2003). The measurements of s_r and L_r , therefore, may not be different for each pathway (Tyree, 2003), and they may not be a function of flow rates since the incorrect driving force is used for the resultant flow. Therefore, the root pressure probe (RPP) may not be measuring real values of s_r and L_r (Tyree 2003).

Physico-chemical properties of the parallel pathways, such as solute-water friction (which is variable), may also alter flow rate and resistance. The effects of friction are usually assumed to be small. However, they have been incorporated into some equations and can affect transport coefficients (Steudle and Frensch, 1989; Steudle, 1997).

4. There are theoretical objections to parameter descriptions of the CTM, as discussed by Tyree (2003). Irreversible thermodynamics predicts relationships between macroscopic variables of the system, independent of internal mechanisms. Properties of system (root) components are not reflected in thermodynamic equations for the whole system (root). The CTM may predict that there is variable L_r across the root but cannot comment about L_r of its components, parallel (i.e. transport pathways) or series. Therefore, although observations show a non-linear relation between flow rates and driving forces, it does not mean L_r is changing, and the CTM does not actually predict changes in L_r with flow rates as concluded from these observations (Tyree, 2003).

On theoretical principles, it is best to have transport coefficients independent of driving forces and flows (Tyree, *pers. comm.*). L_r should be independent of the nature of the driving force (Zimmermann *et al.*, 1992). The transport coefficients for a root – L_r , p_{sr} and s_r – do not need to change with flow to account for a non-linear relation in water flow rate with driving force (Tyree, 2003). The conductivity coefficient is a physical property and should be constant; L_r could only increase with flow rate if a physical change in permeability of membranes to water occurs (e.g. through water channel activity) (*ibid.*).

For modelling purposes, parameters for root components should be treated as constants since we cannot say anything otherwise. L_r for the whole root can still be described by applying transport equations to individual components, which have constant component properties (Tyree, *pers. comm.*).

5. The assumption that water flow between pathways is in equilibrium may not hold in all situations.

While this is a valid assumption, the time-scale for water exchange between cells and their surroundings is of the order of a few seconds. Under large hydrostatic gradients apoplastic flow may be very rapid (if it is true that cell wall L_r increases with hydrostatic flow). Under this situation the assumption may not be met and the CTM model unusable (Steudle and Frensch, 1996).

6. Anatomical assumptions underlying parameter values may not always hold. There is a lack of anatomical data and knowledge of the root tissue. For example cell wall L , and cell wall storage capacity, are not known with sufficient accuracy. Often one assumes s in cell walls to be close to zero. However, this may not be true in the presence of apoplastic barriers, which may increase s and decrease solute diffusion coefficients in the apoplast (Steudle and Frensch, 1996). There is also some water flow through Casparian bands as these do not totally obstruct the passage of water, and it is still debated whether older thickened (suberized) roots contribute to water uptake (Steudle, 2000a). It is difficult to make conclusions about adaptive mechanisms in the dearth of sufficient anatomical knowledge. Yet until we have more developed experimental techniques we have to work on existing assumptions.
7. Only positive pressures in the xylem have been used in calculating root transport parameters. These results might not be representative for the transpiring plant when negative tensions are exerted in the xylem (Steudle *et al.*, 1987). Applying negative pressures to the xylem over long periods of time with the pressure probe is difficult, and only negative tensions up to -2 bar have been obtained (Steudle, 1993; Steudle, 1994). These results did not seem to show any difference in transport properties compared to positive pressures (Steudle, 1994). However, the situation does not seem conclusive, and it is hoped that further developments in measuring techniques will be made.

3 PROJECT AIMS AND OBJECTIVES

3.1 Motivation and approach

Developing a quantitative, integrated model can help clarify some of the above factors in root transport, by observing the effects of their inclusion/ exclusion on parameter values. The ability to determine flow parameters based on biophysical principles gives insight into contributing factors to the rates of solute and water flow in roots. These parameters reflect physical transport properties of the system, and thus also give insight into root physiology.

An assessment of the CTM will be made by comparing parameter outputs of the quantitative model with its predictions, and validating the quantitative model against existing data. A biophysical approach will be taken, using theory from irreversible non-equilibrium thermodynamics (Katchalsky and Curran, 1967) (see section 6). Under this approach membranes are considered a 'black box' and their internal workings are not considered (Kedem and Katchalsky, 1963a).

3.2 System description (proposed)

It is proposed to consider a two-membrane, three-compartment model with two parallel pathways (one apoplastic, one protoplasmic). Some water exchange between the two pathways may occur. The three series compartments will be the xylem, cortex, and external medium; flow through soils are not included. The transport mechanism across

the stele and the cortex are assumed to be the same. The two membranes separating the compartments are the endodermis and exodermis. Only radial transport (not axial) will be considered, between the region immediately external to the root, and the xylem. Boundary layers on the inside and outside of the endodermis, and on the inside of the exodermis, will be included. It can be assumed the boundary layer external to the exodermis is minimal due to stirring of the bathing medium in pressure probe experiments.

Main variables involved are the turgor pressure P , osmotic pressure p , solute concentration C , and volume V , for each parallel pathway. These will vary radially across the root between cell layers. Equations incorporating both permeable and impermeable solutes will be used. It is not clear, at this stage, whether active transport can be adequately incorporated. Friction effects will most likely only be considered at the boundary layers, where there will be solute buildup and unstirred layer effects through solute-solute and solute-water coupling. An isothermal system (i.e. constant temperature) will be assumed.

3.3 Model inputs and outputs (projected)

Inputs

Mean cross-sectional area A of each cell layer
Constant reference volume V_0 for each cell layer
Elastic modulus e for each cell layer

V , C , P of external measuring system at equilibrium
 P change (perturbation) of external medium

Temperature T
Ideal gas constant R
Valence number for ion n .

Outputs

Root reflection coefficient s_r
Root hydraulic conductivity:
- for hydrostatic gradient L_{rh}
- for osmotic gradient L_{ro}
Root permeability coefficient p_{sr}
Volume flux J_v
Solute flux J_s

3.4 **Data requirements (projected)**

For the epidermis and different cortex layers this includes:

P, e, t_h, L_r, s_r

Mean cell diameter, length, number of cells.

For the root:

P, t_h, L_r (for flow into and out of root; hydrostatic and osmotic flows), s_r and p_{s_r} for different solutes.

t_h is the half-time of return to equilibrium, where half-time is defined as the time when “approach to a new equilibrium concentration is half-accomplished” (Philip, 1958a).

3.5 **Objectives**

Only radial variation in the root will be looked at. If time permits, axial variation may be incorporated, however this is not part of the major aims. It will be assumed that water flow between pathways is in equilibrium.

1. Compartmentation

Determine what age and region of the root is to be modelled, which will be ascertained from data availability. Disaggregate components in series (separate serial resistances), by estimating the distribution of resistance from pressure profiles across maize root tissues. Thereby the effective concentration and pressure differences across each compartment can be ascertained.

Apply thermodynamic theory to model flow in the decided compartmentation, which involves serial and parallel components. Investigate how to incorporate combinations of parallel pathways (i.e. exchange between apoplastic and protoplasmic pathways) into these equations.

2. Incorporate a measure for variable resistance of apoplastic barriers in the endo- and exodermis

This involves some assumptions about the contribution of CB and SL on transport processes and parameters. The developmental stages of the endo- and exodermis may need to be taken into account – while they follow the same developmental path the CB frequently occupies a greater region of the exodermal walls than in the endodermis, at least in the earlier stages (Hose *et al.*, 2001).

It has been mentioned previously that apoplastic and protoplasmic resistances should be measured in conjunction. Our lack of current knowledge of water channels makes this difficult. The effects of water channels can probably be ignored in the study of roots grown under experimental conditions where they are not heavily suberized and under extreme stress. It is hoped that composite membrane flow can be described adequately

using a constant L_r of membranes. If not, consider adding another variable parameter into the above to reflect possible water channel activity in membranes, which will vary membrane permeability.

3. *Develop equations for solute-solute and solute-water interactions*

First include non-permeable solutes in the solution, then add permeable solutes. Look at the relative effects of each on osmotic flow. Look at one or two solutes at a time.

For solute-solute interactions, consider how concentrations of one solute affects the driving force of another by changing the osmotic gradient. Look at different permeation and diffusion rates for each solute across a membrane, and the effects of these on the rate of water flow driven by osmotic gradients. Not many people have looked at multiple solutes or solute-solute interactions, which are usually assumed to be small (Tyree, *pers. comm.*). Look at solute-water interactions and how solutes can be dragged along with water, particularly under hydrostatic flow.

4. *Include the influence of tissue geometry on flow, especially in regards to solute buildup at the endo- and exodermis*

Include unstirred layers at the endo- and exodermis. Be sure that this reflects different solute concentration buildup at these regions depending on the direction of flow. Compare also the influence between a still and flowing external medium on uptake.

5. *Examine parameter behaviour*

Examine effects of the above, especially points 3 and 4, on the parameters L_r , s_r and p_{sr} . Examine correlations between the parameters, especially changes in p_s with s_r . Arrive at a conclusion whether L_r and s_r are functions of flow rate, and possible causal factors of observed non-linearities. Examine the effect, if possible, of different ratios of apoplastic and protoplastic flow on L_r , s_r and p_{sr} , perhaps by using a variable parameter denoting their ratio.

Examine thermodynamic theory on L_r , in relation to components and of the overall system. Examine if it is necessary to consider a separate, variable L_r between the protoplastic and apoplastic pathways in light of the above inclusions of solute behaviour and unstirred layers.

Look at how these parameters change under some different environmental conditions (e.g. soil water content, external solute concentrations). Ignore hormonal effects.

6. *Design of tissue model*

Design a model which integrates the above factors into a set of equations to describe transport across the root tissue. Start with the design of each part specified above, then put these together in steps, gradually adding more complex interactions.

7. Implications of results

Analyse how the model outputs compare with existing data and the CTM, and what insights they provide, if any, on possible transport mechanisms within the root. Examine the limitations and validity of these conclusions.

Understand the experimental conditions under which parameters in the CTM were determined. Analyse the impacts of these conditions on conclusions drawn about transport mechanisms, and implications for the predictive scope/ applicability of the CTM and quantitative model. Conclude whether these theoretical transport principles can be applied across different species/ plants. Explore what modifications or additional data may be needed in order for them to be applicable.

4 EXPERIMENTAL TECHNIQUES TO DETERMINE PARAMETERS

The root is a complex tissue, and it is very difficult to take hydraulic measurements of roots. There are only a few experimental techniques available to measure hydraulic parameters at the root cell and tissue levels. General lack of information about root hydraulic architecture makes information on root water transport difficult to assimilate (Russell and Barber, 1960; Steudle, 2000a). Most data is available for young primary roots where the dimensions and shape are well defined, with little information available for entire root systems (Steudle, 1997). Due to anatomical variability within tissues and organs, parameter measurements often average out different developmental stages, and there is still uncertainty about the contribution of different root parts to overall uptake (Steudle, 1997; Steudle and Frensch, 1989).

Pressure probe methods have been widely used to measure transport parameters in plant cells. These are based on known relationships between turgor pressure and transport parameters. Most of them can be classified as ‘pressure relaxation’ (where the pressure decreases from one value to another) or ‘pressure clamp’ (where the pressure is maintained or clamped at a fixed magnitude) methods (Ortega, 1993). The pressure relaxation method may also be called the ‘volume clamp’ technique since after inducing a rapid change in cell volume, the cell volume is held constant to measure the relaxation of cell pressure (Murphy and Smith, 1998). Pressure relaxation yields a plot of pressure with time, while the pressure clamp yields a plot of sap volume in the pipette with time, which may be termed a ‘volume relaxation’ (*ibid.*).

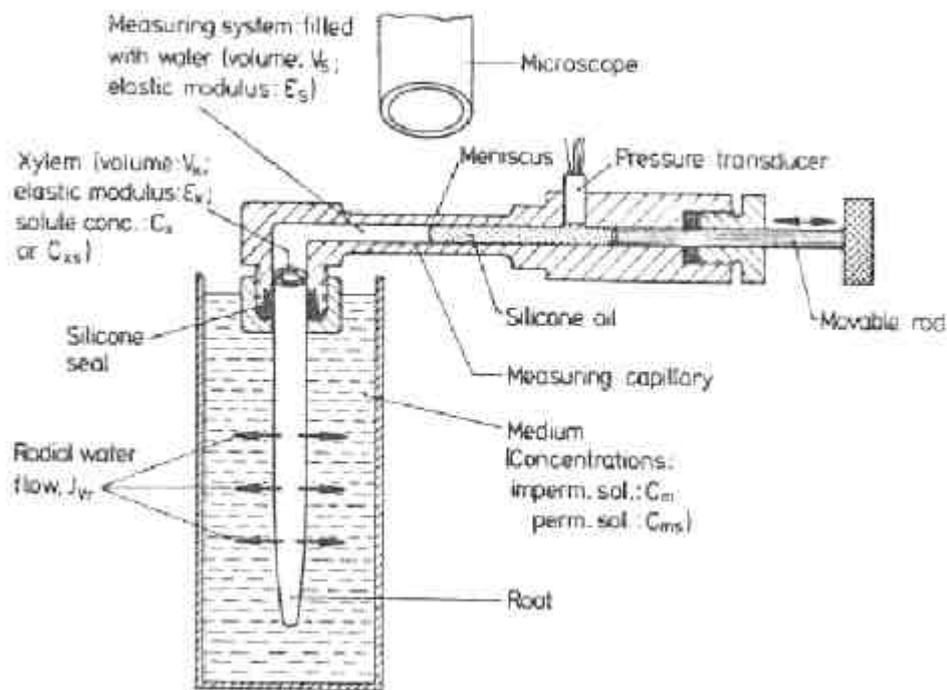
The pressure clamp has two major advantages over the pressure relaxation method: that it can be used on growing cells (Ortega, 1993), and the elastic modulus (a quantity that is difficult to determine accurately) is not required for measurements (Murphy and Smith, 1998; Ortega, 1993). The relaxation times are also longer with the pressure clamp, allowing for more accurate measurements (Murphy and Smith, 1998). However, unstirred layer effects are more of an issue with this technique because gradients are established for a longer time (Tyerman, *pers. comm.*). The cell pressure probe (CPP) and root pressure probe (RPP), which have been used for measurements for the CTM, are pressure relaxation methods.

The CPP and RPP can be used simultaneously for measurements at the cell and root levels, allowing greater insight into transport mechanisms to be obtained (Steudle, 1994; Steudle and Frensch, 1996). The best data set we have for these measurements are from experiments on primary maize roots, although the CPP and RPP have also been used on other herbaceous plants such as rice (Miyamoto *et al.*, 2001), barley (Steudle and Jeschke, 1983), and onion (Melchior and Steudle, 1993).

4.1 The CPP and RPP

The current version of the CPP was developed in the late 1970's, and the RPP (Figure 5) was developed in 1983 (Steudle, 1993). Their advantage over most other techniques is that they can measure all three transport parameters L_r , p_{sr} , s_r at the same time, so that complete sets of transport coefficients are obtainable at the cell and root levels (Steudle, 1997). Driving forces are measurable to high precision under well-defined conditions (Steudle, 1994; Steudle and Frensch, 1996; Steudle and Peterson, 1998), and metabolic states of the roots can also be checked throughout the experiments (Steudle and Frensch, 1996).

Figure 5. Diagram of root pressure probe (Steudle *et al.*, 1987)



The RPP uses excised roots only, from plants grown either hydroponically or aeroponically (Steudle and Frensch, 1996; Steudle and Peterson, 1998; Zimmermann *et al.*, 2000), though the technique can be used in a soil environment as well (Steudle, 1993). The advantage of using excised roots is that it removes the influence of

translocation on uptake, which is useful for studying uptake mechanisms (ref Barber, 1995). Studying intact plants doesn't allow individual processes to be studied in isolation (Russell and Barber, 1960). However, a disadvantage of this is that the rate of the overall process may be controlled by any rate-limiting step, so that conclusions drawn from a single variable alone may be misleading (*ibid.*). Russell and Barber (1960) examined the controversy around active versus passive transport of ions, and concluded that to adequately interpret mechanisms, the effects of all other relevant variables must be examined, and particularly in studies of uptake in the plant-soil system, intact plants should be studied. They cautioned against results from experiments with simple tissue systems as they do not themselves provide an adequate basis for interpreting relationships in the more complex system of the intact plant. Another disadvantage is that turgor and osmotic pressure gradients in the tissue may be disturbed when the roots are excised (Zimmermann *et al.*, 1992).

However, experiments on intact plants are difficult and little detailed data is available. Turgor pressure measurements beyond the cortical layer in intact plants are difficult because further insertion tends to cause leaks and cracks in the tissue (Zimmermann *et al.*, 1992). At 2000, there were no reliable measurements available of the permeability of the endo- and exodermis of intact roots to water (Steudle, 2000b). For modelling which requires detailed data, measurements from pressure-probe experiments on excised roots appears to provide the best data. As long as the above disadvantages are taken note of, conclusions can be drawn when only plants under non-stressed conditions are used. It is important to note that the range of validity of the proposed model in our study is limited to the conditions required by pressure probe experiments (Tyree, *pers. comm.*).

4.2 Plants and nutrient media used in experiments

Maize roots (*Zea mays*) with and without an exodermis have been studied. Those without an exodermis are obtained from plants grown hydroponically (these roots develop no exodermis), and those with an exodermis are obtained from plants grown aeroponically (by a mist cultivation method) (Steudle, 2000b). Aeroponic roots are usually more suberized and develop an exodermis because they have to reduce loss of moisture to the air (Zimmermann and Steudle, 1998; Hose *et al.*, 2001). No difference in suberization of the endodermis has been observed between the two different cultivation methods (Hose *et al.*, 2001). Most of the data is available for roots grown hydroponically (Tyree, 2003), and thus without an exodermis. This is a little unfortunate for the proposed modelling work, which seeks to include the roles of both the endo- and exodermis in root transport.

Measurements on the effects of water channels are difficult. However, research into this is currently being carried out. Genetically modified plants with a higher density or efficiency of water channels are also being explored, which will hopefully provide more insight into their function (Steudle and Frensch, 1996).

For the bathing medium, non-charged solutes commonly used include sugars (e.g. sucrose), polyols (e.g. mannitol), monohydroxyalcohols (e.g. ethanol), amides (e.g. formamide), and ketones (Steudle and Tyerman, 1983). These solutes include both permeable solutes – e.g. ethanol, DMF (dimethyl formaldehyde), and impermeable solutes – e.g. mannitol, sucrose (Steudle and Tyerman, 1983). Charged solutes commonly

used are NaCl and KCl, as well as other nutrient ions (e.g. Frensch *et al.*, 1996; Steudle, 1997; Schneider *et al.*, 1997). Along with solutes used and transport parameters, data tables from root transport studies usually give surface area and length of the root segment used.

The effects of external concentrations and flow rates on the transport parameters can be minimized by stirring of the bathing medium. In experiments on *Chara* (a large-celled algae) it was found that external flow rate did not impact on hydrostatic L_r (only osmotic L_r) showing that bulk flow does not significantly change the concentration gradients at the membrane by sweeping away solutes (Steudle and Tyerman, 1983).

4.3 CPP and RPP measurements

Parameters are obtained from both techniques via pressure relaxations. First an equilibrium state is established, and then a pressure perturbation (ΔP^* in Figure 6) made either by insertion or removal of fluid by movement of the probe (in a hydrostatic experiment) (Figure 6a), or by changing the solute concentration in the external medium (an osmotic experiment) (Figure 6b). A measurable volume then ensues, then a flow of fluid which returns the system to water potential equilibrium (the pressure relaxation stage) (Steudle and Jeschke, 1983; Steudle *et al.*, 1987; Steudle and Frensch, 1989; Steudle and Frensch, 1996). The rate of return to equilibrium is described by the half-time t_h .

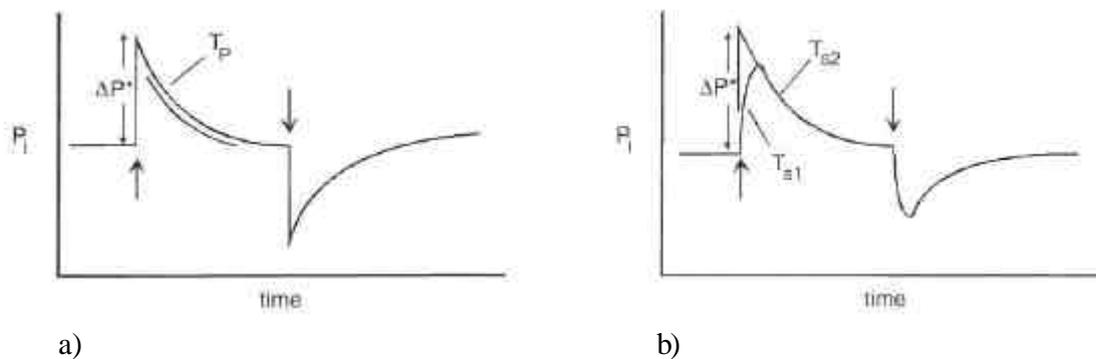


Figure 6. Typical pressure relaxation curves, for a) a hydrostatic experiment, b) an osmotic experiment. P_i is internal pressure; ΔP^* is initial pressure increase; T_p , T_{s1} , T_{s2} indicate half-times (denoted by t_h , t_{h1} , t_{h2} in the text) (Tyree, 2003).

For osmotic flows (Figure 6b) the pressure-time curves are biphasic – after the change in external concentration, rapid water flow ensues in the first stage (‘water phase’) which brings the pressure to a minimum or maximum, after which a slow return to equilibrium occurs in the second stage (‘solute phase’) (Steudle and Frensch, 1989; Steudle *et al.*, 1993). Two half-times are used to describe these phases, with $t_{h1} < t_{h2}$. The time delay for the second phase is assumed to be due to the slow rate of passive diffusion of solutes through the medium and root as the solutes and water reach equilibrium (Steudle and Tyerman, 1983; Steudle *et al.*, 1987; Steudle and Frensch, 1989; Steudle *et al.*, 1993; Frensch *et al.*, 1996). The first phase occurs mainly through bulk flow of water (*ibid.*).

Strictly speaking, the half-times and membrane L_r from the CPP refer to the protoplast rather than the entire cell, although wall rigidity is involved through the volume change (Steudle and Frensch, 1996). Concentration differences driving the root osmotic pressure gradient are usually between the bathing medium and the cell (with the CPP) or xylem sap (with the RPP) (Zimmermann and Steudle, 1998; Zimmermann *et al.*, 2000). With the RPP, problems with external unstirred layers can be minimized by circulating the bathing solution along the root (Zimmermann *et al.*, 2000). For roots with an exodermis, rhizodermal cell walls could not be removed and are included in the term exodermis (Hose *et al.*, 2001).

A polarity of water flow across cell membranes, where the value of L_r is different depending on the direction of flow, has been observed in cells of the *Characeae* (Steudle and Tyerman, 1983; Steudle, 1993). The polarity differs with impermeable and permeable solutes, and seems to depend on concentration and flow rate. Steudle *et al.* (1987) and Steudle and Frensch (1989) have observed a similar polarity of flow across maize roots. The factors contributing to polarity of flow are not yet clear and remain to be resolved (Steudle *et al.*, 1987; Steudle, 1993).

The relative contribution of parallel pathways can be obtained by measuring the parameters for the protoplasmic pathway with the CPP, and subtracting this component from measurements for the whole root (using the RPP) to obtain the apoplasmic component (Steudle and Peterson, 1998). Alternatively, the measured cell L can be used to estimate root L_r without an apoplasmic component, and then the calculated L_r compared with the measured. If flow is mainly protoplasmic both values should be similar, but a substantial apoplasmic component would result in the measured value being larger than the calculated (Steudle, 1994).

4.4 **Parameter calculations used with the CPP and RPP**

4.4.1 **Equations used to calculate parameters**

From the half-time of pressure relaxation, L_r can be calculated provided the elastic modulus e for the cells can be assumed constant and no air bubbles are present (Zimmermann *et al.*, 2000). The equation is of the form (Steudle *et al.*, 1987; Steudle and Frensch, 1989; Zhu and Steudle, 1991; Tyree, 2003):

$$L_r = \frac{\Delta V \ln 2}{\Delta P^i A_r t_h} \quad (1)$$

where P^i = initial change in pressure
 t_h = the half-time of pressure relaxation
 A_r = root surface area
 V = known volume of solution.

For cells, a large variability in A is the main source of uncertainty in L (Steudle and Jesche, 1983).

s_r is obtained from the initial change in osmotic pressure of the medium and the initial change in turgor pressure (Zhu and Steudle, 1991; Schneider *et al.*, 1997; Tyree, 2003):

$$s_r = \frac{\Delta P^i}{RT\Delta C_0} \quad (2)$$

where R = universal gas constant
T = temperature
C₀ = solute concentration in external solution.

ΔP^i is easy to evaluate where $t_{h1} \ll t_{h2}$ (Tyree, 2003). When they are of the same size, a correction is needed to predict the probable ΔP^i by extrapolating the t_h curve back to the initial t_0 (time zero) (*ibid.*) (Figure 6b).

p_{sr} is determined from the second phase in osmotic experiments, by an equation of the form (Steudle *et al.*, 1987; Steudle and Frensch, 1989; Tyree, 2003):

$$p_{sr} = \frac{V_x t_{h2}}{A_r \ln 2} \quad (3)$$

where V_x = volume of the xylem.

The equations for L_r and p_{sr} are strictly only valid for an exponential decay process. Relaxation curves are not true exponentials, however the middle portion is approximately exponential. The computation of L_r by equation (1) has been validated by independent measurements of L_r (Tyree, 2003).

The actual equations used in the calculations can be much more complex than the above, due to correction factors involving change in volume of the xylem, elastic modulus, friction effects, unstirred layers, and time-dependence of the parameters (e.g. Steudle *et al.*, 1987; Steudle and Frensch, 1989; Zhu and Steudle, 1991).

4.4.2 Adjustments for unstirred layers

The elastic modulus of the pressure chamber attached to the root, and the elastic modulus of the xylem, are both used in calculations, although the xylem is much less extensible than the pressure chamber (Steudle *et al.*, 1987). Because measurements are taken between the xylem and the external medium, transport in the cortex is not explicitly defined as they are in more complex two-membrane three-compartment models, where for example an elastic symplastic compartment is assumed (Pickard, 2003). In the CTM, the cortex can be considered as a potential unstirred layer between the two compartments (Steudle and Frensch, 1989).

Adjustments for solute permeability and reflection coefficients have been made in the CTM. Boundary layers on the inside and outside of the endodermis have been considered. Solute permeability p_{sr} has been corrected for the gradient-dissipation effect (section 6.6.3.2 b) with the equation (Steudle and Frensch, 1989)

$$\frac{1}{p_{sr}^{meas}} = \frac{1}{p_{sr}} + \frac{r_e}{D_s} \ln \frac{r_r}{a} \quad (4)$$

where p_{sr}^{meas} = measured permeability coefficient

p_{sr} = true permeability coefficient

r_e = radius of the endodermis

r_r = radius of the root

D_s = diffusivity coefficient (assumed to be the same in the stele and the cortex)

a = radial distance from the root axis to the boundary of the unstirred layer in the stele.

Equation (4) assumes the external unstirred layer extends across the entire cortex. Sweep-away (section 6.6.3.2 a) and gradient-dissipation effects can both affect the reflection coefficient, but the former is assumed small in RPP experiments and s_r is corrected for the latter by the equation (Steudle and Frensch, 1989)

$$s_r^{meas} = \frac{1/p_{sr}}{1/p_{sr} + \frac{r_e}{D_s} \ln \frac{r_r}{a} s_r} \quad (5)$$

where s_r^{meas} = measured reflection coefficient

s_r = true reflection coefficient.

The sweep-away effect would also be small for cells when using the CPP because the amount of water moved is small (Steudle, 1993).

Although adjustments for unstirred layers have been made in some papers, and it has been claimed that “with the root pressure probe these [unstirred layer] effects can be accounted for” (Steudle *et al.*, 1987), they are still a problem and have not been resolved (Tyerman, *pers. comm.*). Equations (4) and (5) have not explicitly been given in most papers regarding the CTM, and it is not clear if these equations are used in all pressure probe experiments. Nor is their validity and usefulness clear – it is difficult to see how the thickness of the boundary layers, required for the determination of a , can be measured.

Unstirred layers have been dealt with in some papers by using an estimation of effective concentrations. For abscisic acid (ABA) transport which occurs primarily via bulk water flow, an ‘apparent’ s_r was used based on an estimation of the concentration of ABA in the endodermis, and by using an average of the external concentration and that of the xylem (Freundl *et al.*, 1998). Because this is an estimation, the mean concentration and s_r may only represent lower limits (*ibid.*).

4.4.3 Limitations of pressure probe calculations

Equations (1) - (3) only apply to passive transport. The ability to calculate parameters with the CPP and RPP are limited where active transport is involved. They can be used for active transport only if changes in the root pressure due to active transport are sufficiently slow such that $t_{h(\text{active})} \gg t_{h(\text{passive})}$. This is generally not the case with permeating solutes where the half-times may be comparable. Changes in solute permeability due to active transport of permeating solutes may be estimated only in the case where there is an observable time lag before the onset of active transport processes (Steudle and Frensch, 1989).

Zimmermann *et al.* (1992) has raised some concerns regarding CPP and RPP measurements. He pointed out that large errors can occur in comparisons between tissue and cellular hydraulic conductivity, due to uncertainties in root geometry in that the extent of water exchange areas are unknown. In the calculation of cellular parameters, it is also unknown how much flow bypasses the plasma membrane and wall through plasmodesmata (Zimmermann *et al.*, 1992). In addition to these concerns regarding lack of anatomical information, there are also the theoretical objections raised previously in section 2.5.

5 MATHEMATICAL THEORY AND EQUATIONS

5.1 Mathematical principles in plant physiology

The principles of irreversible, non-equilibrium thermodynamics in biological systems, primarily developed by Katchalsky and Curran (1967), form the basis of current biophysical equations of transport. These equations are used for tissue or medium-distance transport and involve turgor pressure and concentration differences. Ion fluxes and ion pumps operate at the finer level of membranes, and involve transport kinetics, which will not be looked at here.

Classical thermodynamics primarily deals with systems in equilibrium – a situation where the internal parameters are fully determined by the external ones, and hence can be used as parameters of state (which describe the state of the system). Systems in nature are usually in non-equilibrium, and equations must explicitly include internal parameters and their variation in space and time (Katchalsky and Curran, 1967).

A system in equilibrium does not change with time or space, i.e. there are no net fluxes. Systems in *non-equilibrium* are difficult to define since the state of the system cannot be described in terms of coordinates of the system as a whole, for states are continually changing and a coordinate's value varies from point to point (Zemansky and Dittman, 1981). These systems are dealt with by looking at the *quasi-equilibrium* or quasi-static situation: systems where the rate of change of external parameters are slow enough so that internal parameters are able to adjust their values at every instant to external ones. The system is at all times infinitesimally near a state of thermodynamic equilibrium, which is essentially a sequence of equilibrium states (Katchalsky and Curran, 1967; Zemansky and Dittman, 1981). With many equations it is easiest to begin with a system

in *steady-state*: one where there is change in space but not with time. Once this is done a time-variance can be introduced.

An *irreversible system* is where the work required to bring a system isothermally (at constant temperature) back to its starting point does not equal the work done in the first part of the cycle (Katchalsky and Curran, 1967). All natural spontaneous systems, including biological, are irreversible (Zemansky and Dittman, 1981). While systems in quasi-equilibrium are reversible, a process is only reversible if it is a) performed quasi-statically, and b) there are no dissipative effects (*ibid.*). Energy is dissipated if work is converted to internal energy, either of the system or the external environment. *Dissipative effects* include viscosity, friction, inelasticity. These effects are always present in moving devices (*ibid.*).

Thermodynamic equations for transport have been developed at the cellular level, and the theory is well-established for membrane transport (Katchalsky and Curran, 1967; Barber, 1995). Thermodynamics takes a macroscopic description of the system – the system can be described by external variables which are directly observable and apply to the whole system, such as pressure, temperature, volume, number of moles in solution, conversion of energy etc. Internal workings of the system (such as particle or chemical reaction kinetics) are not considered (Zemansky and Dittman, 1981). This is convenient for studying membranes since the structure and dynamics of cell membranes are not known in detail, and also allows the system to be reduced from a large number of unknown molecular processes to a few macroscopic observables (Katchalsky and Curran, 1967; Zemansky and Dittman, 1981). While ion fluxes are highly dynamic and described by kinetics, at the tissue level and for water thermodynamics is more useful (Barber, 1995).

There have been some objections to the application of irreversible thermodynamics to a complex structure such as the root (Zimmermann *et al.*, 1992; Pickard, 2003). However, they have been applied to composite structures by Kedem and Katchalsky (1963a,b,c) and have been used by many root modellers including Molz and Hornberger (1973), Dalton *et al.* (1975), Fiscus (1975), and Murphy (2000).

The use of a thermodynamic description has a few challenges, including:

- How to extrapolate the mechanisms and descriptions developed for transport between cells, to transport across tissues and organs. Most of the theory has been developed for the cellular and whole plant level, but relatively little for the tissue and organ levels (Steudle, 1997).
- Within the cellular level, assumptions and limitations of established transport equations need to be understood to apply and adapt them suitably to the system being studied. Established thermodynamic equations tend to be broad and generic.
- Established equations are for homogeneous membranes. Biological systems often have homogeneous membranes in series or parallel, so-called “composite” membranes. Composite membranes have different transport properties to homogeneous membranes. For example, homogeneous membranes exhibit a linear relationship between flows and forces, whereas composite membranes

exhibit a nonlinear relationship between forces and flows (Kedem and Katchalsky, 1963b). Rules of linear superposition of properties of individual homogeneous membranes also don't generally hold (*ibid.*).

Another principle widely used in plant physiology is that water flow is analogous to electricity flow. This was suggested in 1948 by van den Honert (Steudle and Peterson, 1998). This theory has some pitfalls and problems of interpretation, and has been extensively analysed by Richter (1973) and Passioura (1984). However, it is still a useful basis of many plant models today.

5.2 Water potential, van't Hoff equation, and elastic modulus

Water potential

In classical membrane transport theory, water flow is driven by water potential ψ (spatial) gradients defined by (Dainty, 1963; Dainty, 1976):

$$\psi = \psi_P - \psi_p \quad (6)$$

where P = turgor pressure
 p = osmotic pressure.

Van't Hoff equation

Ideal dilute solutions can be approximated by the ideal gas law, where the osmotic pressure p is defined as (van't Hoff's formula) (Katchalsky and Curran, 1967):

$$p = nRTC \quad (7)$$

where R = ideal gas constant
 T = temperature
 C = solute concentration
 n = the sum of cation and anion valencies of a completely dissociated salt (Dalton *et al.*, 1975). For non-electrolytes $n = 1$.

Elastic modulus

The elastic modulus e describes the rate of change of cell volume with respect to a change in turgor pressure P , and is defined by (Dainty, 1963; Molz and Ferrier, 1982):

$$\frac{dP}{dV} = \frac{e}{V_0} \quad (8)$$

where e = elastic modulus
 V_0 = constant reference volume (when $\psi = 0$, or $P = 0$).

Shrinking/ swelling of the root tissue is particularly important during water stress or osmoregulation (Steudle and Frensch, 1996). e may be assumed constant for infinitesimal volume changes but is not constant for perfectly elastic cell walls (Philip, 1958b). In general e is a non-linear function of the turgor pressure (Ortega, 1993).

5.3 Diffusion kinetics

5.3.1 Laws of diffusion

The flux of water is assumed to be proportional to the gradient in water potential. This gives rise to a diffusion type of kinetics for propagation of changes in water potential (or water content, turgor pressure), for it follows Fick's first law which states that the flux of some substance is proportional to the appropriate force, i.e:

$$J_j = -D_j \frac{\partial C_j}{\partial x} \quad (9)$$

where J_j = solute flux for solute j
 D_j = diffusion coefficient of solute j
 C_j = concentration of solute j (Nobel, 1974).

The diffusivity D is a general coefficient and can be expressed in terms of other parameters, such as the permeability, elastic modulus, osmotic pressure, tissue geometry, conductivity or resistance, storage or capacitance (Philip, 1958c; Tyree, 1969; Nobel, 1974; Molz and Ikenberry, 1974; Molz and Ferrier, 1982), provided these expressions have the dimensions of the diffusivity coefficient.

Since the continuity equation holds in the system being considered here, membrane transport also follows Fick's second law which is obtained by combining the continuity equation with equation (9), to obtain (Nobel, 1974):

$$\frac{\partial C_j}{\partial t} = -D_j \frac{\partial^2 C_j}{\partial x^2}. \quad (10)$$

Diffusion kinetics are involved in the flow of water although water flow is bulk flow, not diffusional (Steudle and Frensch, 1996). Diffusion plays a more important role whenever solutes are moving more rapidly (e.g. in nutrient uptake, growth) (*ibid.*).

Following the laws of diffusion, path conductance is proportional to tissue cross-sectional area, and inversely proportional to the square of the tissue thickness and number of cell layers crossed. Conductances are additive for the protoplasmic and apoplasmic pathways, but not in the direction of flow (Zhu and Steudle, 1991; Steudle and Frensch, 1996; Steudle, 1997). The rate of propagation also decreases with increased storage capacities of pathways (water storage is about 3-10 times larger for the protoplast than the apoplast), which is determined by the elastic modulus of the cell wall (Steudle and Frensch, 1996).

The rate of propagation of ?? , then, depends on the (Steudle, 1997):

- hydraulic conductivity of cell layers
- tissue geometry (cross-sectional area)
- components of the driving force, and movement of solutes under an osmotic gradient
- water storage capacities of cells (determined by ϵ of cell walls).

The diffusivity term in equations (9) and (10) describing passive components of water transport, can be extended for active solute flow if rates of active uptake into tissue cells are known for given regions (e.g. the root cortex). Rates of active uptake, however, follow a saturation-type of kinetics rather than the above linear relationship, and must be solved numerically (Steudle and Frensch, 1996).

5.3.2 Tissue diffusivity

If local water potential equilibrium is assumed, a global tissue diffusivity can be defined which consists of some or all of the following factors: water capacitance, resistance/ conductivity, permeability, water storage coefficients, elastic modulus, osmotic pressure of the cell at zero turgor, and cell geometry (Molz and Ferrier, 1982). In the absence of local equilibrium, more global (tissue) parameters are required, including separate diffusivities for each pathway (*ibid.*). A review of tissue diffusivity expressions has been made by Molz and Ferrier (1982). A comprehensive expression for tissue diffusivity has also been quoted in Steudle and Frensch (1996).

5.4 Phenomenological and flux equations: parameter definitions

5.4.1 Phenomenological equations

Phenomenological equations can be written which express explicitly relationships between mechanical flows and forces, where the flux J_i is a linear function of all the forces X_k , related through a conductance L (i.e. conducive to flow) (Katchalsky and Curran, 1967):

$$J_i = \sum_{k=1}^n L_{ik} X_k . \quad (11)$$

An alternative expression can be written where the force X_i is linearly related to the fluxes J_k through a resistance R (i.e. resistance to force):

$$X_i = \sum_{k=1}^n R_{ik} J_k . \quad (12)$$

This linearity only holds for sufficiently slow processes when the system is near equilibrium (Katchalsky and Curran, 1967).

5.4.2 Flux equations

The well-known Kedem-Katchalsky equations from non-equilibrium thermodynamics, describing volume and solute fluxes, are based on equation (11) above.

The *volume flux* is assumed to be proportional to the gradient in water potential (Katchalsky and Curran, 1967):

$$\begin{aligned} J_v &= \frac{1}{A} \frac{dV}{dt} \\ &= L_r (\Delta P - s \Delta p) \end{aligned} \quad (13)$$

where J_v = volume flux of water

L_r = hydraulic conductivity (or filtration) coefficient

s_r = root reflection coefficient.

The volume flux is the net flux of solute and water, i.e. (Katchalsky and Curran, 1967):

$$J_v = J_s \bar{V}_s + J_w \bar{V}_w \quad (14)$$

where J_s = solute flux

J_w = water flux

\bar{V} = partial molar volume (volume taken up by one mole of solute).

The *solute flux* is driven by the gradient in osmotic pressure and is coupled to water flow (Katchalsky and Curran, 1967):

$$\begin{aligned} J_s &= \frac{1}{A} \frac{dn}{dt} \\ &= (1 - s_r) C_{av} J_v + p_{sr} \Delta p \end{aligned} \quad (15)$$

where J_s = solute flux

C_{av} = mean concentration of solute in membrane/ tissue

p_{sr} = (passive solute) permeability coefficient.

C_{av} can be defined as:

$$C_{av} = (C'_m + C''_m)/2 \quad (16)$$

where C_m are the solute concentrations adjacent to the membrane (Barry and Diamond, 1984). However, other expressions for C_{av} can be derived (see Barry and Diamond, 1984).

The first term in equation (15) denotes solute flow coupled to the bulk flow of water (where $s=1$ no solutes pass and there is no coupling, where $s=0$ all solutes pass and there is complete coupling). This is the convection or solvent drag term, and does not vanish in the absence of solute gradients (Barry and Diamond, 1984). The second term denotes

flow due to passive diffusion, which depends on the solute concentration gradient and membrane permeability. It should be noted that if solutes are partly transported actively and an active transport term is included in equation (15), then the transport of water is no longer truly passive due to solute-water coupling (see section 6.5 for discussion on active transport).

The Kedem-Katchalsky equations describe the processes of filtration ($L_r \Delta P$) and osmosis ($L_r s \Delta p$), in equation (13), and convection ($(1-s) c_{av} J_v$) and diffusion ($p_{sr} \Delta p$), in equation (15), due to the simultaneous action of pressure and osmotic gradients (Kargol and Kargol, 2003). Use of these equations is restricted to two-component membrane systems, which are sufficiently diluted and well-stirred (Katchalsky and Curran, 1967). They differ in form when applied to each pathway, with fluxes differing in geometry coefficients and parameters, and equations for cell walls (i.e. the apoplast) involve derivatives whereas applied across membranes they involve differences (as used in the equations above) (Molz and Ferrier, 1982).

5.4.3 Time-dependent expressions and half-times

Explicit time-dependent expressions for volume, osmotic pressure, and concentration gradients can be derived, beginning from equation (11) and using various substitutions and approximations for p and P in terms of V (Philip, 1958b). Half-times can easily be obtained from these exponential expressions. The use of half-times seems to have originated in 1933 with a paper by Collander and Bärland (*ibid.*), and has been used since to describe the rate of return of cells to equilibrium.

5.4.4 Flux relations across tissues

The above equations are based on dynamics within a single cell. Diffusion and osmotic processes are different between cells and tissues, because resistances are not confined to tissue boundaries but are distributed within the tissue in cell membranes (Philip, 1958a). Therefore marked differences in concentration or pressure will occur within the tissue during equilibration, in contrast to individual cells where the concentration and pressure within the boundaries are assumed to be the same everywhere (an isotropic distribution) (*ibid.*). Half-time relationships for tissues may then not be exponential, and half-times will certainly vary discontinuously between cells giving step-function propagation curves (*ibid.*).

However, the half-time is a useful descriptive variable, and Philip (1958a) suggests that it still be used if the effect of geometry on half-times are understood, and a parameter describing concentration and pressure propagation be used which is independent of tissue geometry. Philip (1958a) looked at the variation of diffusivity for different geometries in an isotropic tissue, and found that the half-time for equilibration of an individual cell is very sensitive to cell position in the tissue, varying from a small fraction of to over four times the tissue half-time. He also found that the total flux across a single cell, and across cell aggregations for a sheet, cylinder, and sphere, all differed. For all cell aggregations looked at, the rate of approach to equilibrium decreased more rapidly with time than an exponential process.

5.4.5 Definition of transport parameters

The parameters L_r , s_s and p_{sr} are defined by the formulae (Dainty, 1963; Kargol and Kargol, 2000; Kargol, 2001; Kargol and Kargol, 2003):

$$L_r = \frac{J_v}{\Delta P} \quad \text{at } \Delta p = 0, \quad (17)$$

$$s_s = \frac{\Delta P}{\Delta p} \quad \text{at } J_v = 0, \quad (18)$$

$$p_{sr} = \frac{J_s}{\Delta p} \quad \text{at } J_v = 0. \quad (19)$$

Although the broad physical meaning of these equations is clear, they are subject to problems of interpretation. For example, the interpretation of s as a ‘reflection’ coefficient in a porous membrane, and of p_{sr} as a coefficient of ‘diffusive’ solute permeation when it has a dependence on hydrostatic pressure (through J_s), does not seem sufficient. These problems of course impact on the interpretation of terms in the flux equations. The meaning of these parameters has been clarified by Kargol and Kargol (2003), who looked in detail at the influence of solute and water fluxes, hydrostatic and osmotic forces, on each of the parameters, and the relationship of the parameters to each other.

5.5 Active transport

5.5.1 Definitions

The full Kedem-Katchalsky equations have an electrochemical term involving current flux, and a primary active transport term involving rate of chemical reaction (Katchalsky and Curran, 1967; House, 1974). These terms have not been included in the above sections. In this section we will look at these terms and justify their inclusion/ non-inclusion in the solute flux equation in more detail. Water transport is assumed to be passive in plant roots – therefore an active transport term is ignored in the volume flux.

Active transport is a global property of the membrane; terms such as ‘active sites’ and ‘active pumps’ do not necessarily refer to local areas in the membrane (Zimmermann and Steudle, 1978). *Primary active transport* is defined as the ability to generate a gradient with no fluxes except that due to the driving metabolic reaction (Hoshiko and Lindley, 1967). It is linked to a chemical reaction and conversion of energy, and occurs when ions are transported against an electrical gradient (i.e. from a low to high potential difference). Accumulation of ions against a concentration gradient is not a sufficient condition for active transport as is sometimes assumed (Zimmermann and Steudle, 1978). *Secondary active transport* involves a substance moving against a gradient due to entrainment between transport fluxes, without direct entrainment by a metabolic pumping mechanism (Hoshiko and Lindley, 1967). This occurs through the coupling of transport coefficients,

where for example one solute is moved against its own gradient due to the gradient of another solute (Zimmermann and Steudle, 1978).

5.5.2 Electric current

While active transport of ions generates a local electric current, the two terms are not necessarily related. I can be generated through flux interactions (secondary active transport), without input of metabolic energy (primary active transport). Conversely uncharged solutes or water (in which case $I=0$) can be actively transported (Katchalsky and Curran, 1967; Fiscus, 1975).

In previous models of root transport it has been assumed that $I = 0$ for electrolytes and non-electrolytes, but that active transport of both can occur. The I term reflects the transport of ions which, being charged, generates an electric gradient. However, electrolytes can be transported in the absence of a current (Kedem and Katchalsky, 1963a), and the transport of salts in root transport has been treated the same as that of non-electrolytes. The assumption seems to be that bulk movement of ions across the root tissue is electrically neutral, such that ions of highest overall mobility may set up a small electric field which will affect the movement of other ions, but this electric field would be relatively small across a tissue (Newman, *pers. comm.*). Although over short distances (< 2 mm) the electric gradient may have a dominating effect (Tyree, 1969), one can probably assume that the electric gradient is of negligible magnitude across the root tissue and that $I = 0$.

5.5.3 Active transport

The primary active transport term, however, cannot *a priori* be assumed to be zero. Active transport has not been considered in much detail in previous root transport papers. In the few instances where it has been included (Fiscus, 1975; Zimmermann and Steudle, 1978; Steudle, 1993; Steudle *et al.*, 1993; Zimmermann and Steudle, 1998) it has been by the addition of an active flux rate term J^* , assumed to be constant, in the solute flux equation. This lumped term is considered equivalent to the more detailed flux equations (Katchalsky and Curran, 1967), though if $J_s = J(\text{passive}) + J(\text{active})$ the system is required to be linear. This may not actually be the case (Zimmermann and Steudle, 1978), yet it is the easiest way of including active transport. In theory J^* could encapsulate both I and primary active transport, because the maintenance of an electric field is an active process (Katchalsky and Curran, 1967; Newman *pers. comm.*). J^* influences concentration and osmotic gradients via active redistribution of ions, and its effect on selectivity has been considered by Dalton *et al.* (1975). J^* can also affect solute-solute coupling and solute-water coupling (if water flow is active) (Zimmermann and Steudle, 1978). For ideal semipermeable membranes, J^* has been used in place of J_s (Fiscus, 1975; Zimmermann and Steudle, 1998).

The inclusion of J^* in equations can be difficult because one needs to determine if the solutes are actively transported. This is done through experiments involving mathematical tests such as the Nernst equation, which looks at the electro-chemical potential difference across the root, where transport against an electrical gradient implies active transport (Bowling, 1976). This has not been done in the papers reviewed for the CTM model. If it is known a certain ion is transported actively, then one may identify

from the literature the ions involved and typical flux rates (Tyree, *pers. comm.*). From pressure-probe experiments, however, there is no way of measuring the partial fluxes of all ions present (Tyree, *pers. comm.*). With permeating nonelectrolytes, Steudle (1993) has stated that J^* is usually not present.

5.6 Friction, unstirred layers, and other effects

5.6.1 Types of friction

Three basic types of mechanical friction can occur – friction between water and membranes (f_{wm}), between solutes and membranes (f_{sm}), and between solutes and water (f_{sw}). Friction has not yet been considered in the above equations, but coefficients of all three types of friction can appear in the transport parameters. It can be shown that L is inversely proportional to f_{wm} , p_s is inversely proportional to f_{sm} and f_{sw} , and s also contains a term involving f_{sm} and f_{sw} (Katchalsky and Curran, 1967; Steudle and Tyerman, 1983). Friction also forms a part of the diffusivity coefficient (Katchalsky and Curran, 1967).

Frictional coefficients have not been incorporated into most models of root transport (although see Steudle and Frensch, 1989). They have mostly been considered in research on frictional pore models for water channels in *Chara* (e.g. Steudle and Tyerman, 1983; Steudle and Henzler, 1995; Henzler and Steudle, 1995; Steudle, 1997), where frictional effects are included in the reflection coefficient. Frictional effects in root models have been mostly considered in terms of effects of unstirred layers on solute and water movement.

5.6.2 Solvent drag

‘Solvent drag’ is due to friction between solutes and water as they traverse the membrane through the same pathways/ channels. Solute flux due to solvent drag can persist when solute concentrations on both sides of the membrane (immediately adjacent to the membrane) are identical (Barry and Diamond, 1984). Unstirred layers can also alter solute flow such that ‘pseudo solvent drag’ occurs, which has been discussed in detail by Barry and Diamond (1984).

5.6.3 Unstirred layers

5.6.3.1 Definitions

Unstirred (boundary) layers are often a problem with transport measurements. Unstirred layers are slow-moving regions of laminar flow adjacent to the membrane, where diffusion is the only mechanism of transport. The thickness of the layer, d , is defined by (Dainty, 1963; Barry and Diamond, 1984):

$$\left(\frac{\partial C}{\partial x} \right)_{\text{interface}} = \frac{C_{\text{bulk}} - C_m}{d} \quad (20)$$

where C_{bulk} = bulk concentration of the solute
 C_m = solute concentration at the membrane
 d = thickness of the unstirred layer.

The effect of unstirred layers depends on the solute permeability and diffusivity (Dainty, 1963; Barry and Diamond, 1984), since

$$p_s = D_s/d \quad (21)$$

where p_s = solute permeability
 D_s = diffusivity coefficient of solute.

An important observation from equation (19) is that transport of a rapidly permeating solute across a membrane may be partially rate-controlled by the unstirred layer (ref Dainty, 1963; Barry and Diamond, 1984). For small values of p_s the apparent membrane permeability (the measured, uncorrected value) approaches the actual membrane p_s (Barry and Diamond, 1984).

5.6.3.2 Specific unstirred layer effects

Unstirred layers can alter solute behaviour at membranes in a couple of ways, as discussed in the following sections.

a) Concentration or 'sweep-away' effects

A flow of water can 'sweep away' solutes from the membrane surface downstream from the water flow, and concentrate them on the upstream side. This is termed 'concentration polarization', 'convective-flow effect, or a 'sweep-away effect'. This acts to reduce J_v because the flow always perturbs the solutes in a direction that opposes J_v (Barry and Diamond, 1984; Steudle, 1993). That is, the outward convective solute movement is opposed by inward diffusion in the unstirred layer, so that (Dainty, 1963)

$$J_v C + D_s \frac{dC}{dx} = 0. \quad (22)$$

For the planar case with boundary condition $C = C_{\text{bulk}}$ at $x = d$, the solution of this is (Dainty, 1963)

$$C = C_{\text{bulk}} \exp \left[\frac{J_v}{D_s} (d - x) \right] \quad (23)$$

This 'convection-opposed-by-diffusion' can be large, and can cause a time-dependent decrease in J_v which will lead to an underestimation of L_r or p_s (under osmotic gradients) (Dainty, 1963; Barry and Diamond, 1984; Steudle, 1993). For permeable solutes, a time-dependent solute flux in the direction of J_v will be set up, and this will also perturb the solute and water flux ratio and cause an underestimation of s (Barry and Diamond, 1984). Pseudo-solvent drag effects can also arise (*ibid.*).

b) Gradient-dissipation effect

This occurs when a significant fraction of the concentration gradient is across an unstirred layer, and the unstirred layer's diffusional resistance is comparable to or greater than the membrane resistance (Barry and Diamond, 1984). Diffusional pathways would then be long, affecting the actual gradient across the barrier (Steudle, 1993). As a result J_v (under hydrostatic or osmotic gradients) may change with time after formation of the gradient in the unstirred layer. Measurements of L and p_s (under osmotic gradients) can be underestimated by a large amount, and the apparent s can also be affected (which depends on the effective overall permeability of solutes and water) (Barry and Diamond, 1984; Steudle, 1993).

With active transport, the solute concentration at the membrane (in the presence of an unstirred layer) differs from the bulk solution concentration by an amount that increases with the active transport rate (Barry and Diamond, 1984).

c) Current-induced effects

During active transport a third effect of unstirred layers is current-induced effects (see Barry and Diamond, 1984).

Barry and Diamond (1984) have extensively discussed unstirred layers and how they may be measured. Unstirred layer effects means that, ideally, osmotic experiments can only be performed with isolated cells or tissue monolayers (Steudle, 1993). However, this is not the situation with the root tissue, where the effects of unstirred layers must be estimated.

5.6.3.3 Dilution effect

For completion, a note must be made about Fiscus' dilution effect, which is one explanation put forward for increasing conductivity with flow rate. An expression can be derived for hydraulic resistivity made up of two components – one an inverse of hydraulic conductivity, and another term relating the ratio of solute flux to the square of the volume flux, which is the dilution term (Fiscus, 1975). The theory here is that at low flow rates the resistance (conductance) decreases (increases) nonlinearly with J_v due to this term. At high flow rates the dilution term vanishes and the resistance becomes the hydraulic resistance (Fiscus, 1975; Zimmermann and Steudle, 1998). However, Zimmermann and Steudle, 1998) concluded experimentally that this is not the dominant factor resulting in increasing conductivity with flow rate.

5.7 Tissue models and electric analogs

5.7.1 Use of electric analogs

Electric analogs have been applied in root transport models primarily by Molz and Hornberger (1973), Molz (1976), Molz *et al.* (1979), and Molz and Ferrier (1982). The role of ψ is considered analogous to voltage, J analogous to current, resistance analogous to electrical resistance, and water (storage) capacity analogous to capacitance (defined as $dV/d\psi$).

A few different analogs have been reviewed by Molz and Ferrier (1982). They have made an interesting comparison between the usage of differential transport equations with the usage of equations based on electric analogs. Their conclusion was that circuit analogies seem best suited for representing detailed geometry and anatomy, but the inclusion of non-linear behaviour within this is difficult. Differential equations were deemed good for reflecting non-linear behaviour, but not so good for incorporating geometry. Therefore they suggest that for tissues or cell aggregations, differential equations may not work so well (Molz and Ferrier, 1982). However, the type of modelling approach used would depend on what level of detail is incorporated, and what processes are focused on. The applicability of electric analog models has been discussed further by Molz *et al.* (1979).

5.7.2 Review of cell aggregation and tissue models

5.7.2.1 Differential equation approach

Equations for cellular transport are ordinary differential equations (ODE's) involving changes of water potential or volume with time. They can all be written as diffusion equations, and are based on conservation of mass, and empirical relations between volume flux and water potential gradients (Molz and Ferrier, 1982). Equations for modeling cell aggregations or tissues are similar except they use water potential gradients in the apoplast, and water potential differences in the symplast. This results in partial differential equations (PDE's) in two unknowns – the water potential in the apoplast, and water potential in the symplast – with both differentials and finite differences (*ibid.*). If there is local water potential equilibrium between the pathways a single diffusion equation for both pathways (i.e. for the whole tissue) can be derived, in the form of Fick's second law (equation 10), which simplifies the mathematics considerably (Molz and Ikenberry, 1974; Molz and Ferrier, 1982). Equations for tissue models begin with DE's for fluxes at the cellular level, which when solved describe fluxes at the tissue level.

Philip (1958c) looked at transport through cell membranes in cell aggregations. Beginning with expressions for flux rates and volume increases between cells, and making various substitutions for water potential in terms of V , he obtained a second order ODE for dV/dt in the form of Fick's 2nd law (equation (10)). The diffusivity D was dependent on cell geometry, permeability, elastic modulus and osmotic pressure. Philip (1958c) stated that D can be assumed constant for a particular tissue, but more precise equations have a volume-dependent diffusivity, in which case equations assume a 'heat conduction' form (Philip, 1958c). Philip (1958c) looked at isotropic tissues only; for anisotropic systems D would be a second-order tensor.

Molz and Ikenberry (1974) extended Philip's approach to include the cell wall pathway. Through a similar method, beginning with dV/dt between cells and employing expressions relating volume and water potential, they obtained two coupled diffusion equations (one for water potential fluxes within the cell, and one for fluxes within the cell wall). They assumed that only water exchange (not solute exchange) occurs between pathways, and stated that the extent to which this occurs depends on the relative magnitude of resistances encountered (Molz and Ikenberry, 1974). If water potential drops across membranes are assumed small relative to that across the tissue, the

resistance between pathways can be ignored (Molz and Ferrier, 1982). The equations were solved numerically for a one-dimensional case.

Molz and Hornberger (1973) extended Philip's approach to include a diffusible solute (by the presence of s), for transport across cell membranes only, without a separate cell wall pathway. Instead of coupled DE's for water potential fluxes, they obtained two coupled PDE's involving concentration and pressure fluxes. The equations were solved numerically for a one-dimensional case.

5.7.2.2 Electric analog approach

Molz and Ikenberry (1974) and Molz and Ferrier (1982) put their coupled differential equations (DE's) into an electric circuit analog form. Water potential fluxes were expressed in terms of resistances and capacitances (water storage capacity, dV/dt). The final coupled DE's involved diffusivity and time constants expressed in terms of resistances and capacitances, which in turn were defined in terms of quantities from the original coupled DE's. The final equation then involved a diffusivity and time constant for each DE. They solved these DE's for water potential fluxes in a one-dimensional sheet of tissue, and obtained a ratio of water flow between the protoplasmic and apoplasmic pathways (assuming local water potential equilibrium between pathways).

For extension to three dimensions, Molz and Ikenberry (1974) proposed that equations of the same general form can be expected, with coefficients that may be more complicated but which are usually determined experimentally so should pose no problem. A diffusible solute can also be included – however they stated that this is “straightforward conceptually but seems to be complex mathematically.”

In the approach by Molz and co-workers, there are three levels on which global transport parameters in the root tissue can be measured (Molz and Ferrier, 1982). These are:

- a) measurement of fundamental parameters (e.g. L , e , p , s , p , geometry) for single cells, and then the calculation of global parameters
- b) measurement of resistances and capacitances (which contain fundamental parameters) of single cells, and then the calculation of global parameters
- c) direct measurement of the global parameters diffusivity and/ or time constants (which contain fundamental parameters) for the tissue.

The modelling approach taken by Molz and co-workers involves 'nested' parameters/coefficients: a diffusivity D at the tissue level, defined by resistances and capacitances at the cellular level), in turn defined by fundamental parameters at the cellular level.

5.7.2.3 Finite difference approach

Compartment models such as the ones reviewed and developed by Murphy (2000) used equations based on irreversible thermodynamics, applied to fluxes across tissue compartments in series (not between cells). Finite differences and not DE's were used. Beginning from the Kedem-Katchalsky equations for flux (equations (13) and (15)), they

derived expressions for changes in hydrostatic pressure, osmotic pressure, or water potential, rather than expressions for changes in volume. Transport parameters used apply to membranes separating the compartments. The complexity in these models is largely due to the incorporation of complex solute interactions and distributions in the root.

A disadvantage of such models is that parameters for membranes between compartments are often unknown and must be estimated (Murphy, 2000). Predicted overall root parameters are expressed in terms of membrane parameters, which introduces many potential sources of error. This is in contrast to the CTM which only requires one parameter, the elastic modulus, in its calculations of root transport parameters. The model by Murphy (2000) is also based upon the assumption that intercellular transport is via membranes with reflection coefficients which are less than unity, which is supported by data, but for which no theoretical explanation could be given as to why this is so.

5.7.2.4 Classical thermodynamic approach

In his model of root pressure, Pickard (2003) has chosen not to use an approach based on irreversible non-equilibrium thermodynamics. He argued that it is not applicable for the situation of root pressure, where forces and flows are not near equilibrium, and active and time-variant transport play major roles. He also objected to the use of thermodynamics because it assumes linear behaviour in the equations, and thus do not represent a complex system.

Pickard (2003) used finite differences for fluxes between the three compartments xylem, symplasm, and soil water. Two interfaces were incorporated: between the symplast and soil, and between the symplast and xylem. This is similar to other three-compartment models previously developed. In contrast to the CTM formulation, the xylem was made to be at fixed volume and the symplast elastic. Unlike many other models he chose an approach based on classical thermodynamics, and as driving forces at both interfaces used the Gibbs free energy relation (encompassing diffusion and flow through water channels), and active uptake. In addition, hydrostatic pressure at the symplast:xylem interface was modelled by the Navier-Stokes equation. The major quantity of interest was the rate of sap exudation from the xylem. The model seems to require a large range of parameters, some of which can only be estimated (e.g. permeabilities of root xylem parenchyma plasma membranes).

5.8 Issues to consider in developing a tissue model for the CTM

The RPP is able to measure the three fundamental transport parameters on a root tissue level: L_r , s_r , and p_{sr} . The definition of these parameters is based on the Kedem-Katchalsky equations for membrane transport. When used at the tissue level their meaning is no longer clear, for they are 'averaged' parameters for all the cells in the transport pathway. This must be clarified, and considerations of Kargol and Kargol (2003) on the interpretation of these parameters must be examined.

In developing a model for the CTM the aim is to disaggregate the components, working from the bulk root parameters downwards to see what factors contribute to those parameters. This is in contrast with other models discussed in section 6.7.2 which build

the model upwards, beginning with cellular or compartment variables and parameters to predict root parameters. It is not clear what approach should be used for disaggregating these parameters. Perhaps a modelling-up approach must be taken, which incorporates the factors of particular relevance to pressure probe measurements, and testing whether the final outputs are in agreement with the CTM calculations.

In terms of mathematics involved, the approach to be taken is not clear also. Approaches of Molz and co-workers cannot help in these calculations because parameters used in their approach is at the cellular level. However, this is not to say electric analogs may not be usefully applied in some other way, and this is an avenue which should be explored. This seems an elegant method which has been used previously to examine the apoplast and symplast pathways. The finite difference approach used in series compartment models (Murphy, 2000) uses parameters at the membrane level between compartments. Clearly the CTM must be disaggregated into series compartments to incorporate unstirred layers at the endo- and exodermis. Yet how to properly express bulk root parameters which implicitly contain these parameters, without explicitly requiring them, as has elegantly been done by Molz and co-workers, is unclear.

Irreversible thermodynamics is the most commonly used approach in root modelling. Objections to this by Pickard (2003) must certainly be examined, although these objections may well apply to the situation of root pressure and not to root models in general. The issue of a linear system, however, also raised by Zimmermann *et al.* (1992), does need to be examined closely. It is possible another mathematical approach may need to be used if this turns out to be a major limitation. The use of Gibbs free energy and Navier-Stokes relations by Pickard (2003) is also a little-used avenue which may be worth exploring.

6 CONCLUDING REMARKS

This review has discussed explanations given by the Composite Transport Model for parameter behaviour in root transport. While this model can be used to formulate reasonable hypotheses about mechanisms of root transport, there are serious concerns regarding what quantities the pressure probe is actually measuring, the use of separate hydraulic conductivities in parallel pathways which are beyond the scope of application of thermodynamic theory, and the absence of a proper inclusion of the effects of unstirred layers. The simple view of two parallel pathways appears inadequate considering that water exchange between pathways, and transport behaviour at the endo- and exodermis, have not been incorporated into the model. The CTM emphasizes the role of parallel pathways and not serial components, but the latter must be taken into account. It is proposed that the CTM, as it stands, is too simple a model and should be extended.

There is much work to be done in developing numerical, quantitative models for root transport. This includes a quantitative model of the CTM, for without this the value of the hypotheses that emerge from the CTM cannot be properly assessed. A major step in this is to determine what mathematical approach should be used. This depends on the compartmentalization of the root, what detail is to be incorporated and whether the equations can reflect this detail, what data is available for input into the equations, and

whether the equations clearly reflect the physical processes occurring and lend themselves readily to a numerical solution.

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Appendix: List of symbols and abbreviations

Symbol	Units	Meaning
L	$\text{cm sec}^{-1} \text{bar}^{-1}$	hydraulic conductivity, or filtration coefficient
p_s	cm sec^{-1}	solute permeability coefficient
s	dimensionless	reflection coefficient
L_r	$\text{cm sec}^{-1} \text{bar}^{-1}$	root hydraulic conductivity
p_{sr}	cm sec^{-1}	root solute permeability coefficient
s_r	dimensionless	root reflection coefficient
L_{rh}	$\text{cm sec}^{-1} \text{bar}^{-1}$	root hydraulic conductivity under hydrostatic gradient
L_{ro}	$\text{cm sec}^{-1} \text{bar}^{-1}$	root hydraulic conductivity under osmotic gradient
?	bar	water potential
P	bar	turgor pressure
p	bar	osmotic pressure
n	mol	number of mole of solute
C	mol cm^3	solute concentration
R	$\text{cm}^3 \text{bar mol}^{-1} \text{K}^{-1}$	universal gas constant
T	K	absolute temperature
C_{av}	mol cm^3	average concentration of solute in cell membrane
C_m	mol cm^3	solute concentration adjacent to membrane
C_{bulk}	mol cm^3	bulk concentration of solute
τ_h	sec	half-time of pressure relaxation
d	cm	thickness of unstirred layer
A	cm^2	surface area of interface in direction of flow
V	cm^3	volume
\bar{V}_s	$\text{cm}^3 \text{mol}^{-1}$	partial molar volume of solute
\bar{V}_w	$\text{cm}^3 \text{mol}^{-1}$	partial molar volume of water
e	bar	elastic modulus
D_s	$\text{cm}^2 \text{sec}^{-1}$	diffusivity coefficient of solute
f	dimensionless	coefficient of friction
I	amp	electric current
J_v	cm sec^{-1}	volume flux of water per unit area
J_s	$\text{mol cm}^{-2} \text{sec}^{-1}$	solute flux per unit area
J_w	$\text{mol cm}^{-2} \text{sec}^{-1}$	water flux per unit area
J^*	$\text{mol cm}^{-2} \text{sec}^{-1}$	solute flux per unit area due to active transport

Abbreviation	Meaning
CTM	composite transport model
CB	Casparian band
SL	suberin lamellae
ODE	ordinary differential equation
PDE	partial differential equation
DE	differential equation