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REVIEW ARTICLE

The exodermis: a variable apoplastic barrier

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Abstract

The exodermis (hypodermis with Casparian bands) of plant roots represents a barrier of variable resistance to the radial flow of both water and solutes and may contribute substantially to the overall resistance. The variability is a result largely of changes in structure and anatomy of developing roots. The extent and rate at which apoplastic exodermal barriers (Casparian bands and suberin lamellae) are laid down in radial transverse and tangential walls depends on the response to conditions in a given habitat such as drought, anoxia, salinity, heavy metal or nutrient stresses. As Casparian bands and suberin lamellae form in the exodermis, the permeability to water and solutes is differentially reduced. Apoplastic barriers do not function in an all-or-none fashion. Rather, they exhibit a selectivity pattern which is useful for the plant and provides an adaptive mechanism under given circumstances. This is demonstrated for the apoplastic passage of water which appears to have an unusually high mobility, ions, the apoplastic tracer PTS, and the stress hormone ABA. Results of permeation properties of apoplastic barriers are related to their chemical composition. Depending on the growth regime (e.g. stresses applied) barriers contain aliphatic and aromatic suberin and lignin in different amounts and proportion. It is concluded that, by regulating the extent of apoplastic barriers and their chemical composition, plants can effectively regulate the uptake or loss of water and solutes. Compared with the uptake by root membranes (symplastic and transcellular pathways), which is under metabolic control, this appears to be an additional or compensatory strategy of plants to acquire water and solutes.

Key words: Exodermis, plant roots, barrier, variable resistance, solutes, water.

Introduction

The words that are used to describe objects and ideas have a potent influence on the way we think about them. The word 'barrier' is used in the title of this piece, but it has been done with some misgivings since a barrier can be thought of as something that excludes completely the passage of material. It is perhaps this notion that has given rise to some rather discordant discussions about the function of the exodermis. The point that the authors wish to make in this article is that the exodermis is more like a resistor through which currents of different materials can pass. Moreover, its resistance is variable so that the current passing through it can change in response to environmental conditions. During root development, the selectivity of the exodermis may change, just as it does in the endodermis (Clarkson, 1993; Steudle and Frensch, 1996). A barrier or resistance may be approached from either side, it can be used to keep intruders out or to keep captives in. The research perspective may condition the way in which the barrier is approached. In this article it is shown, for instance, that the exodermis may play a crucial role in retaining the phytohormone abscisic acid (ABA) *within* the apoplast of the cortex. Conversely, there is much evidence that an exodermis may provide a substantial peripheral resistance to the *entry* of water and

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solutes into the apoplast. The physical conditions at the root surface have a clear role in determining the magnitude of the exodermal resistance. Both the presence of a permanent film of liquid water and the oxygen partial pressure seem to affect the resistance, and at the extremes of growth under conditions of drought, high salinity, or low oxygen, make the exodermis an absolute barrier in the strict sense (North and Nobel, 1991; Azaizeh and Steudle, 1991; Colmer *et al*., 1998; Martre *et al*., 2001).

The access of materials from the surroundings to the interior surfaces of root cells is obviously influenced by the exodermis. To this extent, it is an interesting structure to those who study the pathways followed by materials as they move through the root cylinder towards the stele. In the absence of apoplastic barriers, the extraprotoplastic space (or free space) of the cortex provides a path of relatively low resistance along which water and charged and uncharged solutes may move. This space is part of the root apoplast. Where access to this space becomes restricted by an exodermal resistance, a greater proportion of the total flux of a material may be absorbed at the root periphery and pass from cell to cell via the symplast. Substances that cannot move by this pathway are likely to be more severely hindered by the presence of the exodermis.

Roots in nature are rarely in a uniform environment and one should anticipate that there may be gradients or heterogeneity in the resistance of the exodermal layer in a given plant. Moving along the length of a root, the relative contributions of the two main pathways to the radial transport of materials may change due to the maturation of the exodermis (Melchior and Steudle, 1993; Frensch *et al*., 1996). Thus, although the transport properties of a root system can be averaged, they are unlikely to be uniform, as frequently assumed in models of water and nutrient uptake by roots in soil.

Many of the examples in this article are drawn from the authors experience with ABA (Freundl *et al*., 1998; 2000; Hose *et al*., 2000). This phytohormone has a special role in registering the imminence of drought in the soil and as a message bearer between the root and the shoot (Davies and Zhang, 1991). In these respects, the flux of ABA from roots to shoots is of particular importance. The rate of water movement through the root tissues, particularly in the apoplast, may affect the delivery of ABA to the leaves. It has been pointed out that changing concentrations of ABA in the xylem are likely to occur as transpiration rates change during the day/night cycle or during a period of progressive water stress (Else *et al*., 1994, 1995; Jackson *et al*., 1996). Some evidence is presented that suggests that the release of ABA from root cells responds positively to increased water flow through the apoplast and that, in turn, the hydraulic conductivity of cell membranes and water flows across roots are increased by ABA. As a further point, it is possible that the presence of a sustained increase in the ABA in the root apoplast may promote an increased resistance to water flow across the exodermis by inducing its formation (Kollatukudy and Agrawal, 1974). Hence, for more than one reason the coupling between the flows of water and ABA should affect root-to-shoot signalling (Steudle, 2000*a*, 2001). Evidence is presented of how changes in growth conditions affect the structure (anatomy) of the exodermis in relation to both its chemical composition and transport properties (Zimmermann *et al*., 2000). The structure of apoplastic barriers in the exodermis which would allow some passage of water and solutes is discussed (Schreiber *et al*., 1999).

The occurrence and formation of the exodermis

Roots are designed for the uptake of water and nutrients across the apoplast and the symplast. At the same time, they should prevent infection by pathogens and must exclude harmful or even toxic substances. Furthermore, *loss* of water, nutrients and phytohormones into the soil solution has to be reduced by variable resistances in special cell layers in the root cylinder. The variable resistance is achieved by the interplay between several components of the complex anatomy of roots. Due to their composite structure roots exhibit a composite transport barrier (Steudle and Peterson, 1998; Steudle, 2000*a*, *b*).

Casparian bands, composed of lignin and suberin and located in the primary walls of endodermal and exodermal cells, are linked structurally to the plasma membranes in former tissue. A band plasmolysis in the exodermis has not been reported frequently. It is reported from the short cells of the dimorphic type (*Allium cepa* L.) where the deposition of the suberin lamellae is delayed, and from maturing exodermal cells of *Zea mays*. Perhaps, the explanation for this scarcity of information is that suberin lamellae form at a much earlier stage in the exodermis than in the endodermis often appearing along with, or shortly after the development of Casparian bands. The Casparian band, when attached to the plasma membrane, appears to direct flows of materials across cell membranes, and clearly provides an opportunity to exercise selectivity in the fluxes of materials through these cell layers. The occurrence and formation of endodermal layers, that separate the cortex from the stele, have been extensively investigated. By contrast, there has been less attention given to the functional consequences of the formation of the exodermis that separates the cortex from the surrounding medium. Although the presence of the structure is very common (Perumalla *et al*., 1990) the significance of the exodermis is neglected in most standard textbooks. In an extensive survey of more than

200 angiosperm species only 6% of all investigated plants lacked a hypodermis and only 3.5% of them had a hypodermis without Casparian bands (Perumalla *et al*., 1990; Peterson and Perumalla, 1990). A hypodermis with clearly suberized bands is what is taken as the definition of an exodermis. The great majority (91%) of all investigated angiosperms showed a clearly suberized exodermis with Casparian band. Of these 13% had more elaborated bi- or multi-seriate exodermae (Peterson, 1991).

Exodermis with Casparian bands was found in roots of hydrophytic, mesophytic and xerophytic species and in members of primitive as well as advanced families. Some investigations (Brundrett *et al*., 1990; Damus *et al*., 1997) showed that seedless vascular plants and gymnosperms develop no exodermis with the exception of some species from the Selaginellaceae.

The Casparian band can be developed very close to the root tip. In aeroponically grown maize, 30 mm above the root tip a complete exodermal layer could be detected (Fig. 1A). In roots elongating more slowly, the band may mature within 5 mm of the tip (Peterson, 1988). The Casparian band of the exodermis frequently occupies the whole of the radial and anticlinal walls (Fig. 1A, B). In this respect, it differs from endodermal cells where the band is restricted, at least in the early phase of state I, to the mid region of the walls.

Maturation of the Casparian band (state I) in the exodermis is followed by the formation of a suberin lamellae (state II, Fig. 1C). There may be further deposition of cellulosic secondary walls (state III): for example, *Arundinaria japonica*, *Sorghum vulgare*, *Primula auricula*, and *Saccharum officinarum* (von Guttenberg, 1968). Two kinds of exodermis are known:

(i) The uniform exodermis, where all cell walls become suberized in state II. This type of exodermis may form just one layer (e.g. *Zea mays*, *Helianthus annuus*) but may be multiseriate, for example, 20 layers in *Ananassa macrodontes* (von Guttenberg, 1968).

(ii) The dimorphic exodermis. Here the exodermis is made up by long, suberized cells and short cells in which the deposition of suberin lamellae is delayed, sometimes indefinitely (e.g. *Allium cepa*, *Asparagus officinalis*). It is agreed that short cells probably act as passage cells for water and nutrient ions. Dimorphic exodermal layers are

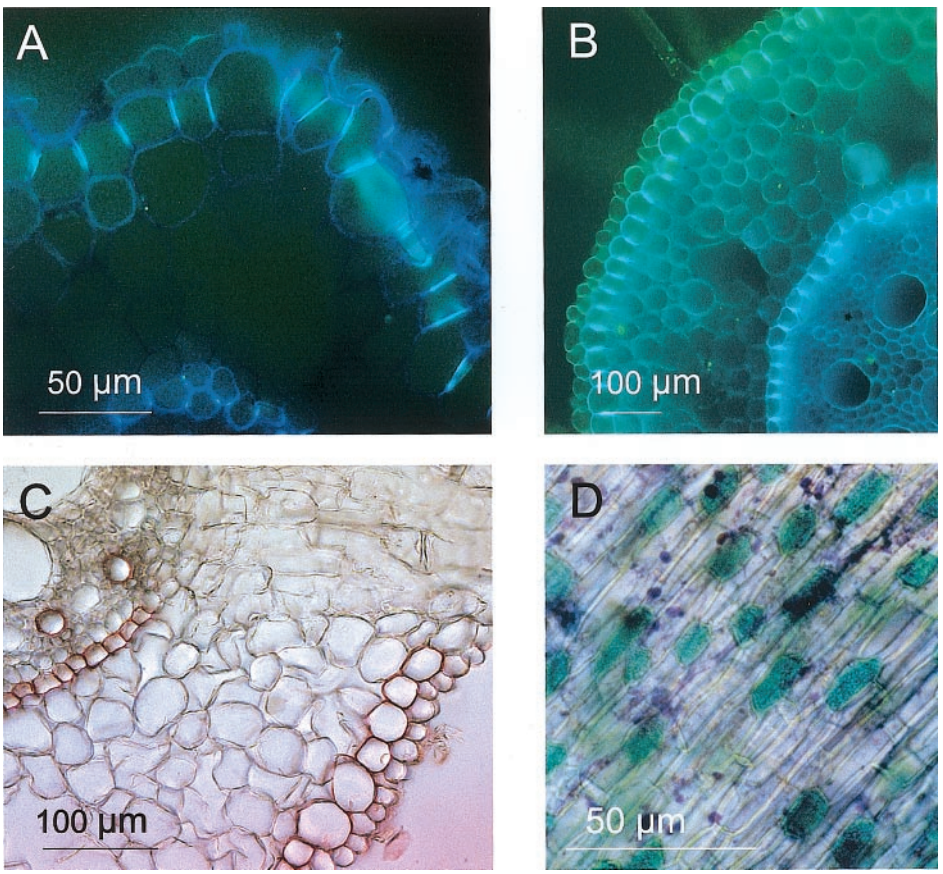

Fig. 1. (A–C) Cross-sections of 11-d-old maize primary roots grown for 7 d in aeroponics. Fresh sections have either been stained with berberin hemisulphate/aniline-blue (Brundrett *et al*., 1990) (A, B) in order to stain Casparian bands or with Sudan III (C) in order to stain the suberin lamellae. Cross-section (A) was taken at a distance of 30 mm from the root tip, section (B) at a distance of 300 mm above the root tip and (C) at the root base. (D) Dimorphic exodermis of the poikilohydric plant *Chamaegigas intrepidus* stained with toluidine-blue. Short cells are stained blue.

typical for xerophytic plants and for the resurrection plant *Chamaegigas intrepidus* (Fig. 1D; von Guttenberg, 1968; Hickel, 1967; Shishkoff, 1987).

The existence of plasmodesmata in the outer and inner tangential walls of exodermal cells suggest that a symplastic path is present. In the short cells of the dimorphic exodermis of *Allium cepa*, plasmodesmata remain in place even in older parts of the root, while those of the long cells become disrupted during the deposition of suberin (Ma and Peterson, 2000, 2001). In the uniform exodermis of seminal roots of *Zea mays*, intact plasmodesmata were found even in exodermal cells where the walls were suberized (Clarkson *et al*., 1987). Also in the branch (lateral) roots of *Zea mays*, plasmodesmatal canals passed straight through suberin lamellae (Wang *et al*., 1995). In both the short cells of *Allium cepa* and in *Zea mays*, there were more frequent plasmodesmata in the inner tangential wall (adjoining the cortex) than on the outer tangential wall of the exodermis (Clarkson *et al*., 1987; Ma and Peterson, 2000). A greater frequency of plasmodesmata was found in the inner tangential wall of the endodermis in *Hordeum vulgare* (Clarkson *et al*., 1971), but not in *Allium cepa*, where the frequencies on the two walls was similar (Ma and Peterson, 2001). The existence of unequal numbers of plasmodesmata in the outer and inner walls of the exodermis of *Allium* and *Zea* suggests that there may be a confluence of symplastic and apoplastic streams. Plasmodesmata have been recorded in the lateral, or branch, roots of field-grown *Zea mays* that are probably responsible for a large part of the total uptake of nutrients and water (Wang *et al*., 1995). The frequency of plasmodesmata was similar in the exodermis and the endodermis suggesting that the transport capacities of both barriers are adapted to each other.

Chemical composition of exodermal cell walls

In the past, modifications of apoplastic barriers in roots (e.g. endodermal and exodermal cell walls) have been analysed by applying histochemical staining techniques in combination with microscopic techniques (Wilson and Peterson, 1983). The approach allows the documentation and description of structural changes in cell walls, which are due to chemical cell wall modifications but modifications themselves are not well characterized by histochemistry. An insight into the true chemical composition of modified cell walls can be obtained only by directly analysing the compounds occurring in the cell walls. This became possible for the endodermis and exodermis when an enzymatic method for the isolation of root cell walls forming apoplastic barriers had been developed (Robards, 1976; Karahara and Shibaoka, 1992; Schreiber *et al*., 1994; Schreiber, 1996). Separate exodermal wall preparations were obtained which were used for a detailed chemical analysis. Since rhizodermal cell walls were generally resistant to cell wall degrading enzymes and could not be removed mechanically from the exodermal cell walls, cell wall samples from the outer root apoplastic barrier were composed of **rh**izo- and exodermal **c**ell **w**alls together (RHCW; Zeier and Schreiber, 1997). Thus, in the following, the term exodermis always includes the rhizodermis. Solvent-extracted cell wall samples were degraded by specific depolymerization reactions as described in the literature for the degradation of various structural cell wall polymers such as carbohydrates, structural cell wall proteins, lignin (Lapierre *et al*., 1991), and suberin (Kolattukudy and Agrawal, 1974). After extracting, purifying and concentrating monomers from the reaction mixtures, they were analysed by gas chromatography coupled to a flame ionization detector or to a mass selective detector (Zeier and Schreiber, 1998).

In all RHCW samples isolated from nine plant species, the four different biopolymers mentioned above were detected (Schreiber *et al*., 1999; Zeier *et al*., 1999*a*). Carbohydrates were the most abundant substance class (20–50%) indicating that a significant fraction of the cell wall carbohydrates was efficiently protected from being digested by the enzymes due to the presence of further non-carbohydrate polymers such as cell wall proteins, lignin and suberin. Thus, the spatial arrangement of carbohydrates and non-carbohydrate polymers in exodermal cell walls must be very close to each other. There was a pronounced difference in the amounts of structural cell wall proteins and lignin detected with monocotyledonous species (*Allium cepa*, *Cli*via *miniata*, *Agapanthus africanus*, *Aspidistra elatior*, *Iris germanica*, and *Monstera deliciosa*) compared to dicotyledoneous species (*Pisum sativum*, *Cicer arietinum* and *Ricinus communis*). Dicotyledonous species contained a large amount of structural cell wall proteins (17%) and only traces of lignin (0.4–1%). On the other hand moncotyledonous species possessed relatively high amounts of lignin (8.3–9%) and low amounts of structural cell wall proteins (2–4%). It appears that there are different strategies for reinforcing outer cell walls at the root/soil interface and to protect them from microbial attack and decomposition. In all RHCW samples linear long-chain aliphatic suberin monomers and esterified aromatic suberin compounds, like ferulic and coumaric acids, were detected with varying abundance (0.5–5%). Aliphatic suberin is thought to be much less permeable for water and polar solutes than aromatic suberin. Hence, the fact that the former compound was detected in all investigated samples suggested that rhizodermal and exodermal cell walls represent a protective, apoplastic interface separating the root cortex from the soil as it is generally described for suberized surfaces (Kolattukudy *et al*., 1975). Wax-like compounds were not detected in

exodermal cell wall isolates. They are well known to occur in plant/atmosphere interfaces where they form water-tight barriers such as the leaf cuticle and suberized periderm (Espelie *et al.*, 1980; Schönherr and Ziegler, 1980; Soliday *et al.*, 1979; Schönherr, 1982; Vogt *et al.*, 1983; Kerstiens, 1996; Riederer and Schreiber, 1995). It is established that the transport-limiting barrier of leaf cuticles and suberized periderms is based on the existence of solid-crystalline waxes deposited to the polymers (Riederer and Schreiber, 1995). It may be concluded that, in the absence of waxes, the resistance of the suberized exodermis towards water and dissolved molecules should be lower than those of above-ground interfaces.

The development of suberin in isolated exodermal and endodermal cell walls along the length of maize (*Zea mays*) roots was investigated in plants growing in various conditions (Zeier *et al.*, 1999*b*; Hose, 2000; Zimmermann *et al.*, 2000). From microscopic investigations it is known that corn roots grown in humid air were strongly suberized even in the very young root zones compared to roots grown in hydroponics (Enstone and Peterson, 1998). When the chemical composition of exodermal cell wall isolates of maize roots grown in aeroponics was analysed, significantly higher amounts of aliphatic suberin were detected compared to roots grown in hydroponics (Fig. 2A). No significant differences were detected in the composition of the aromatic suberin of the exodermal cell wall isolates (Fig. 2C). Absolute amounts of aliphatic and aromatic suberin were higher in endodermal than in exodermal cell walls (Fig. 2C, D). Again there was no difference in the composition of endodermal cell wall preparations between the two different cultivation methods. Thus, different growth conditions affected the chemical composition of exodermal cell walls that formed the direct interface between the roots and their surroundings. The internal apoplastic barrier at the endodermis remained unaffected. As a consequence of increased amounts of suberin deposition, properties of the exodermis as an apoplastic transport barrier were altered, as will be shown below. Comparing roots of sunflower and maize, it was evident that there is significantly more aliphatic suberin in the sunflower exodermis (Fig. 3), which, in turn, could be related to significantly lower rates of radial transport of ABA and water in roots of this species compared to maize.

Passage cells

In an excellent review by Peterson and Enstone (1996) the occurrence of passage cells in both the endodermis and exodermis has been described. A common source of confusion is the mistaken belief that passage cells lack Casparian bands (von Guttenberg, 1968). They do not. In both the endo- and exodermis the differentiation of the Casparian band is an early and more or less synchronous event in all cells. Once it is formed, all cells appear to form an attachment between the plasma membrane and the Casparian band in those species where this has been tested by band plasmolysis (Bonnett, 1968;

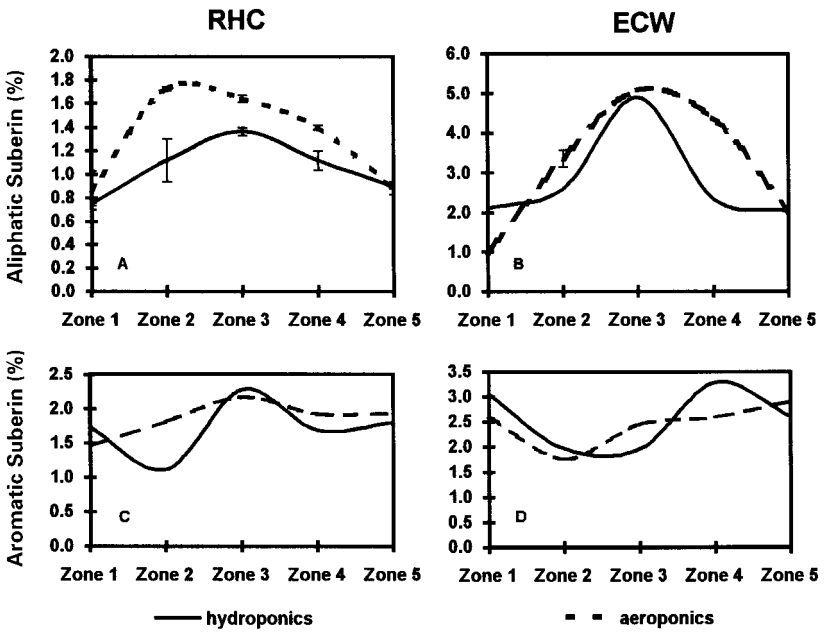

Fig. 2. (A–D) Amounts of aliphatic (A, B) and aromatic (C, D) suberin from hypodermal (RHCW; A, C) and endodermal (ECW; B, D) cell walls from 11-d-old seedlings of maize as % of the dry weight of the cell wall preparations. Plant have been grown for 7 d either in hydroponics (-) or in aeroponics (- -). Primary roots were divided into five equal parts (Zone 1: root tip; Zone 5: root base). Mean ± range of two independent experiments of 30 plants.

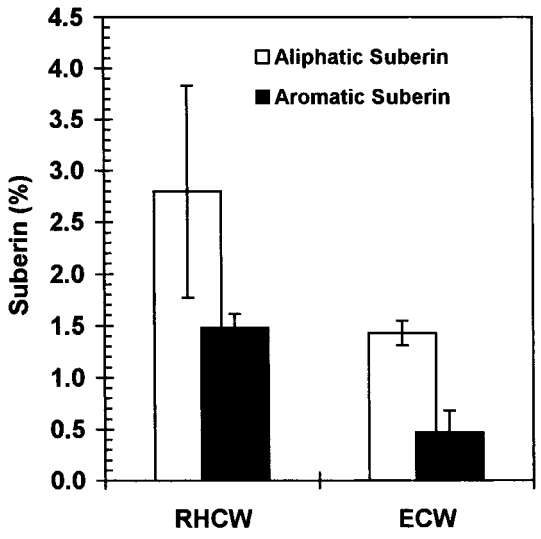

Fig. 3. Amounts of aromatic (■) and aliphatic (□) suberin of the hypodermal (RHCW) and endodermal (ECW) cell walls of 11-d-old roots of *Helianthus annuus* L. in % of dry weight of the cell wall preparations. Mean ± range of two independent experiments of 30 plants.

Peterson and Emanuel, 1983; Clarkson, 1993; Enstone and Peterson, 1997).

In the dimorphic type of exodermis, passage cells have a Casparian band but lack suberin organized into lamellar structures. The delayed suberization is restricted to the short cells and these were termed 'Durchlaβzellen' ('passage cells') (von Guttenberg, 1968). He noted that their position was not related to any underlying tissue or tissue pattern, unlike the endodermis where passage cells are seen most frequently opposite to xylem poles. Short cells are found in a very regular pattern in certain species, especially in the family of the Liliaceae, for example in *Haemanthus puniceus*, *Agapanthus umbellatus* and *Allium cepa* (von Guttenberg, 1968; Wilson and Robards, 1980; Stasovski and Peterson, 1993). In a number of orchid species, the outer tangential walls of short cells have numerous wall ingrowths that greatly expand the surface area of the plasma membrane and, thus, resemble transfer cells (von Guttenberg, 1968). In other tissues, the presence of transfer cells indicates the location of large fluxes of material into, or out of cells. In the exodermis, it is reasonable to assume that short cells represent principal points of symplastic entry into the underlying cortex. However, experimental evidence on this point is lacking. Short cells with wall ingrowths have also been found in older parts of onion roots that have been exposed to damp air rather than being kept immersed in hydroponic culture (Wilson and Robards, 1980, Clarkson, 1993). Short cells with massive wall elaborations are also found in the resurrection plant *Chamaegigas intrepidus* (Fig. 1D; Hickel, 1967). It is paradoxical that short cells in onion, that are initially lacking suberin lamellae, should prove to be very drought resistant. Stasovski and Peterson found that they were some of the last survivors of peripheral cells during prolonged water stress (Stasovski and Peterson, 1993).

Xerophytic plants

The role of suberization for the hydraulic conductivity of roots of desert plants has been investigated (North and Nobel, 1991, 1995; Martre *et al.*, 2001). Hydraulic conductivity of roots of *Agave desertii* declines to varying extents during soil drying (2-fold for older nodal roots, 10-fold for young nodal roots and 20-fold for lateral (rain) roots. Increases of suberization in the exodermis, endodermis and cortex cells adjacent to the endodermis occurred in response to drying and were paralleled by a decrease of radial hydraulic conductivity, pointing again to a protective role of the exodermis, as far as water loss is concerned.

Roots of xerophytic plants often have a dimorphic exodermis with short cells, and these are also found in the aerial roots of orchids. Stasovski and Peterson exposed onion roots to dryness over a period of up to 200 d (Stasovski and Peterson, 1993). The epidermis and the root apices did not survive this desiccation treatment whereas the short cells of the dimorphic exodermis, part of the cortex and the stele stayed alive thus maintaining a symplastic connection between exodermis and stele. This points to an important role of the short cells of the dimorphic exodermis for the transport of materials, as already suggested for xerophytes, aerial roots and roots of the Asclepiadaceae (von Guttenberg, 1968). The structure was also found in roots of the poikilohydric angiosperm *Chamaegigas intrepidus* (Fig. 1D) (Hickel, 1967; Heilmeier and Hartung, 2001). The short (passage) cells of *Chamaegigas* exhibited a characteristic pad (or cap) adjacent to their outer cell wall. The pad resembled a sponge in tissue sections and consisted of cellulose incrusted with silicic acid and small amounts of lignin. It was free of suberin. According to Hickel the pads are used to close passage cells during dehydration (Hickel, 1967). They act as valves slowing down water loss. During rehydration the pads take up water, swell and reversibly open the passage across short cells for radial transport. The findings support the view of Stasovski and Peterson that short cells (passage cells) of the dimorphic exodermis play an important role during the desiccation tolerance of roots of xerophytic plants (Stasovski and Peterson, 1993).

Wetland plants

Many wetland plants are able to develop root systems that grow into oxygen-depleted mud or water. The apical meristems of such roots receive their oxygen supply from

aerenchyma, a tissue developing large continuous air channels in the root cortex (Jackson and Armstrong, 1999). The water at the bottom of ponds and pools in marshes may itself be nearly hypoxic. So there will be a risk that oxygen delivered from the shoot by axial diffusion in the lacunae of the aerenchyma will be lost to the anaerobic environment before it reaches the root tips. In all plants from such habitats, the exodermis is well developed even in species with weak or irregular aerenchyma development (e.g. *Caltha palustris*: Seago *et al.*, 2000), and may have one or more additional layers of thickened cells interior to it.

The exodermis and underlying layer of heavily lignified sclerenchyma of *Oryza sativa* (rice) is a constitutive feature (Morita and Abe, 1999). Both layers are present in hydroponically and aeroponically grown roots and in upland (dryland) and paddy rice varieties (Miyamoto *et al.*, 2001). The hydraulic conductivity of the adventitious roots of rice (Lp_r) was found to be around 6×10^{-8} m s^{-1} MPa^{-1} (Miyamoto *et al.*, 2001). This is low in comparison with maize roots, even when they possess an exodermis (e.g. in maize 4×10^{-7} m s^{-1} MPa^{-1}; Hose *et al.*, 2000), but is comparable to root Lp_r measured under hydrostatic driving force in *Lotus japonicus* (4×10^{-8} m s^{-1} MPa^{-1}) that lacks an exodermis (Henzler *et al.*, 1999). It is also similar to the root Lp_r of species in which a cell-to-cell rather than an apoplastic transport of water dominates such as barley and bean (Steudle and Jeschke, 1983; Steudle and Brinckmann, 1989). There are indications that the low root Lp_r in rice is due to a high resistance to water flow at the strongly developed endodermis and/or outer root parts with the exodermis and sclerenchyma (Miyamoto *et al.*, 2001). However, figures of the relative contribution of endo- and exodermis and aerenchyma have not yet been worked out, neither has the hydraulic resistance of the cell-to-cell path been estimated by measuring the hydraulic conductivity of individual cells (cell *Lp*). Because of the strong suberization and lignification, these measurements are hard to perform. Nevertheless it appears that lignification and suberization of outer root parts represents an adaptation that effectively limits the outward diffusion of oxygen (Colmer *et al.*, 1998; Armstrong *et al.*, 2000). This, however, may result in a low capacity of rice to take up water. Rice may tend to optimize radial transport of water, nutrients and oxygen in such a way that oxygen loss is reduced, but uptake of water and nutrients is still sufficient to supply the shoot. Thus, even when sitting in flooded fields, rice shoots may suffer from water shortage (Hirasawa *et al.*, 1992, 1996). The problem could be even more severe for varieties grown in the upland. It has to be solved by breeding for varieties which grow under conditions of low soil water (Miyamoto *et al.*, 2001). When the apoplastic transport component is small, there could be some improvement if it was possible to modify the capacity of the cellular pathway by increasing the density or activity of water.

Uptake and lateral transport of solutes

It is perhaps unfortunate that early attempts to analyse transport functions in relation to root anatomy made use of the seminal root system of *Hordeum vulgare* (Robards *et al.*, 1973; Clarkson and Robards, 1975). This is because such roots do not have an exodermis, the principal apoplastic barrier being located at the endodermis where the Casparian band, and its attachment to the radial cell walls, minimizes unrestricted access of water and solutes to the stelar tissues. In such a simple root design, water and solutes can potentially move apoplastically through the walls of the epidermis and cortex as far as the Casparian band. The major flows of materials are then constrained to pass across the endodermal plasma membrane; this permits selectivity functions to be exercized. Nutrient ions and other solutes may enter the endodermal protoplast via channels or be actively transported by carriers. This access will be strongly restricted by the development of suberin lamellae. Most of the investigated angiosperm species differ from the simple structure of barley seminal roots because they develop an exodermis in which there is also a Casparian band. Recent evidence suggests that, even in barley the nodal (adventitious) roots develop an exodermis in hydroponic cultures (Lehmann *et al.*, 2000). It might be anticipated there would be a drastically decreased surface area for the uptake of solutes if opportunities to enter the symplast were restricted to the epidermis (Kamula *et al.*, 1994). This raises the crucial question of the permeability of the apoplast in exodermal layers. Does the exodermal Casparian band, for example, block the pores in the apoplast to the same extent as is usually supposed in the case of the endodermis?

Whether or not a substance is transported apoplastically is determined by its physico-chemical properties and by those of cell walls. Walls consist of a porous network of cellulose fibrils, hemicellulose, pectins, and glycoproteins (Marschner, 1995). Interfibrillar spaces, the pores of the cell wall network, have a calculated diameter of 3.5–14 nm (Strugger and Peveling, 1961; Carpita *et al.*, 1979). Since low molecular weight solutes such as hydrated ions would have diameters of only a few tenths of a nanometre, there could hardly be a selection due to size in the absence of apoplastic barriers. However, there are fixed negative charges in the wall matrix (carboxylic groups of the pectins) that will tend to repel anions, but which exchangeably bind cations. Hence, the diffusivity of cations, namely of those bearing two charges such as Ca^{2+}, should be somewhat restricted. Uncharged polar and even ionic dyes with substantial molar weights have often been used to trace apoplastic water movement

(Strugger, 1939; Varney *et al*., 1993). These attempts are doomed to failure because the mobility of the small water molecule differs substantially from that of these solutes (Hülsbruch, 1944; Steudle and Peterson, 1998).

Impregnation of endodermal and hypodermal walls with suberin and lignin should reduce the diameter of interfibrillar spaces. Depending on the polarity of deposits, water and hydrophilic substances may be repelled from inner surfaces of interfibrillar pores of walls. There are, to date, no data about pore diameters in suberized walls. Quantitative chemical measurements of suberin present in isolated exodermal layers from different species showed that about 0.4% to 5% of the total dry weight consisted of suberin (Schreiber *et al*., 1994; Schreiber, 1996; Zeier and Schreiber, 1997, 1998; Zeier *et al*., 1999*a*, *b*; Zimmermann *et al*., 2000; Degenhardt and Gimmler, 2000; Hose, 2000). Suberin makes walls lipophilic but may not completely interrupt interfibrillar passages, at least for small uncharged solutes and water. This idea is supported by experiments that showed dye permeation through suberized walls of epidermal cells (Peterson *et al*., 1978). What needs to be known is more information about the diffusivity of permeants (such as water, nutrient ions and polar uncharged solutes of interest) in walls in the presence of apoplastic barriers. Detailed knowledge about the porous structure of walls would then allow predictions about interactions between water and solutes in the presence of an hydraulic (transpirational) water flow across the barriers and how solutes could be dragged with the solvent (water).

Apoplastic transport of water

Water is a small molecule with a diameter of 0.28 nm (Stein, 1986). It may pass relatively unhindered through the root apoplast. To some extent it may also move across apoplastic barriers such as Casparian bands in the endo- and exodermis (Steudle and Peterson, 1998; Steudle, 2000*a*, *b*; 2001). Seminal roots of young maize plants grown in hydroponics with no developed exodermis (Enstone and Peterson, 1998; Zimmermann *et al*., 2000) had a root hydraulic conductivity (Lp_r) higher by factors of 1.5 to 3.6 in hydrostatic experiments than those grown aeroponically which developed an exodermis (Zimmermann and Steudle, 1998; Zimmermann *et al*., 2000). The decrease in Lp_r could be closely related to the increased amount of aliphatic suberin in the hypodermis (Zimmermann *et al*., 2000). Changes in root Lp_r were not caused by changes in hydraulic conductivity at the level of root membranes (cell *Lp*). No differences were found in the *Lp* values of cells from roots cultivated either aeroponically or hydroponically and root Lp_r was similar to cell *Lp*.

The conclusion drawn from these results was that, at least under the hydrostatic pressure gradients which occur during transpiration, a significant amount of water passes the root apoplast in addition to that passing along the cellular pathway. However, this does not mean that nutrient ions were not filtered off at apoplastic barriers in the root's exo- and endodermis. Puncturing the endodermis of young maize roots had a substantial effect on the permeability of ions and on the root reflection coefficient, but did not affect water permeability (hydraulic conductivity) of the roots (Steudle *et al*., 1993).

The apoplastic passage of water and solutes?

During transpiration, there is high density of radial water flow across root tissues. This flow is shared between the apoplast and the cell-to-cell pathways according to their hydraulic conductance. In the classical view, the former component becomes zero at the endodermis. According to this view, water and solutes move independently across the root (Miller, 1985; Engels, 1999). Hence, increasing transpiration progressively dilutes the solution entering the xylem vessels. The situation is known from intact plants, but could also be obtained with excised roots when gradients of hydrostatic pressure were applied by suction at the root base with a vacuum pump, or by pressurizing root systems (Fig. 4; Fiscus, 1975; Rüdinger *et al*., 1994; Zimmermann and Steudle, 1998; Hose, 2000).

When water and dissolved solutes can move through the walls in Casparian bands, then movements would no longer be independent of each other. The additional apoplastic transport component could compensate to some extent for the dilution of solutes delivered via symplastic transport. This possibility is now considered in relation to the movement of ABA and other substances from the root tissues into the xylem sap.

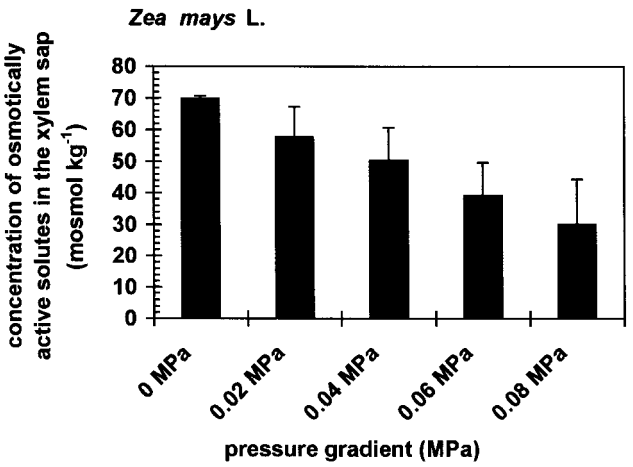

Fig. 4. Concentration of osmotically active solutes in the expressed xylem sap of 11-d-old maize plants. With increasing the sub-atmospheric pressure which led to an increased water flow the concentration of osmotically active solutes has been diluted. Mean ± SD, $n = 5$–7 roots.

ABA

The role of (+)-*cis-trans*-abscisic acid (ABA) as a root-to-shoot stress signal is well established (Blackman and Davies, 1985; Davies and Zhang, 1991; Hartung *et al.*, 1999). The concentration of ABA in the xylem sap is thought to be the signal that is carried up to the shoot with the transpiration stream. When ABA is produced in the root during drought stress at a reduced water uptake, the concentration in the xylem, i.e. the signal going to the shoot, should be high. It would be switched off by dilution of xylem sap, when the availability for water in the soil improves, which raises transpiration. In this model, the apoplastic transport of ABA (delivered by root cells), contributes to the signal strength by an interaction with the water flow.

The sesquiterpene ABA is a weak acid with a pK_a of 4.8. It is rather lipophilic in its protonated form but can also be dissolved in water. The molecular weight is 264 Da. The question arose, whether ABA has the structural prerequisites for passing the cell wall pores by solvent drag or a lipophilic barrier by dissolution. The latter view is supported by the fact that the reflection coefficient is small under acid conditions (Freundl *et al.*, 1998). It was also found that PEG 1000 with a molecular weight of 950–1050 can freely pass through the cell walls of living plants (Carpita *et al.*, 1979). The dimensions of interfibrillar spaces are in the range of 4 to 14 nm (Strugger and Peveling, 1961; Carpita *et al.*, 1979). The mobility (diffusion coefficients) of different lipophilic compounds has been measured in recrystallized, epicuticular waxes of *Fagus sylvatica* and *Picea abies* (Schreiber *et al.*, 1996). The mobility of ABA has been fairly high, even in reconstituted waxes of these leaves. Thus, ABA might also be able to pass cell wall pores of the apoplast of plant roots even in the presence of apoplastic barriers. Recent measurements on excised roots of sunflower and maize indicate that this is the case (Freundl *et al.*, 1998, 2000). Excised roots with the mesocotyls (maize) and with the hypocotyls (sunflower) still attached to them, were fixed to a capillary by a seal. Increasing the pressure difference in the attached capillary with a vacuum pump resulted in increased xylem sap flows through the roots. ABA supplied in concentrations of 20 to 500 nM in the root medium, could be detected in the xylem sap. Despite the increase in radial water flow of 180% ABA concentrations in the xylem (c_x^{ABA}) remained constant or even increased (Fig. 5). The observation that after increasing radial water flow J_{Vr}, the ABA concentration in the xylem was never diluted, led to the conclusion that ABA must be transported largely by solvent drag in the apoplast, whereby concentration polarization effects at the endodermis may have led to the increase in concentration as compared with the external concentration. Hence, the dilution effect caused by the water uptake was compensated or even overcompensated (Freundl *et al.*, 1998). A strong correlation between water- and ABA flow also appeared when comparisons were made between maize roots grown in aeroponic culture (with an exodermis) or in hydroponics (roots lacking an exodermis). In the presence of an exodermis, both the transport of ABA, J_{ABA} (Freundl *et al.*, 2000) and the water flow, J_{Vr} (Zimmermann and Steudle, 1998; Zimmermann *et al.*, 2000) were similarly retarded (by factors of 3 and 2–4, respectively, Fig. 6). Consequently, the ABA concentrations in the xylem sap and the reflection coefficient of ABA (σ_{ABA}) appeared to be similar in exodermal and non-exodermal maize roots (Freundl *et al.*, 2000). It appeared that apoplastic ABA-transport provides a

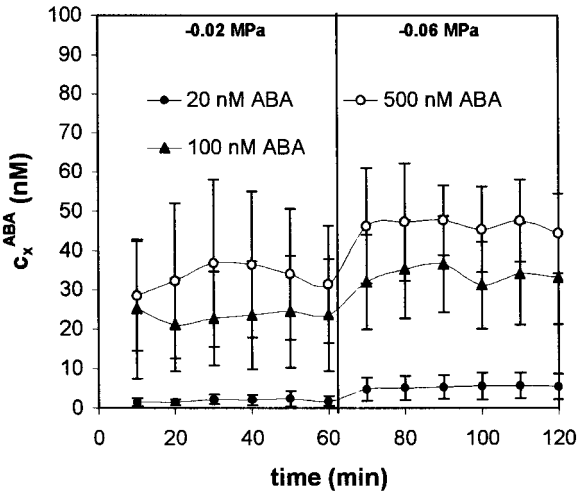

Fig. 5. ABA concentrations in xylem sap (c_x^{ABA}) from 11-d-old maize plants grown in aeroponics. ABA was applied to the nutrient solution at concentrations of 20 nM (-●-), 100 nM (-▲-) and 500 nM (-○-). After increasing the pressure difference from −0.02 MPa to −0.06 MPa, c_x^{ABA} was not diluted; it may have increased. Mean ± SD, $n = 6$–9 roots.

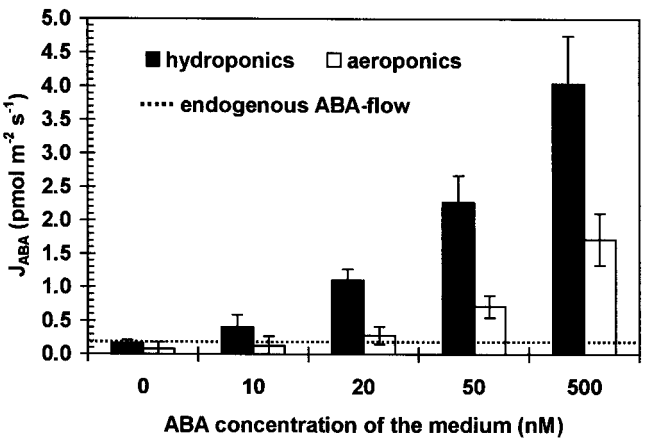

Fig. 6. Flows of ABA (J_{ABA}) driven by a pressure gradient of −0.06 MPa from roots supplied with different ABA concentrations in the medium (0–500 nM) (redrawn from Freundl *et al.*, 2000). J_{ABA} in hydroponically grown maize roots (filled columns) are consistently higher than in aeroponically grown roots (open columns). Mean ± SD, $n = 3$–9 roots.

stress-signal from roots to shoots even in the presence of an exodermis that reduces root Lp_r. Since the apoplastic transport component was coupled to transpiration, the signal is also coupled to transpiration in some kind of a feed forward mechanism.

There were big differences in the apoplastic transport of ABA detected during the investigation of two species, maize and sunflower. ABA concentrations in the xylem sap were much larger in maize (Freundl *et al*., 1998). The origin of the difference between the two species may be related to the fine structure of the roots, namely the porosity of Casparian bands (see above). In the exodermis of sunflower, aliphatic suberin is more abundant (by a factor of 2–3) compared to maize roots grown in aeroponics. This may result in a lower apoplastic flow across the root walls of this species. Thus, the symplastic pathway should dominate the overall radial transport of ABA.

An apoplastic transport of ABA, dependent on the transpiration stream was also found in roots of *Populus tremuloides* (aspen) seedlings (Wan and Zwiazek, 2001). However, at present no data of an aspen root chemistry in relation to transport functions are available.

ABA glucose ester

It has been postulated by different authors that ABA conjugates may act as an additional root-to-shoot signal (Munns and King, 1988; Netting *et al*., 1992; Munns and Sharp, 1993). One of the conjugates was identified as the ABA glucose ester (ABA-GE). Concentrations of ABA glucose esters increased under stress conditions in the xylem sap of sunflower and rice plants (Bano *et al*., 1993, 1994). ABA glucose ester is synthesized in the cytoplasm of root cells, but may also be reabsorbed from the soil solution. According to Sauter and Hartung the ABA glucose ester in the xylem sap comes from the root symplast rather than from the soil, although it could be detected in soil solutions in concentrations of up to 30 nM (Sauter and Hartung, 2000).

The authors showed that ABA-GE is not transported into the xylem apoplastically with the transpiration stream. The suberized parts of root cell walls (endo- and exodermis) appeared to be perfect barriers for the hydrophilic ABA-GE.

Further examples of apoplastic transport

The transpiration stream through the xylem transports cytokinins, as well as ABA, from the root to the shoot. It is of interest to see if there is any evidence of an apoplastic component of the total cytokinin flux from roots (after being transported symplastically up to the more mature part of the roots) that can be related to water flow and solvent drag. There are not many reports on this subject. This may be because of the complex and sophisticated analytical procedures involved in quantitative cytokinin measurement. In order to evaluate the root-to-shoot transport of cytokinins, Beck and Wagner measured the concentration of the principal cytokinin in the xylem sap of *Urtica dioica* (Beck and Wagner, 1994). The cytokinin level tended to increase during the night but fell during the first part of the photoperiod. This sequence could be attributed to a relatively constant rate of release of cytokinin from the root cells leading to an accumulation in the root (apoplast) at night that was swept away when transpiration increased during the day. It was clear, however, that even when transpiration was great in the afternoon a similar concentration of cytokinin was maintained in the xylem sap as was found in the early part of the night when transpiration was low. The authors showed that the release of cytokinin from the root cells was linked directly to the water flow across the root; an almost linear relationship was found between cytokinin flux to the xylem and water flow over the whole range of normal transpiration rates, presumably because dilution of apoplastic cytokinin increased the concentration gradient of cytokinin across the plasma membrane. Such a result clearly implicates an apoplastic flow of cytokinin in the root tissue.

An even clearer demonstration of the maintainence of xylem cytokinin concentration was reported by Dieleman *et al*. in experiments with *Rosa hybrida* (Dieleman *et al*., 1997). Concentrations of zeatin riboside in the bleeding sap of *Rosa hybrida* were not significantly different in the light and dark periods of the diurnal cycle. As with ABA, the close relationship between water- and cytokinin-fluxes to the xylem indicated a substantial apoplastic transport component of the total cytokinin flow.

In a recent paper (White, 2001), that made use of *Arabidopsis thaliana*, a very strong relationship between transpiration and Ca^{2+} fluxes to the shoot was shown. In this species, fluxes across roots showed some remarkable features. Most importantly, there was no competition or interaction between Ca^{2+}, Ba^{2+} and Sr^{2+} transport to the shoot (White *et al*., 2000). The delivery of Ca^{2+} to the leaves was the same in the presence as in the absence of equimolar amounts of the other divalent cations. The author estimates the capacity of Ca^{2+} channels and pumps and concludes that they were not sufficient to provide the amounts of Ca^{2+} found in the shoot. Hence, a substantial apoplastic bypass flow was postulated. The conclusion was supported by the absence of a saturable Ca^{2+} transport which would be expected if a transport protein were involved. These results (White, 2001) are at variance with those of other authors. If they apply in other species, they would change the current picture of the independent movement of water and nutrients in roots which is some kind of a dogma in root physiology.

In earlier literature, Lazeroff and Pitman, working with *Hordeum vulgare*, also described significant effects of

transpiration on Ca^{2+} flows to the shoot, but the effects were not as extreme as those observed in *A. thaliana* (Lazeroff and Pitman, 1966). Some caution is necessary. From a study of 21 species and varieties of common soil compost-grown experimental plants, some of which develop exodermae and some that do not, Atkinson *et al.* concluded that, neither Ca^{2+} uptake nor its distribution was closely related to water uptake or transpiration (Atkinson *et al.*, 1992). The result emphasizes that there can be significant differences in the relative importance of apoplastic flows of Ca^{2+} into the xylem that depend on the anatomy of roots of a given species, and on the culture conditions.

In *Zea mays*, careful manipulation of water fluxes by controlling rates of leaf growth, and by adding osmotica to the solution bathing excised root systems showed that water fluxes into the xylem could be reduced to a greater extent than Ca^{2+} fluxes leading to a significant increase in the $[Ca^{2+}]$ of the xylem sap (Engels, 1999). In these experiments, there was no support for the idea of substantial Ca^{2+} fluxes across the endodermal apoplast. On the contrary, a case was made for a metabolic regulation of Ca^{2+}.

The findings of White (White, 2001) are at variance to results obtained with the root pressure probe that have been obtained in the past two decades (Steudle *et al.*, 1987; Steudle and Frensch, 1996; Steudle and Peterson, 1998; Steudle, 2000*a*). The results indicate that roots behave like osmometers although they are not as perfect as cells, and allow some leakage of solutes such as nutrient salts. This, however, does not seriously compromise the function of roots in taking up nutrients and keeping them within the stele. In this picture, the stress hormone ABA plays a special role in that a substantial apoplastic bypass is possible (up to 10% of the ABA concentration offered to the root medium was dragged into the xylem with the water). This is comparable to the contribution of apoplastic transport of NaCl found by Flowers and co-workers for rice (Yeo *et al.*, 1987; Yadav *et al.*, 1996), but is much larger than that given for the apoplastic tracer PTS which bears three negative charges (see below; Zimmermann and Steudle, 1998). The physicochemical basis of these differences in selectivity are not yet understood. They should be found in the chemical composition and fine structure of apoplastic barriers (see above).

The transport of silicon (silicate) from roots to shoots is important for the structure of many leaves and their trichomes. As with the phytohormones discussed above, it is clear that there are both symplastic and apoplastic components of the total flux into the root xylem. Experimental work shows that there are important species-dependent differences in the balance between the two pathways. The clearest case where apoplastic transport dominates the overall transport is in *Triticum aestivum* (wheat) where the seemingly direct relationship between transpiration rate and silica deposits in leaves was proposed as a basis for integrating the long-term water use by leaves (Hutton and Norrish, 1974). The distribution of silica within the aerial part of the plant is also determined by the transpiration rate of organs (Jones and Handreck, 1965). In roots of *Sorghum bicolor* that had been grown in hydroponics, substantial opaline deposits of silica were found in the walls of endodermal cells external to the Casparian band, suggesting an ultrafiltration of apoplastically moving silica/silicate (Hodson and Sangster, 1989). Similar, but less pronounced silica deposits were recorded exclusively in the endodermis in seminal roots of *Oryza sativa* L., rice (Lux *et al.*, 1999). In neither *Sorghum* nor *Oryza* were silica deposits seen in the exodermis. This implies that in the zones examined (up to 200 mm from the root tip in *Oryza*) the exodermis did not have the same ultrafiltration properties for silica as the endodermis.

In rice, which is relatively sensitive to salinity, a considerable apoplastic contribution to the Na^+-flux to the shoot was observed previously (Yeo *et al.*, 1987; Yadav *et al.*, 1996). They found great variations between individual plants but the Na^+-flux to the shoot was always well correlated with the flux of the apoplastic tracer PTS (tri-sodium-8-hydroxy-1,3,6,-pyrenesulphonate) suggesting that there are apoplastic bypasses despite the well-developed exodermis in this species. Most interestingly, plants grown with supplementary silicate grew better at higher levels of salinity which correlated with a reduced uptake of sodium (Yeo *et al.*, 1999). This suggested to the authors that colloidal precipitates of silica in the apoplast (apoplastic barriers?) could have reduced its permeability, perhaps, by blocking off pores. Plants were grown, however, in well-aerated nutrient solution and it is possible that the variation was due to accidental opening of apoplastic bypasses due to the penetration of the root cylinder by lateral roots (Peterson and Moon, 1993). In maize this should have major effects on root pressure development and reflection coefficients of solutes (Miller, 1985; Steudle *et al.*, 1987; Steudle and Peterson, 1998; Henzler *et al.*, 1999).

Sorbitol

The exodermis provides a major resistance to hydrophilic substances that are not actively transported into cells (e.g. sorbitol), not only during transpiration, when they may be moved apoplastically by solvent drag, but also under non-transpiring conditions. Sorbitol absorption by excised lengths of maize root (the apical 100 mm) that had an exodermis (aeroponically cultured) was lower by a factor of four compared to similar lengths of roots grown in hydroponics that lacked an exodermis (Fig. 7).

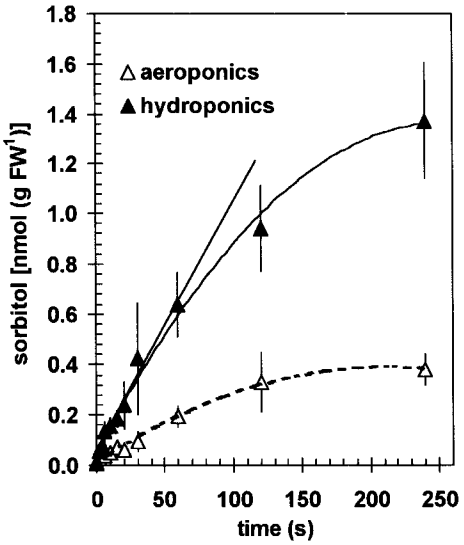

Fig. 7. Time-course of uptake of [^{14}C]-sorbitol into apical (100 mm) lengths of maize plants grown hydroponically (-▲-) or aeroponically (-△-). The cut ends of the roots were sealed with paraffin. The pH of the medium was 5.5 and it contained 10 μM sorbitol. The net uptake rate of sorbitol was calculated from the initial linear phase. Mean ± SD, $n = 4$ roots.

Dyes

The diffusion of fluorochromes, such as PTS or berberine hemisulphate, that can be transported only apoplastically, is restricted by the occurrence of both an endo- and an exodermis (Peterson *et al*., 1978, 1980; Peterson, 1988; Moon *et al*., 1986; Enstone and Peterson, 1992*a*, *b*).

Different authors have used PTS or berberine as a tracer for apoplastic water flow, based on the assumption that they are as mobile as water in the cell walls, including their lipophilic barriers (Hülsbruch, 1944; Hanson *et al*., 1985; Skinner and Radin, 1994). Zimmermann and Steudle, however, concluded that PTS dragged along with the water in the apoplast to the xylem does not trace the flow of water within the root apoplast (Zimmermann and Steudle, 1998).

Aloni *et al*. found that, for different dicotyledonous and monocotyledonous species under transpiring conditions, some berberine was moved across the cortex by solvent drag, but did not pass the endodermis (Aloni *et al*., 1998). While such tracers may give some impression of where water is flowing in a tissues they are unlikely to give a real picture. They are certainly not quantitative since their transport rate in the apoplast is considerably slower than that of water (Aloni *et al*., 1998; Zimmermann and Steudle, 1998).

The exodermis of roots imposes a barrier to solutes that enter the root apoplast from the soil solution mainly due to the solvent drag of the water stream under transpiring conditions. Solutes that are too hydrophilic or have a molecule size that is too big to pass through the interfibrillar spaces of exodermal apoplastic barriers are confronted with a perfect filter. For solutes that can enter cells by diffusion or by an active transport mechanism and are then transported mainly symplastically, the suberin lamellae of the exodermis and endodermis will, at least, reduce the usable membrane surface for uptake or slow down the access to that surface.

The exodermis—a protection against loss

Under natural conditions, especially when no hydrostatic gradient induces a transpiration stream through the plant, the exodermis seems well designed to prevent loss of water and stored solutes from the root tissue into the soil solution.

Loss of water

Under stress conditions, when stomata are closed, water will not move into the root by a hydrostatic pressure gradient, although some water uptake may occur due to solute deposition in the xylem and the development of root pressure. On the contrary, if the water potential of the soil is lower than that of the roots, water can be lost to the rhizosphere by diffusion. This process plays a role during hydraulic lift when water absorbed at depth is released at night into dry layers of soil near the surface. Direct evidence of hydraulic lift was provided by Caldwell *et al*. who found that heavy water absorbed deep in the soil profile by the roots of *Artemisa tridendata* was released in the upper soil profile and was reabsorbed in measurable amounts by shallow-rooted *Agropyron* species (Caldwell *et al*., 1991). Further references to this phenomenon can be found (Tyree *et al*., 1995; Tinker and Nye, 2000).

Taleisnik *et al*. grew different plant species under conditions of water shortage (Taleisnik *et al*., 1999). They compared the ability of roots to retain water by plants which have an exodermis (e.g. *Zea mays*, *Helianthus annuus*, *Allium cepa*) with those which do not (*Pisum sativum*, *Vicia faba*, wheat). Rates of water loss were slower in exodermal than in non-exodermal roots. Hose measured water loss from initially turgid, 40 mm long apical portions of roots from maize cultivated in aeroponics and hydroponics, as well as 60 mm long maize root segments cut 50 mm above the root tip (Hose, 2000). Cut ends were sealed with paraffin wax. The loss of root water into the air was determined by measuring the relative water content (*RWC*) of the root tissue at 25 °C and 60% relative humidity. Root apical portions and hydroponic root segments that did not develop a complete exodermis lost 90% of root water within 75 min. Exodermal, aeroponic maize root segments retained more than 50% of their root water after 75 min of air-drying (Fig. 8). Thus, it was concluded that the exodermis can limit water loss into the rhizosphere. As well as providing a significant resistance to water uptake under transpiring

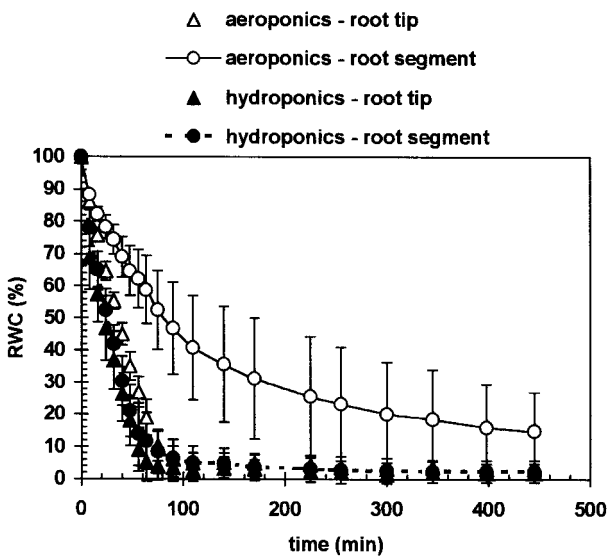

Fig. 8. Relative water content (*RWC*) of 40 mm long root tips (△, ▲) and root segments (○, ●; 50 mm up to 110 mm above the root tip) from maize primary roots grown hydroponically (filled symbols) and aeroponically (open symbols). Air drying started at time 0. Mean ± SD, $n = 3$ roots.

conditions, water loss from the root tissue involves both the 'cell-to-cell'-pathway and a significant apoplastic transport component (Peterson *et al.*, 1993; Steudle *et al.*, 1993; Steudle and Frensch, 1996; Taleisnik *et al.*, 1999). Hence the exodermis is able to protect the root tissue from damage during severe water shortage without eliminating water loss altogether. Regulated hydraulic lift will secure the physiological functions of the root and the whole plant in a drought environment by making mineral nutrients available for absorption (Caldwell *et al.*, 1991).

Loss of ABA

In roots, ABA is synthesized in the cytosol of all root cells but the strongest capacity is in the non-vacuolated cells of the root tip. According to the model of Slovik and Hartung, ABA is then distributed between the apoplast and the symplast according to the difference in pH between the two compartments (Slovik and Hartung, 1992*a*, *b, c*; Slovik *et al.*, 1995).

If stomata are closed, ABA in the cell walls will not be dragged into the xylem vessels with the transpirational water stream. In such circumstances, it could be lost to the rhizosphere by diffusion. Loss of ABA into the soil solution would have a large influence on the hormone concentrations in all parts of the plant, as predicted by model calculations (Slovik *et al.*, 1995). Formation of an exodermis, however, might reduce the leakage of ABA out of the cell walls into the rhizosphere.

Hose performed an efflux compartmental analysis for ABA (as described earlier by Behl and Hartung, 1987) in 60 mm long root segments (50 mm up to 110 mm above the root tip) from maize plants cultivated either in aeroponics or in hydroponics (Hose, 2000). The ends of the segments were sealed with paraffin to eliminate solute exchange at the cut surfaces. Root segments from aeroponically grown maize had developed a complete exodermis, while hydroponically-grown roots had not. The amount of suberin in the isolated hypodermis from whole root segments with an exodermis was greater, by a factor of 1.5, than from segments of non-exodermal roots. The size of the apoplastic compartment was smaller by a factor of 0.27 in roots with an exodermis compared to non-exodermal roots. The compartment derived from the wash-out kinetics estimates the volume within the cell wall that is freely accessible to external ABA (Fig. 9).

In an earlier study (Peterson, 1987), a ratio of the freely accessible cell wall volume for SO_4^{2-} was determined in sealed onion roots with an exodermis compared to unsealed cortical tissue in which the stele was removed (and the endodermis disrupted). This ratio was found to be between 0.16 to 0.26. Peterson also determined that the ratio of cell wall surface in onion roots outside to inside the exodermal Casparian band is 0.25 to 0.26. This suggests strongly that the freely accessible apoplast for SO_4^{2-} is restricted to the cell volume exterior to the exodermis. Peterson concluded, therefore, that a hypodermal Casparian band is an impermeable barrier for SO_4^{2-}, a conclusion that implies that SO_4^{2-} uptake by onion roots occurs at the root periphery followed by radial transport in the symplast (Peterson, 1987).

Degenhardt *et al.* examined the ABA concentration in the soil solution extracted from soil around *Zea mays* and *Vicia faba* seedlings grown in an alkaline substrate (municipal solid waste bottom slag; pH of the soil solution was 8.0) (Degenhardt *et al.*, 2001). According to the anion trap mechanism, the weak acid ABA should be trapped in the alkaline soil solution if there is no barrier retaining it within the root tissue. Roots of *Vicia faba*, unlike those of maize, do not develop an exodermis even in soil culture (Perumalla *et al.*, 1990; Degenhardt *et al.*, 2001). They released more ABA into the soil solution than exodermal maize roots. ABA concentration in the rhizosphere of *Vicia faba* was greater by factors of 3 to 6 (Degenhardt *et al.*, 2001).

Loss of preloaded sorbitol

Compartmental wash out analysis has been done with maize roots loaded with [^{14}C]-sorbitol (Hose, 2000). Differences were found in the size of the apoplastic compartment in exodermal, aeroponic root segments and in non-exodermal, hydroponic root segments. Sorbitol can be regarded as a good tracer for the apoplast because its membrane permeability is very low. A puzzling anomaly in the wash-out curves for both sorbitol (Fig. 10) and ABA occurred with aeroponically grown roots (with

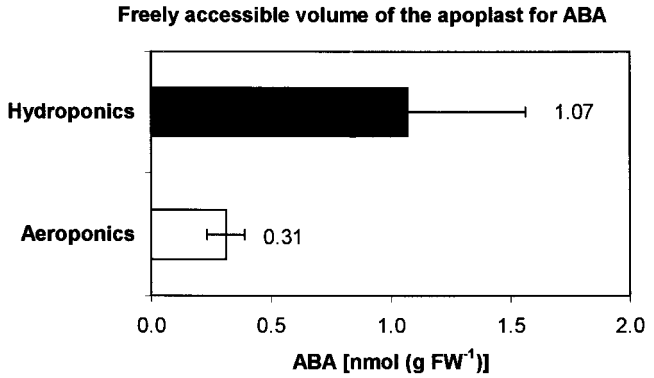

Fig. 9. Freely accessible volume of the apoplastic compartment in non-exodermal (hydroponics) and exodermal (aeroponics) maize root segments for ABA. Mean ± SD, $n = 3$ roots.

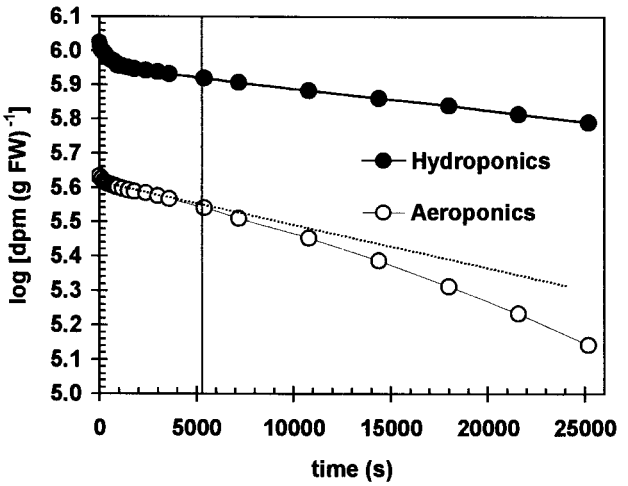

Fig. 10. Typical efflux curves of maize root segments for sorbitol grown hydroponically (-○-) and aeroponically (-●-). Sorbitol-concentration in the medium was 10 μM.

exodermis). After 5000 s the rate of tracer loss began to increase and continued to speed up for the following 6 h. This behaviour was not seen in hydroponically grown roots lacking an exodermis. It appeared as if the barrier properties of the exodermis were great at first but, as the period after excision increased, the permeability of the barrier to sorbitol and ABA also increased, releasing material from the interior of the root. This cannot be explained mechanistically at present.

The freely accessible apoplastic volume for sorbitol in maize roots was smaller than that for ABA by factors of 7 and 10 for roots grown in aeroponics and in hydroponics, respectively. This is probably related to the much more lipophilic nature of the protonated ABA molecule that can pass through pores with hydrophobic linings and can be taken up subsequently into the symplast. A comparison of the size of the apoplastic compartment for sorbitol between roots from aeroponics and hydroponics showed that the ratio of exodermal roots to non-exodermal roots was only 0.18 (Fig. 11), indicating that

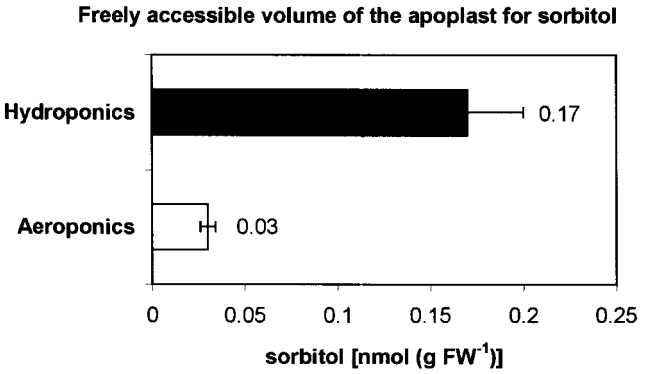

Fig. 11. Freely accessible volume of the apoplastic compartment in non-exodermal (hydroponics) and exodermal (aeroponics) maize root segments for sorbitol. Mean ± SD, $n = 3$ roots.

the exodermal Casparian band of maize strongly impedes the diffusion of apoplastic solutes such as preloaded sorbitol from the root tissue into the rhizosphere.

The presence of a hypodermal barrier will, therefore, minimize losses of phytohormones, water and other substances to the surroundings by diffusion, while not eliminating them completely, and thus maintain a greater apoplastic concentration particularly when the flows of water through the apoplast are low, as would be the case in a root in drying soil. For ABA, this would be of importance in the later phases of the response to drought when stomata are closed. Restriction of outward diffusion would maintain the strength of the root ABA signal. In the early phase of the perception of stress, when the roots release ABA but the stomata are still open, ABA would move preferentially to the xylem by solvent drag in the transpiration stream. In such circumstances, the barrier properties of the exodermis are less important.

Adaptations of the hypodermis to unfavourable environmental conditions

Root systems have developed a variety of strategies that enable them to react to stressful changes in their environment. A number of studies have shown alterations of root architecture and structure induced by different unfavourable conditions (Neumann *et al.*, 1994; Shannon *et al.*, 1994; Setia and Bala, 1994; Tsegaye and Mullins, 1994; Plaut *et al.*, 1997). In addition to changes in external root morphology there are also changes in the fine structure of the root.

When the basal 50 mm of hydroponically-grown maize roots were exposed to damp air for 3–5 d, but not desiccated, the permeability of the exodermis to water and to ^{86}Rb (a tracer for K^+) was decreased by an order of magnitude relative to old parts of the root that were only a few mm away from the impermeable zone, but that had remained immersed in the nutrient solution (Clarkson *et al.*, 1987). The results have been confirmed and extended more recently (Enstone and Peterson, 1998).

It is suggested that the signal for the change in permeability might be the increased oxygen partial pressure. Adventitious roots of maize and the prop roots of other species (Gill and Tomlinson, 1975) often develop in the air and it must be important to minimize the risk of desiccation.

It has been shown that salinity promotes suberization of the hypodermis and endodermis paralleled by a development of the Casparian strip closer to the root tip than in non-saline roots (Shannon *et al*., 1994). In cotton seedling roots, the formation of an exodermis can be induced by salinity (Reinhardt and Rost, 1995). The effects of different external stress factors like salt stress (NaCl), osmotic stress (PEG) and heavy metals stress (Cd) on the chemical composition of exodermal cell walls of hydroponically grown corn showed that the qualitative composition of the exodermal suberin and lignin was not altered (Zeier, 1998). But in all cases an 1.5–3-fold increase in absolute amounts of suberin and lignin was observed relative to the control. Increased rates of suberization and lignification in the exodermis may lead to a greater resistance to the entry of materials into the root apoplast.

Degenhardt and Gimmler used municipial solid waste bottom slag to grow maize under various abiotic stresses (high pH, high salt and high heavy metal content) (Degenhardt and Gimmler, 2000). They analysed the structural and chemical adaptations of the cell wall of various root tissues. Slag-grown plants had higher amounts of lignin in endodermal cell walls when compared to control plants and a higher proportion of H-type lignin in the cell walls of the hypodermis. However, the amount of aliphatic suberin in both endo- and hypodermal cell walls was not affected by growing plants on slag. In maize roots grown hydroponically or aeroponically, suberin amounts in the hypodermis were raised by factors of 1.2 to 5 (Zeier *et al*., 1999*b*; Zimmermann *et al*., 2000; Hose, 2000). The higher amount of suberin in the hypodermis of soil-grown roots compared to roots from a hydroponic or aeroponic system points to the likelihood of the exodermis, in natural conditions, imposing a major limiting resistance to entry into the root apoplast. The incrustation of the cell walls with lignin is reported to provide mechanical stability for root architecture. The tight binding of lignin to the cellulose-fibrils gives stabilizing properties to the cell walls (Richter, 1996). H-enriched lignin is regarded as a response to stress (Monties and Chalet, 1992) but is also associated with compression wood in gymnosperms (Campbell and Sederoff, 1996). A higher proportion of H-units produces a more condensed lignin because of more inter-monomeric C–C linkages.

Such functional adaptations of the hypodermis are not envisaged as a sealing off of the root tissue to the external solution, because lignin is not an absolute barrier to water or even to fluorochromes (Degenhardt and Gimmler, 2000). The hydroxyphenyl components of the hypodermis will contribute to the hardening of the cell walls. The roots responded to cultivation in slag with mechanical strengthening.

Changes in the effectiveness of apoplastic barriers may be induced by phytohormones. It was found that a 2.5-fold increase in exodermal suberization was induced in corn roots when ABA was applied externally in the hydroponic solution. Kinetin and IAA did not have an effect on the suberization (Zeier, 1998). This result highlights the important role of ABA as a stress hormone mediating between external stress factors as they were described above and the reaction of the plant. This relationship between ABA and an enhanced suberization has also been described in biochemical studies investigating the formation of wound suberin in potato slices (Soliday *et al*., 1978). Application of ABA led to increased rates of suberization which, in turn, significantly reduced rates of water loss from the potato slices. Thus, ABA seems to be an important trigger in suberization in plants.

Conclusions

From the reviewed literature, it is clear that the exodermis represents a barrier of variable resistance to the flow of both water and nutrients to internal root cells and conducting elements. Variability is brought about largely by changes in structure and anatomy of developing roots, and these changes are frequently seen in the exodermis. The extent and rate at which its walls become suberized respond to stress conditions such as drought, aeration, heavy metal or nutrient stresses. As Casparian bands and suberin lamellae form in the exodermis, permeability is reduced, but rarely reduced to zero. Passive selectivity may be increased, and, under these conditions, the cell-to-cell path for both water and solutes becomes more important, relative to the apoplastic bypasses that exist in the exodermis. These bypasses do not compromise seriously the semi-permeable nature of the root system. There is considerable evidence that the root still behaves like an osmometer, but its 'passive selectivity' (as expressed by the reflection coefficient) is less than that of, for example, cells which behave like nearly perfect osmometers. The composite transport model of the root established by Steudle and co-workers implies that the passive selectivity of roots can be relatively low in some circumstances (reflection coefficients smaller than unity). Recent findings that roots have a very low selectivity for nutrient ions such as Ca^{2+} are at variance with earlier and recent findings, and need to be checked.

Root selectivity should depend on the permeability of apoplastic barriers; where there are frequent bypass

channels, selectivity will obviously be weak. Experimentally, it is difficult to make unequivocal measurements of these bypasses. In this review attention has been drawn to the uncertainty about the actual porosity of apoplastic barriers such as Casparian bands itself and the suberin in the walls of the exodermis. There is some suggestion that what one can simply observe in the electron microscope, concerning suberin lamellae, does not tell the whole story as far as the permeability of the exodermal walls is concerned (Clarkson *et al*., 1987). If one takes the conventional view about the impermeability of suberin lamellae, it is hard to see how anything can enter the apoplast for most of the length, say, of a maize root growing in soil. One's instincts, if nothing else, suggest that such a design would not serve the purposes of an absorbing structure well. Apoplastic bypasses are implicated in the data on the permeability of maize roots to ABA and water. They indicate a substantial coupling (solvent drag) between the flows of water and solutes in roots. As the exodermis develops, both the water and solute flow are reduced, but are by no means eliminated. Nevertheless, for ABA, the roles of the exo- and endodermis are less pronounced in retaining ABA than one would expect by comparison with, for example, the apoplastic dye PTS. A more pronounced reduction of solute permeability in the presence of an exodermis has been obtained during elution of hydrophilic organic compounds from the root apoplast. The differential behaviour of ABA and sorbitol points to the importance of the chemical nature of the solute (polarity, size) as well as the porosity of the barrier in the determination of what will pass across it and at what rate. Broad generalizations about the barrier properties of the exodermis are unwise in the face of rather small amounts of experimental evidence. It is most important to realize that most descriptions of the functional anatomy of roots have come from main root axes, and yet in a 4-week-old cereal plant (e.g. barley) more than 85% of the root length is made up from laterals (Russell and Clarkson, 1976). Clearly, the focus of studies of the apoplast needs to be widened to include these. In particular, it is important to learn whether or not stressful conditions in the soil affect main axes and branch roots in the same way.

Measurements of the chemical composition of apoplastic barriers in the exodermis of corn roots indicate a significant increase of aliphatic suberin that correlates with changes in transport properties (root hydraulic conductivity). In the presence of an exodermis, water flow is substantially reduced. More detailed data are required to correlate changes in chemical composition with those in transport.

In wetland plants, the exodermis and additional fibre tissue in the outer parts of the root function as a barrier for oxygen diffusion out of the aerenchyma into the surroundings. This may cause problems, however, with the water uptake because the exodermis lowers the hydraulic conductivity of the root, as in rice. The problems are similar to those encountered in reconciling water loss with inward diffusion of CO_2 across leaf surfaces; some effective working compromise has to be reached that will balance inflows and outflows at an optimal level.

Overall, the interacting elements of the root anatomy need to be accounted for in any transport model of material flows across the root exodermis. The composite transport model (Steudle and Peterson, 1998) attempts to do this and points to the importance of switching between the pathways through which transport occurs. Switching may depend on physiological and environmental constraints as well as on driving forces. It may be thought of as analogous to the opening and closing of stomata that optimize transpiration and gas exchange. In the physical switching of root hydraulics, the existence of gradients in hydrostatic pressure play an important role. These are provided by transpiration according to the cohesion–tension theory and allow for a supply of water to the shoot according to demand (Steudle, 2000*a*, 2001). Roots do not have any obvious counterpart to the stomatal guard cells. The model incorporates the possibility that both the transport capacity of the apoplast and of the cellular path can be modified. The strategy of plants appears to be 3-fold. It may involve (i) the induction or repression of the transport capacity of root membranes, for instance by changes in the activity of aquaporins or nutrient transporters, (ii) the constriction of the apoplast pathway by hydrophobic barriers, or (iii) simply the promotion of root growth to increase its surface area. Each of these processes has a different time scale, but only the first of them is likely to be reversible.

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