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Root Endodermis and Exodermis: Structure, Function, and Responses to the Environment

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ABSTRACT

Roots of virtually all vascular plants have an endodermis with a Casparian band, and the majority of angiosperm roots tested also have an exodermis with a Casparian band. Both the endodermis and exodermis may develop suberin lamellae and thick, tertiary walls. Each of these wall modifications has its own function(s). The endodermal Casparian band prevents the unimpeded movement of apoplastic substances into the stele and also prevents the backflow of ions that have moved into the stele symplastically and then were released into its apoplast. In roots with a mature exodermis, the barrier to apoplastic inflow of ions occurs near the root surface, but prevention of backflow of ions from the stele remains a function of the endodermis. The suberin lamellae protect against pathogen invasion and possibly root drying during times of stress. Tertiary walls of the endodermis and exodermis are believed to function in mechanical support of the root, but this idea remains to be tested. During stress, root growth rates decline, and the endodermis and exodermis develop closer to the root tip. In two cases, stress is known to induce the formation of an exodermis, and in several other cases to accelerate the development of both the exodermis and endodermis. The responses of the endodermis and exodermis to drought, exposure to moist air, flooding, salinity, ion deficiency, acidity, and mechanical impedance are discussed.

Key words: Endodermis; Exodermis; Structure; Drought; Mycorrhizae; Casparian bands; Suberin lamellae

INTRODUCTION

Plant roots have two ''physiological sheaths'' that play important roles in basic root function and protection against stresses. These sheaths are the endodermis and exodermis. Some fundamental information regarding these layers has been known for a long time. However, it is only recently that significant new insights regarding their structure, chemistry, and functions have come to light. Recent publications attest to the effect of environmental conditions on these layers. Currently the foundation is being laid for an understanding of their roles during times of stress, and research in this area will have practical applications in plant resistance to drought, pathogens, organic contaminants, heavy metals, and possibly salinity. Details of permeability of the exodermis have been reviewed recently

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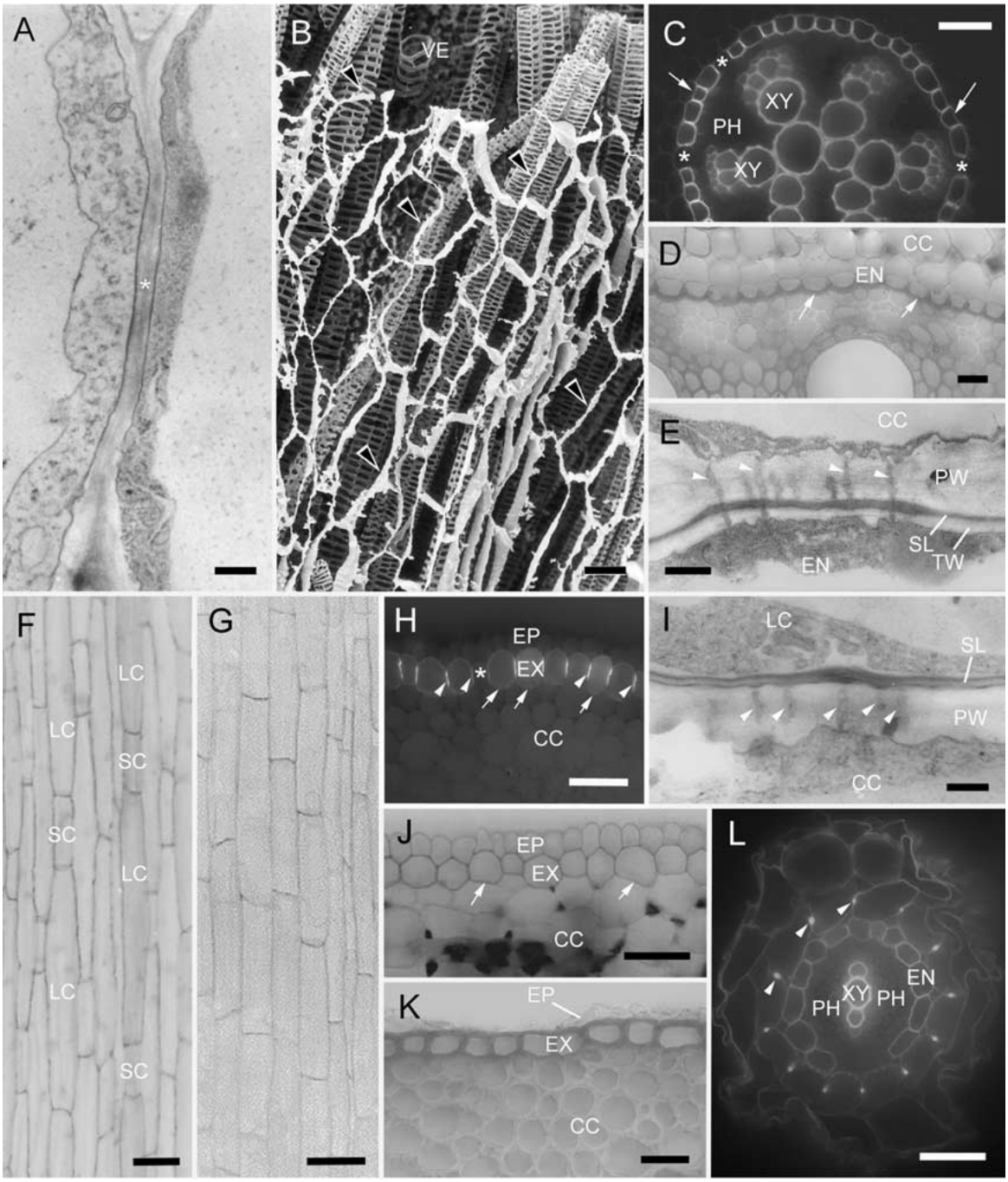

(Hose and others 2001); the present will focus primarily on ion uptake, structure-function relationships, and environmental responses.

Structure and Function

The root endodermis and exodermis are structurally specialized layers. By definition, their cells possess Casparian bands and may also develop suberin lamellae and thickened, tertiary walls. The endodermis and exodermis each have several known functions.

Endodermis

All vascular plants normally develop an endodermis in their roots, an exception being *Lycopodium* (Damus and others 1997). The endodermis, derived

Figure 1. Structure of root endodermis and exodermis. Cross-sectional views of tissue unless otherwise indicated. CC, central cortex; EN, endodermis; EP, epidermis; EX, exodermis; LC, long cell; PH, phloem; SC, short cell; VE, vessel; XY, xylem. Bars = 0.5 μm (**A**, **E** and **I**) or 50 μm (the rest). (**A**) Casparian band (asterisk) in the endodermis of onion root. Transmission electron micrograph. Tissue fixed with glutaraldehyde-acrolein and osmium tetroxide and stained with uranyl and lead. (**B**) A 'fish-net' of endodermal Casparian bands (arrowheads) enzymatically isolated from a *Clivia miniata* root. Secondary walls of xylem vessels (VE) are also retained. Digested whole mount observed with a scanning electron microscope. (Photo courtesy Dr. Lukas Schreiber) (**C**) Onion root endodermal cells have formed suberin lamellae (arrows) except passage cells (asterisks). Autofluorescence observed with UV illumination. (**D**) Tertiary walls (arrows) in the endodermis of maize nodal root. Much heavier thickening at the inner tangential side than at the outer tangential side. Plant grown in the field and sample taken near the soil surface of a root. Tissue treated with phloroglucinol-HCl for lignin. (**E**) Endodermal cell from onion root. Suberin lamellae (SL) and tertiary wall (TW) are present internal to the primary wall (PW). Plasmodesmata (arrowheads) remain intact. Some plasmodesmata are not included as whole in this section and thus appear truncated. (Reproduced from Ma and Peterson 2000 with permission from Protoplasma). (**F**) Longitudinal view of dimorphic exodermis of onion root. Short cells alternate with long cells. Negative image of a fluorescence micrograph. Root segment treated with 50% sulphuric acid for 12 h and the exodermis-epidermis isolated. Photo made with the autofluorescence from the exodermal cell walls. (**G**) Longitudinal view of uniform exodermis from maize root. Cells are all elongate and are similar to each other. Negative image of a fluorescence micrograph. Section observed under UV illumination. (**H**) Onion root exodermal long cells have both Casparian bands (arrowheads) and suberin lamellae (arrows). Short cell (asterisk) does not have suberin lamellae. Section stained with berberine-aniline blue and observed with a fluorescence microscope. (**I**) Exodermal long cell in onion root has developed suberin lamellae (SL) which severed plasmodesmata (arrowheads). (Reproduced from Ma and Peterson 2000 with permission from Protoplasma). (**J**) Exodermis of maize root (3 weeks old, 150 mm long), with suberin lamellae (arrows). Section stained with Sudan red 7B and observed with bright-field optics. (**K**) Tertiary walls in the exodermis of maize root. Thickening greater at the outer tangential side than at the inner side. Epidermis is dead and collapsed. Photo taken from the same section as in (**D**). (**L**) Phi-thickenings (arrowheads) in the cortical layer external to the endodermis of *Brassica napus* root. These thickenings are localized cellulosic enlargements of primary walls. Autofluorescence was induced with UV illumination.

from the ground meristem, is the innermost layer of the cortex and is characterized by the formation of Casparian bands in the anticlinal walls of its cells.

Casparian Bands. The Casparian band is a wall modification that appears electron-dense in a transmission electron microscope (Figure 1A). This is due to the incrustation of lignin and, to a lesser extent, suberin into the framework of the primary wall (Schreiber and others 1994; Schreiber 1996). The plasma membrane tightly adheres to the wall so that, upon treatment with a hypertonic solution, band plasmolysis occurs in endodermal cells (Bonnett 1968). In most plants, Casparian bands mature within 10 mm of the root tip. They rarely occupy more than 1/3–1/2 of the anticlinal walls. Because endodermal Casparian bands are such a consistent feature of roots, they can be expected to have at least one essential function. This is generally understood to be prevention of the apoplastic passage of ions from the cortex to the stele. The evidence that Casparian bands are, in fact, effective barriers to apoplastic ion movement is widespread and convincing, beginning with the classic work of de Rufz de Lavison in 1910. Since then, the endodermal Casparian band has been shown to block the free apoplastic passage of various ions, heavy metals, and fluorescent dyes (Robards and Robb 1972; Nagahashi and others 1974; Singh and Jacobson 1977; Moon and others 1986; Peterson and others 1981; Peterson 1987; Cholewa 2000; Bücking and others 2002). The consequence of this blockage is that in a healthy root any ion not in the symplast is prevented from entering the root stele and, thus, the shoot system (Figure 2A). The proteins (pumps, carriers, and channels) in the plasmalemmae of the root epidermal and cortical cells are the arbiters of which ions will enter the symplast and move into the stele (Figure 2B, white arrows). The second (and much less studied) control point for determining which ions are delivered to the shoot is the movement from the symplast to the apoplast in the stele (Figure 2B, black arrows). This step is a critical part of the sequence that keeps the concentration of ions low in the root core symplast, setting up the gradient that favors centripetal movement by diffusion through plasmodesmata from the epidermal and cortical cell cytoplasms (Figure 2B). Once the ions have been released into the cell walls of the endodermis, pericycle and stelar parenchyma, they are free to diffuse into the lumena of the tracheary elements (tracheids or vessel members) and so be carried to the shoot in the transpiration stream (Figure 2B). It is assumed that diffusion into the tracheary elements occurs mainly by way of the (nonlignified) pits.

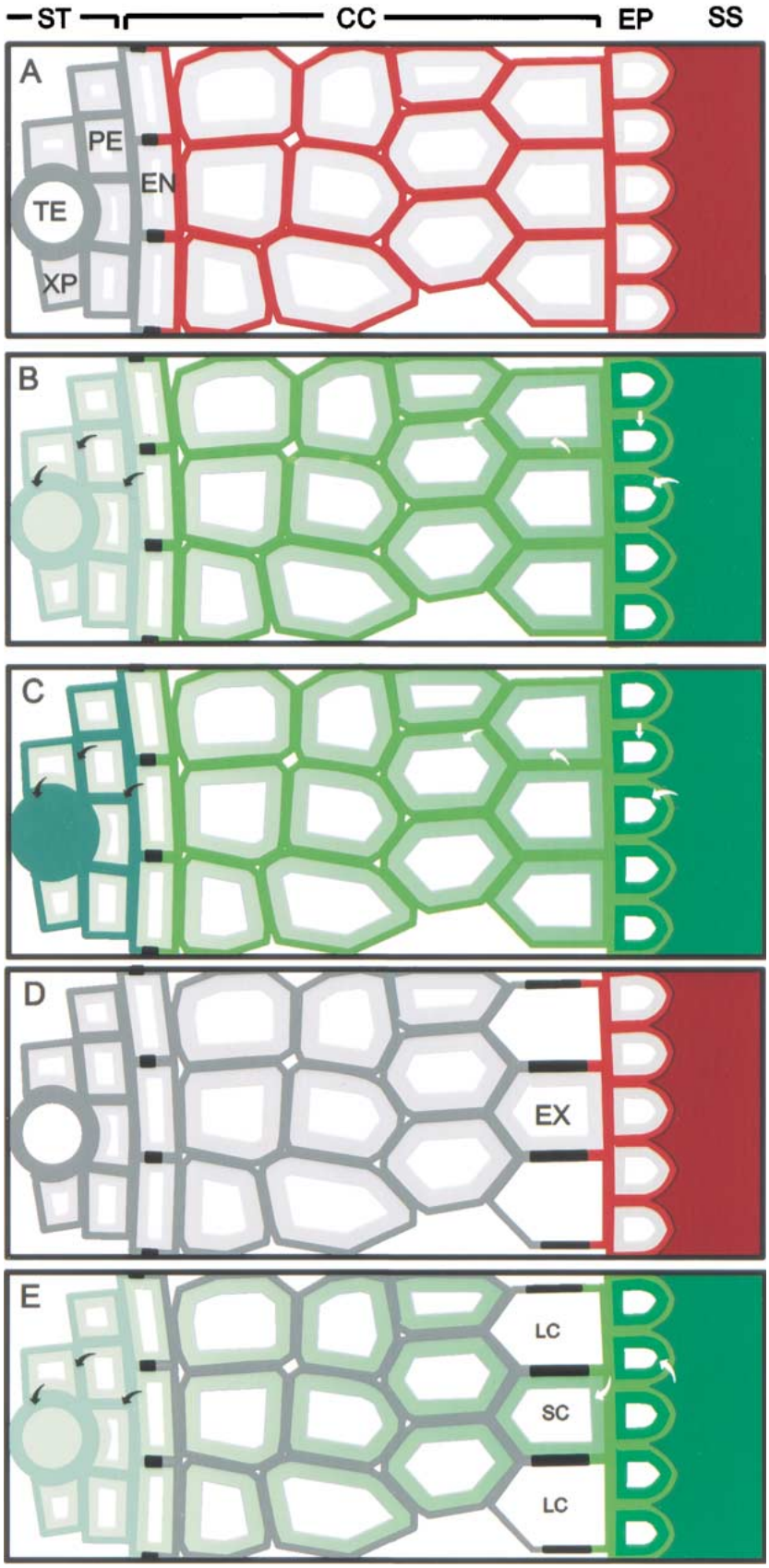

Figure 2. A series of diagrams of partial cross-sections illustrating the movement of apoplastic ions (red) and symplastic ions (green) within roots. Intensity of green signifies ion concentration. ST, stele; CC, central cortex; EP, epidermis; SS, soil solution; TE, tracheary element; XP, xylem parenchyma; PE, pericycle; EN, endodermis; EX, exodermis; LC, long cell; SC, short cell. Casparian bands are indicated by black areas in the walls. Walls not contacted by ions, dark grey; cytoplasm not contacted by ions, light grey; vacuoles and lumena of tracheary elements and mature exodermal long cells not contacted by ions, white. (**A**) Root with a mature endodermis and immature or absent exodermis. Apoplastic ions (red) enter walls of epidermis and cortex. Their farther movement is blocked by the Casparian bands of the endodermis. (**B**) Root with a mature endodermis and immature or absent exodermis. Original plant was transpiring. Symplastic ions (green) initially enter the walls of epidermis and cortex (as in **A**) but also pass (white arrows) through the plasmalemmae into the cytoplasm of the epidermis and cortex. They move symplastically into the stele, where they are shunted (black arrows) from the cytoplasm of endodermis, pericycle, and xylem parenchyma cells into the apoplast. The ions diffuse into the lumen of the tracheary element. Concentrations of ions are low in the lumen due to removal of material by the transpiration stream. (**C**) Root with a mature endodermis and immature or absent exodermis. Original plant was not transpiring. Ion entry into the stele is as in (**B**) but the concentrations of ions are high in the stele because they are not removed by the transpiration stream. Diffusion of the ions out of the apoplast of the stele is prevented by the Casparian bands of the endodermis. (**D**) Root with mature endodermis and exodermis. Apoplastic ions (red) enter the walls of the epidermis and the outer tangential walls of the exodermis. They are prevented from entering the cortex by the Casparian bands of the exodermis. (**E**) Root with a mature, dimorphic exodermis. Symplastic ions (green) enter the walls of the epidermis and outer tangential walls of the exodermis as in (**D**). Ions are taken up (white arrows) into the cytoplasm of epidermal cells and short cells of the exodermis. They are transferred symplastically from the latter into the cortex and then into the stele.

A second role of the Casparian bands of the endodermis, one that is highly beneficial if not essential, is to prevent backflow of ions from the apoplast of the stele to the apoplast of the cortex. When the plant is transpiring, ions are swept out of the root stele and their concentrations in the apoplast are kept low (Taiz and Zeiger 1998). However, under conditions of little or no transpiration, ions may accumulate in the apoplast of the stele faster than they are removed by the transpiration stream. Then the concentration gradient in the apoplast favors diffusion back into the cortex and, ultimately, the soil solution. This backflow is prevented by the Casparian bands of the endodermis (Figure 2C), the ion-trapping function of which has been documented experimentally (Peterson and others 1993). One consequence of ion buildup in the apoplast of the stele is establishment of root pressure. This is known to provide a means of dissolving air embolisms in the xylem conduits and thus helps maintain xylem function (see Tyree and Sperry 1989). There are other examples of situations in plants where backflow in the apoplast is prevented by Casparian band-like structures. These include the nectar secretion system, well studied in *Abutilon megapotamicum* (see Gunning and Hughes 1976), and salt and other glands (Figure 4.10 in Lüttge and Higinbotham 1979). In all cases, material destined for outside the plant is secreted into the apoplast and is prevented from diffusing back into the plant tissue by strategically placed wall modifications

The effect of the endodermis on mycorrhizae is well known if not completely understood. Ectomycorrhizal fungi grow intrusively between the epidermal and cortical cells of the mature root regions by digesting the middle lamella between the cells (see Smith and Read 1977). These fungi are unable to pass the Casparian band and thus are prevented from entering the stele. It is assumed that the hyphae cannot separate the anticlinal walls between adjacent endodermal cells because the wall and middle lamella are impregnated with lignin and suberin. Circumstantial evidence strongly supports this idea. When roots are treated with a strong acid (Priestley and North 1922) or with the wall-degrading enzymes cellulase and pectinase (Schreiber and others 1994), the unmodified walls of the endodermis and neighboring cells disintegrate but the areas of endodermal walls and middle lamellae that house the Casparian band do not, leaving an intact structure that resembles a microscopic fish net (Figure 1B). These results indicate that even enzymes secreted by wall-degrading fungi do not digest the Casparian band and are unable to separate endodermal cells from each other. Thus, it is not surprising that ectomycorrhizal fungi find their passage into the stele blocked. More mysterious is the exclusion of endomycorrhizal hyphae from the stele of the root. These fungi can penetrate cellulosic walls (see Smith and Read 1977) and would seem to be capable of entering the stele through the unmodified tangential walls of the endodermis. Yet for unknown reasons they refrain from doing so.

Suberin Lamellae. In the great majority of plants, suberin lamellae are deposited as secondary walls after Casparian bands are mature in the endodermis. The major biopolymers of the lamellae are suberin and, in lower amounts, lignin (Zeier and others 1999a, b). Suberin lamellae are characterized by their alternating electron-lucent and -dense layers observed by transmission electron microscopy. These layers are hypothesized to consist of a suberin poly(aliphatic) domain and a suberin poly(phenolic) domain, respectively (Bernards 2002). Some endodermal cells near the protoxylem poles that do not develop suberin lamellae are termed ''passage cells'' (Figure 1C). As the root grows older these cells may form suberin lamellae (and sometimes tertiary walls) and thus their number decreases. In very old zones of roots of some species (especially monocotyledonous) there may be a complete lack of passage cells.

Unlike Casparian bands, suberin lamellae are not formed in every root, nor are they formed in every cell of the endodermis in roots where they do develop. Therefore, one can surmise that their function(s) are useful but not essential. Suberin lamellae form a hydrophobic covering around the cell (except in those regions occupied by plasmodesmata) and presumably prevent the uptake of ions from the apoplast into endodermal cells. However, considering the relatively large potentially absorptive surface area of the cortical cell membranes in most species, the loss of this endodermal absorbing surface is theoretically not significant. For example, in a 1 mm segment of onion root, the combined surface area of the membranes of the epidermis and cortex is 91 mm^2 (Kamula and others 1994) whereas the surface area of the endodermal plasmalemma located on the cortical side of the Casparian bands (assuming a stele diameter of 0.29 mm and an anticlinal wall length external to the Casparian band of 4.5 μm) would be 1.2 mm^2. Thus, investment of all endodermal cell membranes with suberin lamellae brings about a loss of only 1% of the total membrane surface area available for absorption. In practice, the actual effect of suberin lamella development on ion uptake by the plant is strongly dependent on the ion under consideration. The transfer of calcium and

magnesium to the stele is severely curtailed by suberin lamellae deposition in barley (*Hordeum vulgare*: Robards and others 1973) and maize (*Zea mays*: Ferguson and Clarkson 1976b). However, the transfer of phosphate in barley (Clarkson and others 1968) and potassium in *Cucurbita pepo* (Harrison-Murray and Clarkson 1973) is not adversely affected. Clearly, it is not possible to generalize about the effects suberin lamellae may have on ion transport. Why should the endodermal suberin lamella exert such strikingly different effects on Ca^{2+} and Mg^{2+} vs $PO_4^{3-}$and K^+ and delivery to the stele? As pointed out by the researchers involved (see also Clarkson 1993), the strong effect of suberin lamella development on Ca^{2+} and Mg^{2+} movement can be explained by assuming a predominantly apoplastic transfer of these ions through the epidermis and central cortex, and uptake into the symplast at the outermost membrane of the endodermis. PO_4^{3-} and K^+, on the other hand, would enter the symplast primarily in cells external to the endodermis and simply continue their transport in this compartment into the stele. It is important to note that the plasmodesmata of the endodermal cells remain intact and functional during this and even later stages of development in all species thus far investigated (Figure 1E) (Clarkson and others 1971; Ma and Peterson 2000; Ma 2000).

Suberin lamellae, as a consequence of their hydrophobicity, should have a major effect on the path of water flow in roots (detailed in Peterson 2003). It is expected that water flow will be routed through the passage cells of the endodermis that, when present, are usually conveniently situated near the xylem poles (Figure 1C) (Peterson and others 1993).

Such a common modification as suberin lamellae would be expected to have positive adaptive functions. These become evident when the root is suffering water shortage or is under attack by pathogens. During drought stress, the epidermis and cortex of the root will die, leaving the endodermis to function as the outermost layer of the root (Clarkson and others 1968; Jupp and Newman 1987). Although the ion-absorbing capabilities of these roots may be minimal (actually this has never been tested), the pericycle and vascular tissues are protected from drying out. When one considers the entire root system in a soil that dries from its upper surface, this modification would allow a continued vascular connection between the shoot and its deeper, absorbing roots. Further, when local conditions improve, the pericycle/endodermis could regenerate new lateral roots for the system. Thus, the plant does not lose its anchorage, its connection to the rest of the root system, or its capacity to regenerate. Roots of some woody species die back to the endodermis in regions of primary growth, even under benign growing conditions (Richards and Considine 1981; McKenzie and Peterson 1995). The protective properties of the endodermis with its Casparian bands and suberin lamellae would also come into play with these roots.

Tertiary Walls

Although many species, particularly woody dicotyledons, do not develop wall modifications beyond suberin lamellae in their endodermis, most monocotyledonous and a few dicotyledonous plants continue to elaborate these walls, making thick, tertiary layers (Figure 1D). The thickening is usually asymmetrical, being thinner on the outer tangential wall than on other walls (see Figure 17.5 in Esau 1965). The major component of tertiary walls is cellulose, which may be impregnated with lignin (Esau 1953). Lignin can also be a major component of last-deposited tertiary wall materials (Scott and Peterson 1979). Suberin is frequently absent or present in extremely low levels (Zeier and Schreiber 1998; Zeier and others 1999a, b).

This wall has been assumed to aid in mechanical support of the root, but this has not been proven (or even tested) experimentally. The compression and tension stresses experienced by shoots due to wind and the unsupported weight of their branches, for example, are less severe in the soil. It may be that the most common stress experienced by most roots is tension due to water stress that would lead to collapse of their tissues.

In all plants examined to date, pits occur in the tertiary walls, and the plasmodesmata located in primary pit fields remain intact and functional (Figure 1E) (Clarkson and others 1971; Ma and Peterson 2001; Ma 2000). Thus, the transfer of ions to the stele through the symplast will continue throughout the life of the endodermis.

Exodermis

The exodermis is a special type of hypodermis that develops Casparian bands in the anticlinal walls of its cells (Peterson and Perumalla 1990). An exodermis can be either dimorphic or uniform (Figure 1F, G). A dimorphic exodermis has long and short passage cells alternate along the root axis, as in *Hoya carnosa* (Olesen 1978) and onion (*Allium cepa*) (von Guttenberg 1968), whereas a uniform exodermis consists of elongate cells that are uniform in shape,

as in maize and sunflower (*Helianthus annuus*) (von Guttenberg 1968). The suberin and lignin contents of the exodermal/epidermal walls are lower than those of the endodermal walls (Schreiber and others 1999; Zeier and others 1999a, b).

Casparian Bands and Suberin Lamellae. An exodermal Casparian band typically spans nearly the entire dimension of an anticlinal wall (Figure 1H). In some species (for example, *Phragmites* and *Typha*) with a multiple exodermis, the tangential walls between the individual layers also develop Casparian bands (Seago and others 1999b; Soukup and others 2002). The overall morphology of the combined Casparian bands is an H-shape as seen in a cross-section of the root. The technique devised by Brundrett and others (1988) has been routinely used to detect Casparian bands.

As in the endodermis, the Casparian band of the exodermis is a barrier to the apoplastic movement of ions and fluorescent dyes (Figure 2D) (Peterson and others 1978, 1982; Peterson 1987; Cholewa 2000; Lehmann and others 2000). Consequently, in roots where the exodermis is mature, the filtration of ions from the soil solution occurs much nearer the root periphery than in cases where the exodermis is absent or immature, and the ions enter the cortical walls. In roots with a mature exodermis, the cells of the central cortex are not exposed to the contents of the soil solution.

The significance of the location of this apoplastic barrier depends on the charge of the ion and on the mechanism of water entry into the apoplast. If it is by diffusion alone and the ions are negatively charged, they should remain in the water free space at the same or lower concentration as in the soil solution (see Sattelmacher 2001). Positively charged ions are concentrated in the Donnan free space by attractions to negative charges of the cell walls (see Sattelmacher 2001). If water moves into the root by bulk flow, it will carry ions with it. These can be screened out at the sites of Casparian bands, and their concentrations increase near the endodermis of nonexodermal species (Aloni and others 1998). Thus, having the screening occur in the exodermis near the root surface prevents the concentration of potentially toxic materials in the cortex.

If the exodermis is performing the ''apoplastic screening'' function for the root, why have species with an exodermis retained an endodermis? One reason is connected with the sequence in which these two layers mature. In a rapidly growing root of most species, the endodermis matures within a few millimeters of the root tip whereas the exodermis matures several centimeters from the tip. Thus, without the endodermis, there would be a substantial ''unprotected'' area of the root. The other major function of the endodermis, that of retaining ions in the stele apoplast, would not be so efficiently performed by the exodermis because of its position near the edge of the root. Without the Casparian band of the endodermis, the apoplastic volume of the cortex would fill with ions, some of which may be reabsorbed by the cortical cells, setting up a futile cycle.

The effect of the Casparian band of the exodermis on the growth (and subsequent localization of mycorrhizal fungi) is similar to that of the endodermis in some respects. Ectomycorrhizal associations are typically a feature of gymnosperms, a group that does not have an exodermis (Damus and others 1997), so the Hartig net will form throughout the cortex. There are, however, a few exodermal angiosperm species with ectomycorrhizae. In these cases, the Hartig net is confined to the epidermis and this tissue can become palisade-like in conformation (Massicotte and others 1987). Endomycorrhizal fungi treat the endodermis and exodermis differently. As indicated above, the hyphae do not grow through the cells of the endodermis, but they do grow through the passage cells of the exodermis (Gallaud 1905; Smith and others 1989; Esnault and others 1994; Imhof and Weber 1997; Matsubara and others 1999) and form their typical arbuscules and vesicles in the central cortex. It has been suggested that the endodermis and exodermis form two borders of an isolated apoplastic system in which ion and nutrient exchanges occur between the mycorrhizal fungus and the cortical cells of the root (Smith and others 1989).

Unlike the endodermis, in which the two processes are temporally and spatially separated, the exodermis develops suberin lamellae during or immediately after the Casparian bands are deposited (Peterson and others 1982; Perumalla and Peterson 1986; Ma and Peterson 2001). In some species (for example, *Typha*: Seago and others 1999b; *Phragmites*: Soukup and others 2002), precursors of the suberin lamellae were found prior to Casparian band development. In the roots studied in detail so far, it seems the root *system* does not completely seal itself off with suberin lamellae. This is achieved by different methods, depending on the type of exodermis. Onion often lacks lateral roots and has a dimorphic exodermis in which initially the long cells make suberin lamellae and the short (passage) cells do not (Figure 1H). Once suberin lamellae are laid down, plasmodesmata are severed (Figure 1I) and the long cells will die (Walker and others 1984; Ma and Peterson 2000). With time, some of the short cells do develop suberin lamellae, but even

when severely drought-stressed, some short cells remain without lamellae (Kamula and others 1994). These cells are among the last to die from extreme and prolonged drought (Stasovski and Peterson 1993). In species with a uniform exodermis such as maize (Figure 1J) and sunflower, the deposition of suberin lamellae is patchy and the exodermis tends to mature late (Kroemer 1903; Enstone and Peterson 1992, 1997). Also, suberin lamellae (and tertiary walls, if present) do not interrupt the exodermal plasmodesmata in rice (*Oryza sativa* L.) (Clark and Harris 1981) and maize (Clarkson and others 1987; Wang and others 1995). Thus, both the dimorphic and uniform exodermis have cells without suberin lamellae. The difference between these two types of exodermis is that in the dimorphic type the cells without lamellae do have Casparian bands, whereas in the uniform type they do not.

A consequence of maturation of the Casparian bands and suberin lamellae in the exodermis is a reduction in the membrane surface area available for ion absorption. Whereas in a nonexodermal root the cells of the epidermis and cortex are potentially involved (Figure 2B, C), in a root with a dimorphic exodermis this area is reduced to the membranes of the epidermis and the outer membrane of the short cells of the exodermis (Figure 2E, white arrows). According to Kamula and others (1994), in onion this reduction is about 84%. In a root with a fully developed uniform exodermis, the absorptive membranes would be further limited to those of the epidermis. This reduction in absorptive surface area seems to be a negative consequence of the maturation of the exodermis. Even ions like $PO_4^{3-}$and K^+ that are taken up by the cells near the root surface (Grunwaldt and others 1979; van Iren and Boers-van der Sluijs 1980; Kochian and Lucas 1983) are affected. In maize, Ferguson and Clarkson (1975) found that the amount of phosphate delivered to the stele was reduced by 77% when suberin lamellae developed, and histoautoradiography indicated that a reduced amount of PO_4^{3-} entered the central cortex (Ferguson and Clarkson 1976a). Calcium delivery to the stele of onion was reduced by 32% by maturation of the exodermis (Cholewa 2000). From the few studies correlating ion uptake with exodermal maturation, it seems that ion uptake is significantly reduced but not eliminated in root regions with either a dimorphic or uniform exodermis. So many other factors are involved with ion uptake (for example, vitality of the cells, numbers and activity of various transport proteins on the membranes of living cells) that such effects cannot be ascribed to only one anatomical feature of the root.

As with the endodermis, positive attributes of the exodermis become apparent under stress conditions. During drought, the epidermis often dies but the cortex does not (Stasovski and Peterson 1993) so that the exodermis becomes the outermost protective layer of the root. As the growth rate of the root declines, the exodermis matures ever closer to the root tip (Perumalla and Peterson 1986; Seago and others 1999b) and can even extend under the root cap when the root has ceased growing (Figures 130 and 131 in Brundrett and others 1990).

Tertiary Walls. In the exodermis of those plants in which the development of suberin lamellae does not sever the plasmodesmata, the cells may remain alive and develop a tertiary wall similar to that of the endodermis (Wang and others 1995). Usually these walls are asymmetric, and their shape and lignification again suggests a mechanically strong layer (Figure 1K). Such a function could be especially important in the exodermis as collapse of the cortex would lead to a loss of contact between the root and soil. An investigation of the mechanical properties of an exodermis with tertiary walls would be a good project for a biophysicist.. Olesen (1978) described interesting wall modifications in the short cells of *Hoya carnosa*. A wall thickening or pad develops from the outer tangential wall and its fine structure reveals small pores. The author proposed that when water is available, these channels open, allowing water into the root, but when water is unavailable they close up and prevent its loss from the root. Such valve-like structures also warrant more investigation.

Death of the Epidermis. In the field, the epidermis often dies and is degraded by microorganisms, leaving few traces behind (Figure 1K) (McCully 1999; Walker and others 1984). This layer also dies even under less than optimal conditions in the laboratory (Barrowclough and Peterson 1994; Enstone and Peterson 1998); then the exodermis becomes the outermost part of the root. Such a root region has little remaining plasmalemma surface area through which to admit ions into the symplast. In onion, maturation of the exodermis reduces the available plasmalemma surface area to 16% of the original; death of the epidermis further reduces it to 0.23% of the original (Kamula and others 1994). In species with a uniform exodermis, in root regions where the exodermis is mature, there would theoretically be no plasmalemma surface area available for ion absorption. Without the protective epidermis, the antimicrobial properties of the exodermis become important in preventing pathogen entry into the root, and the phenolic components of suberin are thought to be especially important in

this regard (see review by Bernards 2002 and references therein).

Effects of Environmental Conditions

In the laboratory, plants are growing under environmental conditions that are very likely to be more uniform and optimal for growth than conditions in nature. A substantial body of evidence is accumulating to show that roots of various species are adapted to their natural habitat, and can accommodate to changes either by altered growth rates and/or by changing the rates of development of critical structures.

Cultural Environments

In addition to a normal decrease in growth rate that often occurs with aging (especially in determinate roots), root systems grown under varying cultural conditions can exhibit significant differences in the development of the endodermal and exodermal layers, including suberization (that is, the formation of suberin lamellae). For example, compared with results obtained in aeroponic culture, maize growing in hydroponic culture exhibited delayed deposition of endodermal and exodermal Casparian bands and suberin lamellae (Zimmermann and Steudle 1998; Zimmermann and others 2000; D.E. Enstone and C.A. Peterson unpubl.). Greater levels of suberization occurred in vermiculite culture compared with either hydroponics or aeroponics (D.E. Enstone and C.A. Peterson unpubl.). On the other hand, in two legume species (lupin and chickpea) there was little difference in the development of the endodermis between hydroponic and aeroponic conditions (Hartung and others 2002). A few endodermal cells formed suberin lamellae opposite phloem fibers (not xylem as claimed in the paper) in aeroponically grown chickpea, and none at all formed in lupin. Legumes are nonexodermal (Perumalla and others 1990) and none of these culture conditions induced an exodermis to develop. The addition of polyethylene glycol 6000 to hydroponic culture in amounts to produce an osmotic potential of −400 kPa greatly reduced the growth rate of maize roots compared with normal hydroponic and vermiculite cultures (Perumalla and Peterson 1986). When root zones of similar age (as determined by extrapolation from presented data) from this study are compared, it is clear that the endo- and exodermal layers matured earlier in the water-stressed treatment.

Localized Environmental Conditions

Root systems of plants growing in the field experience extremely variable microenvironments as they encounter fluctuating levels of water, nutrients, toxins, temperature, and other factors in the soil volume they explore. Most experimental protocols apply a uniform growth condition to the root system and do not take into account the possible effects of localized environmental differences on root anatomical development. There are a handful of studies that look at the effect of microclimate on root system anatomy.

Application of localized environmental conditions in many cases will influence the development of endodermis and exodermis in those zones. Onion root bases not consistently covered by hydroponic solution formed transfer cells with suberin lamellae in the exodermis (Wilson and Robards 1980). Portions of hydroponically growing maize roots elevated above the solution into an enclosed humid chamber suberized both layers while the submerged portions of the root system and that of the controls remained largely unsuberized (Enstone and Peterson 1998). Zonal treatments applied to the mid-root system have provided variable results. In these systems, roots grew through a soil-based potting medium that was divided into 3 layers by water-impermeable but root-penetrable wax barriers. Maize roots formed in either dry or wet middle layers flanked by moist layers showed no difference in endodermal and exodermal development compared with controls (Watt and others 1996). In contrast, the desert native *Agave deserti* growing through a wet middle layer flanked by dry layers had reduced suberization and lignification in the endodermis compared to the endodermis in the dry zones. The only comparison performed for the exodermis was to look at the number of cell layers in the hypodermis which were nonsignificantly fewer in the wet middle zone (North and Nobel 2000). Directly beneath the succulent leaves was an interesting, basal 40-mm root zone that exhibited reduced endodermal suberin lamella development; the authors point out that this zone is ideally positioned to absorb water from light rain showers, providing the plant with an ecological advantage. The zone is believed to provide a lower resistance pathway for water uptake in the event of rain showers and the location is shallow, shaded by the shoot, and the design of the leaves channels intercepted rainfall to that location.

In a previous paper, North and Nobel (1998) showed that *Agave* had developmental plasticity related to sources of water. The endodermis in basal

regions of the roots grown under dry conditions had fewer suberin lamellae than in middle regions of the root. The authors discuss the ability of the roots to facilitate water uptake by altering their developmental schedule in local zones of higher water content (condensation under stones, for example) where the roots are less suberized. In contrast to the results of North and Nobel (1998, 2000), Perumalla and others (1990) observed that *Agave americana* has a uniseriate exodermis. It is fairly common for many species to develop sub-exodermal layers modified with suberin or lignin (rings of lignified sclerenchyma, for example), but these do not have Casparian bands or, necessarily, suberin lamellae. These structures normally occur in *Pontederia*, *Nelumbo* (Seago and others 2000a; Seago 2002) and *Phragmites* (Soukup and others 2002), and apparently provide structural support. It would be useful to examine *Agave* grown under the above experimental conditions (North and Nobel 1998, 2000) to obtain more detailed information about these additional layers and to determine if the anatomical differences are related to variations between species or growth conditions.

In another documented microclimate effect, the epidermis and cortex of chickpea roots died back to the endodermis, except where the root had grown through a dense chunk of peat moss (Spaeth and Cortes 1995). In these locations, the epidermis and cortex were preserved. Unfortunately, the authors did not have the resources to perform histochemical tests so it is unknown if there were differences in the structure of the endodermal cells within and without these microenvironments. Some of the chickpea roots were able to retain their cortices throughout two cycles of imposed drought stress and the authors speculated that perhaps chickpeas are exodermal (Spaeth and Cortes 1995). The results of Hartung and others (2000) from aeroponically grown chickpea makes this hypothesis unlikely. However, it is possible for stressful conditions to induce an exodermis in normally, non-exodermal roots, two instances being barley grown with mechanical impedence (Lehmann and others 2000) and cotton grown under high salinity (Reinhardt and Rost 1995b). Given the variability of root responses to microclimate, it is necessary for researchers to document anatomical changes induced in a specific species by a specific stress.

Major Environmental Stressors

Drought. A generalized response to less-than-optimal conditions in the rhizosphere tends to be a reduction in root growth rate and a greater proportion of the root with a mature endodermis and exodermis. This holds true for drought stress. Cruz and others (1992) applied severe drying stress to sorghum root systems and found that they were very short and that the endodermis and exodermis progressed to deposition of tertiary walls very close to the apex. Control sorghum roots were much longer and the development of these layers was more spread out along the root length. The authors proposed that the enhanced endo- and exodermal development was a water conservation measure, as the stressed roots exhibited slowed water uptake and blocked the movement of apoplastic fluorescent dyes. Taleisnik and others (1999) worked with a variety of exodermal and nonexodermal species (including sorghum) and found that the mature exodermis slowed the initial rate of water loss from root segments exposed to air, supporting Cruz and others' (1992) idea.

North and Nobel (1996) examined the effect of drying on the desert inhabitant *Opuntia ficus-indica*. Endodermal Casparian bands were present close to the root tip, and the point at which 50% of the endodermal cells had suberin lamellae was also closer to the root tip than in the control. This may be a function of a lower growth rate, but it is not possible to tell from the data provided. In these experiments, radial hydraulic conductivity of the endodermis was reduced when the roots experienced drying stress (North and Nobel 1996). Perumalla and others (1990) list *Opuntia* as exodermal, but the authors (Nobel and North 1996) only detected slight lignin deposits in the hypodermal layer prior to periderm formation. Tests of radial hydraulic conductivity indicated that water movement into the stele decreased in roots grown with drying stress and additionally that the endodermis formed a more efficient apoplastic barrier than the periderm, which was eight layers thick. A closer look at this species, along with *Agave*, to determine the exodermal development (if any) under different growth conditions would be extremely helpful.

Onion adventitious and maize seminal seedling roots develop extensive suberization in both the endo- and exodermal layers under drying stress. However, the end result in terms of tissue survival is very different in the two species (Stasovski and Peterson 1991, 1993). In onion, which possesses a dimorphic exodermis, the tips of the adventitious roots died, but the cortical tissues behind the tip and the lateral root primordia within them were conserved. The exodermis matured right up to the dead tip of the water-stressed roots. The long cells suberized but remained transiently alive; eventually,

the long cells became symplastically isolated and died (Stasovski and Peterson 1993; Peterson and Waite 1996; Ma and Peterson 2000). Many of the short cells remained unsuberized. At the end of the drought period, water presumably entered via the passage cells and the root system began to regrow by breaking the dormancy of the lateral primordia. In contrast, root tips of droughted maize seedlings remained alive throughout the stress period. The exodermis, even though suberized, died by 35 d and the underlying cortex partially collapsed. Upon rehydration, the existing roots of the surviving seedlings recommenced elongation. Stasovski and Peterson (1991) postulate that the die-back pattern of maize seedlings was similar to that of non-exodermal species because of the young age of the plants. The authors (1993) provide a concise literature review to earlier work on drought stress in exodermal and non-exodermal species. Their work raises a number of related questions. Would the response of maize vary in roots of different orders of branching or in root systems of older plants? Are these drought response patterns typical of many species? Is there a consistent difference in die-back patterns between exodermal and non-exodermal plants experiencing drought stress?

Noldt and others (2001) compared fine root anatomy of two timber species in Brazil. The exodermis of *Carapa* had heavily modified walls forming two different cell types. ''U-shaped cells'' had thick, lignified tertiary walls with a prominent suberin lamella whereas ''Pad'' cells had a very heavy thickening on the outer tangential walls only and no suberin lamella. The latter may be a form of transfer cell as well as passage cell. In contrast, the more lightly modified exodermis of *Swietenia* had thinner tertiary walls and suberin lamellae in some cells and the remainder were thin walled passage cells. The authors suggest that the heavy wall modifications contribute to the great drought resistance of *Carapa* compared to *Swietenia*. If further studies support this idea, it may be possible to optimize species choices for timber plantations by considering the root anatomy and the plantation site. It may also be possible to direct breeding strategies or crop development projects to modify exodermis development and, hence, tree survival in less favorable locations.

Salinity. As with drought-stressed roots, the response of root systems to salinity is a reduction in growth rate and the appearance of endodermal and exodermal suberization closer to the root apex (see Shannon and others 1994; Reinhardt and Rost 1995a, b). An analysis of growth rates from the data presented by Reinhardt and Rost (1995b) for cotton seedlings reveals that this effect is due to both a reduction in growth rate and an accelerated maturation of Casparian bands and suberin lamellae. This accelerated maturation only occurred in young seedlings; older seedlings were not affected. Higher salinity (200 mM NaCl) levels also induced an exodermis in this typically non-exodermal plant (Perumalla and others 1990) at the base of the root and in the transition zone to the hypocotyl (Reinhardt and Rost 1995b). This exodermis may have some protective function against water or solute loss or pathogen ingress but only in a limited area of the root (Reinhardt and Rost 1995b).

Walker and others (1984) compared two *Citrus* species that differed in salt exclusion ability and that were grown at various salinity levels. Salinity reduced the growth rate in both species compared to control, but more so in the salt-sensitive one. This is the probable cause of the deposition of suberin lamellae in both endodermis and exodermis closer to the root tip in plants exposed to NaCl; it is not possible to determine if suberization of the layers was accelerated with the data presented. In both species, the epidermis died off in controls as well as in treated plants, leaving the hypodermis as the outermost layer. The description of the hypodermis indicates a dimorphic type of exodermis. The long cells suberized and died. The endodermis also largely suberized and lignified, initially leaving passage cells. Eventually, the passage cells also suberized, and the endodermis sealed off and died. Salinity did not appear to have any effect on the sequence of development in the *Citrus* species nor was it clear if the endodermis or exodermis had any role in salt tolerance (Walker and others 1984).

Taleisnick and others (1999) found that while root hydraulic conductance decreased in *Chloris gayana* grown in 300 mM NaCl, there was no difference in exodermal development compared to control roots. Root segments from these plants lost water more rapidly than those from control plants. Again, in tomato, Peyrano and others (1997) found that 100 mM NaCl did not alter the levels of lignin or suberin in the roots, although the hydraulic conductance of the salt-exposed plants was lowered. It is possible that a change in aquaporin function contributed to the observed change in hydraulic conductance (Peyrano and others 1997).

The endodermis and exodermis are potentially important for plant resistance to this major environmental and agricultural stress. Further study on their development under saline conditions would be appropriate.

Flooding. Flooding, the exposure of root systems to too much water, is primarily a stress of reduced oxygen concentration and diffusion in the rhizosphere (for example, see reviews by Nilsen and Orcutt 1996; Drew 1997; Drew and others 2000). With adequate diffusional exchange with the atmosphere and convective movement in the water around the root zone, oxygen supply to the root system can be sufficient for continued root growth, although boundary layer effects will occur at the root surface to slow gas diffusion into and out of the roots. Hence, many species thrive in aerated nutrient culture. However, in nature, many flooding situations result in stagnant conditions and the rhizosphere quickly becomes hypoxic or anoxic as a result of respiratory activity by roots and soil microorganisms. Species that survive under temporarily or permanently flooded conditions usually cope by developing root aerenchyma, porous cortical tissue with enhanced gas exchange with the atmosphere in which the shoot grows (see Nilsen and Orcutt 1996 and references therein). In those species that develop aerenchyma in response to flooding, ethylene is believed to play a role in its formation, probably by triggering programmed cell death in specific cortical cells (Drew and others 2000; Gunawardena and others 2001).

A hypoxic or anoxic rhizosphere not only imposes a stress on the root system by failing to deliver oxygen, it can also act as an oxygen sink, drawing oxygen out of root tissues along a concentration gradient. This radial oxygen loss (ROL) has been examined using both oxidation indicator dyes and oxygen microelectrodes. Many wetland native plants possess a ''barrier'' to ROL in the proximal portions of their roots. Growth in a stagnant solution can induce such a barrier in rice (Colmer and others 1998), *Carex acuta* and *Juncus effusus* (Visser and others 2000), and a partial barrier (of variable effectiveness) in *Caltha palustris*, *Ranunculus sceleratus*, and *Rumex palustris* (Visser and others 2000). McDonald and others (2001) tested the development of porosity (aerenchyma formation) and barrier to ROL in nine species of the tribe Triticeae in the Poaceae grown under stagnant conditions and found that most did not form a strong barrier. The exception was *Critesion marinum*, a close relative of cultivated barley, which is also in the Triticeae. According to Lehmann and others (2000), most barley roots have an exodermis. McDonald and others (2002) surveyed a number of Panacoid and Festucoid grasses and two Cyperaceae species grown in aerated and stagnant nutrient cultures. Many of these species developed a strong ROL barrier after growth in stagnant solution. Unfortunately none of the authors tested for the presence of an exodermis in any of the species examined. The survey performed by Perumalla and others (1990) indicated that a single examined member of the Cyperaceae possessed an exodermis, as did tested species of the tribes Oryzeae, Paniceae, Andropogoneae, and Maydeae of the Poaceae. Members of the Poaceae tribes Triticeae, Festuceae, and Aveneae did not possess an exodermis, at least in the roots examined (Perumalla and others 1990; but see Lehmann and others' 2000 study of barley roots, discussed below, for a correction).

In another study, the flooding-sensitive species *Brassica napus* did not develop aerenchyma or a ROL barrier (Voesenek and others 1999). A brief examination of young, vermiculite-grown *Brassica napus* cv. ''Bounty'' roots revealed a two-layered central cortex with phi thickenings in the inner layer, an endodermis with suberin lamellae (Figure 1L), and a prompt transition to secondary growth. No exodermis was present. If this result is confirmed with a more rigorous study, it could explain the lack of an observed ROL barrier in this species. To provide maximum information on the root systems, ROL studies need correlative, anatomical observations.

Armstrong and others (2000), using an oxygen microelectrode, performed an excellent correlative study of the oxygen profile across *Phragmites* roots and the anatomy of the regions probed. They pinpointed the location of the ROL barrier to the epidermis and the suberized-lignified hypodermis, and specifically to the outermost layer of the hypodermis. Soukup and others' (2002) detailed anatomical follow-up of *Phragmites* grown under stagnant and aerated conditions demonstrated that the outer two to three layers of the hypodermis is an exodermis (with H-shaped Casparian bands). Subjacent is a two-layered ring of sclerenchyma. Armstrong and others (2000) further showed that where the exodermis/hypodermis remained immature — overlying regions of emerging lateral roots — there was significantly increased oxygen egress from the roots. It seems clear that the suberized exodermis constitutes the ROL barrier or at least a significant portion of it. Support for this finding may be found in the work of Jacobsen and others (1998) who used iodine vapor to trace the pathway of oxygen diffusion into nodules of two legume species. The vapor moved through lenticels and unsuberized tissues, but was not able to cross endodermal layers with Casparian bands and suberin lamella, indicating that these layers do form a barrier to gas diffusion. The survey of species with regard to presence of exodermis conducted by Perumalla and others (1990) provides a starting point to perform correlative anatomical investigations of those species

which have already been tested for a ROL barrier. Such studies would clearly define the role that the exodermis plays in regulating the internal oxygen status of plants growing in wetland environments or under flooded conditions.

The value of the ROL barrier to the plant is the maintenance of internal oxygen concentration in root systems growing in a hypoxic or anoxic environment. Aerenchyma provides a relatively low resistance, long-distance conduit for oxygen movement into the root system. A peripheral barrier allows the retention of this oxygen within the root, allowing bottom-rooted species to penetrate into oxygen-deficient substrata in which it would otherwise not be able to survive. The expression of the barrier can be constitutive or facultative. The wetland native *Phragmites* forms a suberized exodermis regardless of whether it is cultured in aerated or stagnant conditions (Soukup and others 2002). On the other hand, maize, which is relatively flood intolerant, responds to stagnant conditions within days by substantially increasing exodermal suberization compared to the amount formed in aerated nutrient solution (D.E. Enstone and C.A. Peterson unpubl.). This is a strategy that should help it survive short-term flooding stress. Gibbs and others (1998) monitored the effects of maize growth in hypoxic conditions with respect to the radial and longitudinal transport of water and solutes, and appear to point out that Colmer and others' work (1998) on hypoxia inducing a ROL barrier in rice could have the same effect on the endodermis. Exodermal plants will typically suberize both their endodermis and exodermis when exposed to drought and also high salinity conditions (see Drought and Salinity, above). If this effect occurs with hypoxia, then the stele of the root system is likely to become anoxic despite a reasonably well-aerated cortex. In fact, in maize, the opposite effect occurs. As this species suberizes its exodermis in response to stagnant growth conditions, it simultaneously reduces the amount of endodermal suberization, compared to aerated controls (D.E. Enstone and C.A. Peterson unpubl.). This coordinated action would have the advantage of reducing oxygen outflow to the rhizosphere while increasing diffusion into the stele. It is important to see if this result is borne out in other species, as it indicates that the exodermis and endodermis, despite the similarities in their appearance and the overlaps in their functions, can react in opposing ways to deal with specific tissue needs.

Other Environmental Challenges. A number of environmental factors in addition to those already discussed occur that can lead to changes in endodermal and exodermal development. Wilson and Robards (1978) found that seminal roots of barley seedlings growing with mechanical impedance had apparently accelerated endodermal development even after taking into account the reduction in growth rate. Interestingly, they found a zone at the base of the impeded roots in which the endodermis had Casparian bands only, although a few millimeters distal to the base up to 85% of the cells were more modified. This is reminiscent of North and Nobel's (2000) observations of an unsuberized basal region in drought-stressed *Agave*. Also in barley, Lehmann and others (2000) noted that mechanical impedance can induce an exodermis near the base of the normally non-exodermal roots.

Nutrient status and toxins in the environment will also influence the development of the layers. Pozuelo and others (1984) found that maize seedlings grown in magnesium-deficient culture were more heavily suberized in both exodermis and endodermis than seedlings grown in a balanced medium. Armstrong and Armstrong (2001) demonstrated that exposure of *Phragmities* to acetic acid or to ''cocktails'' of lower monocarboxylic acids resulted in rapid morphological, anatomical, and physiological responses. Root length decreased, and lignification and suberization of the exodermis occurred closer to the apices of the adventitious roots compared with untreated controls. Concomitantly, a barrier to ROL associated with this lignification also formed closer to the apex (Armstrong and Armstrong 2001). However, although root growth measurements were taken, anatomical and ROL measurements were related to tissue distance from the apex, so it is not clear if these changes were accelerated in the treated plants. In the environment, development of the exodermis protects the root from the phytotoxins; however, the simultaneous cessation of oxygen supply to the rhizosphere creates a more hostile environment which can lead to the death of root system, a further release of organic acid, and thus an increase in phytotoxins in the rhizosphere. This can set up a cycle of dieback that has been observed in wetlands (Armstrong and Armstrong 2001).

Genetics vs Environment

Seago and others (1999b) speculated that the development of the exodermis and the aerenchyma in wetland species were correlated; in roots of *Typha,* exodermis development precedes aerenchyma development. It is a logical assumption, given that aerenchyma is responsible for the internal delivery of oxygen from the shoot and the exodermis is the

putative ROL barrier, conserving this oxygen for root use. This developmental relationship also occurs in *Phragmites* (Soukup and others 2002). However, subsequent studies revealed that this correlation is not maintained in other wetland species. The small floating aquatic *Hydrocharis* forms aerenchyma but has no exodermis (Seago and others 1999a). The roots of *Hydrocharis* reach 750 mm or longer, but the riverine habitat combined with a free movement near the water surface may reduce the oxygen diffusion gradient out of the root. *Caltha,* an inhabitant of seasonally flooded wetlands, forms an exodermis but not aerenchyma (Seago and others 2000b). And *Pontederia* and *Nymphaea,* bottom-rooted species with emergent leaves, both have aerenchyma which develops before maturation of the exodermis (Seago and others 2000a, b). Finally, in flood-sensitive maize, exodermis formation precedes aerenchyma formation in aerated hydroponic and in vermiculite culture, but under stagnant conditions aerenchyma forms much earlier than the exodermis (D.E. Enstone and C.A. Peterson, unpubl.). These more recent data strongly suggest that the signals for aerenchyma development are separate from those for exodermal development. It will be very interesting to discover the nature of these signals.

Despite the significance of the endodermis and exodermis to the root, very little is currently known about the genetic controls governing their development. Nor do we have a clear understanding of the influence of the environment in modifying this genetic control. Most angiosperms have an exodermis. Members of the Fabaceae and the Malvaceae, however, are not typically exodermal (Perumalla and others 1990). It would be interesting to know if all species of these families are non-exodermal. Further, is the lack of exodermis correlated with nodulation, including the actinorhizal species (for example, *Alnus*: see Wall 2000)? The Festucoideae subfamily of the Poaceae is also recorded as non-exodermal (Perumalla and others 1990); however, Lehmann and others (2000) have demonstrated that the seminal and first generation nodal roots of barley (*Hordeum vulgare*) are non-exodermal while the later generation nodal roots possess an exodermis. It would be useful to know if the other festucoid grasses also have this diversity in their root systems. Additionally, we also now know that cotton, a member of the Malvaceae, has an inducible exodermis under certain stress conditions (Reinhardt and Rost 1995b), as does barley (Lehmann and others 2000). Genetically modified, *Bt* cotton also forms an exodermis (B. Wheat and J.L. Seago Jr, personal communication). Therefore, at least in some species, the genetic coding is present for exodermis formation given the right stimuli. To the best of our knowledge, none of the Fabaceae observed under stress culture conditions differentiated an exodermis (see, for example, Hartung and others 2002). How many ''non-exodermal'' species are capable of forming an exodermis under extreme stress? How much variability can occur within families regarding the presence of the feature? Of 43 species of ferns and fern allies investigated (Damus and others 1997), only three species of *Selagenella* possessed an exodermis. Why did the other two species of *Selagenella* in the survey not develop an exodermis? Were they lacking the genetic coding or the environmental cues? It would be valuable to target key plant families and extend the previous survey results (Perumalla and others 1990) to most or all species in these families and examine the various root orders within these species. There is an enormous amount still to be learned about the formation and development of the endodermis and exodermis from molecular to environmental aspects.

Note added in proof

Since this review was written, Colmer (2003) has reviewed the current knowledge on gas exchange, aerenchyma formation, and the ROL barrier in plants that deal with either a transiently or permanently water-saturated environment.

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