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**AN EXPERIMENTAL STUDY OF THE DEVELOPMENT OF PLANT
RHIZOSHEATHS**

by

Michelle Watt, B. Sc. (Hons.)

**A thesis submitted to
the Faculty of Graduate Studies and Research
in partial fulfilment of
the requirements for the degree of**

Master of Science

Department of Biology

Carleton University

Ottawa, Ontario

May, 1994

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**"AN EXPERIMENTAL STUDY OF THE DEVELOPMENT OF PLANT
RHIZOSHEATHS"**

submitted by Michelle Watt, B.Sc. (hons.)
in partial fulfilment of the requirements for
the degree of Master of Science

Thesis Supervisors

Chair, Department of Biology

Carleton University

Frontispiece

Table 1.1. Rhizosheath formation reported on plants of mesic and xeric habitats

<u>Habitat</u>	<u>Species</u>	<u>Source</u>
mesic	<i>Triticum aestivum</i>	Sachs, 1865
xeric	<i>Aristida pungens</i>	Price, 1912
xeric	<i>Aristida plumosa</i>	Arber, 1934
xeric	desert grasses	Goossens and Stapelberg, 1933
xeric	<i>Oryzopsis hymenoides</i>	Wullstein and Pratt, 1981
mesic	<i>Zea mays</i>	Vermeer and McCully, 1982
xeric	<i>Zygochloa paradoxa</i>	Buckley, 1982
mesic	cereal grasses	Duell and Peacock, 1985
mesic	<i>Secale montanum</i> , <i>Triticum aestivum</i>	Goodechild and Myers, 1987
mesic	C4 grasses	Wenzel <i>et al.</i> , 1989
xeric	<i>Calamovilfa longifolia</i>	Brejda <i>et al.</i> , 1989
xeric	<i>Ferocactus acanthodes</i> , <i>Opuntia ficus-indica</i>	North and Nobel, 1992

Setaria spp. thrive during the hottest periods of summer in the field plot at the Experimental Farm. The young nodal roots are concentrated in the upper soil and are covered in large, stable rhizosheaths. In the morning, these sheathed roots and the soil immediately surrounding them is wet, while the bulk soil is very dry. Clearly, during the night, in the absence of transpiration, water in deep soil layers has been taken up by the older roots and has moved into the young nodal roots, from where it has exuded into their rhizosheaths.

ABSTRACT

Rhizosheaths are encasements of aggregated soil found on the younger regions of all grass roots. Rhizosheaths are very large on grasses of xeric habitats. To quantify the effect of soil water content on rhizosheath formation, corn primary roots were grown through a 30-centimeter-deep, prepared soil profile with a central, horizontal, "dry" (9% water content), or "wet" (20% water content) layer (4 cm thick), sandwiched between damp soil (15-17% water content). Rhizosheaths formed in dry layers were five times the volume of the subtending portion of the root, while those formed in wet layers were only one and a half times the root volume. Rhizosheaths developed in dry soil were more resistant to removal from the root compared to those developed in wet soil. Portions of roots developed in dry layers had four and a half times more root hairs than portions from wet layers. The surfaces of hairs and the epidermis proper of root portions from dry soil appeared more hydrophobic than those from the wet soil, however, the suberization of the endodermal and hypodermal apoplastic barriers was similar. A survey of field-grown grasses and dicotyledonous plants showed that root-cap mucilages that bind soil, and numerous root hairs, are components common to sheath-forming plants. Drier soil enhances stabilization of the rhizosheath by these components. There is strong evidence, however, that the initial step in the production of the rhizosheath is the exudation of water from the root surface to hydrate the mucilages. Extensive and stable rhizosheaths may function in nutrient acquisition by roots, especially in dry soils.

ACKNOWLEDGMENTS

I am very grateful to have done this thesis with the support and guidance of Dr. McCully and Dr. Canny. While encouraging imaginative ideas, they provide an example of excellent, careful science. Dr. Torrance and Dr. Topp gave me help with the soils part of the project. I am thankful to Karsten and Rebecca, and to Cathy, Linda, Wayne, Zhongmin and Kathleen. Without the help of all these people, I would not have finished on time. Thanks also goes to my family for their continual encouragement, and to Paul, for his love, patience and kindness.

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CHAPTER 1

GENERAL INTRODUCTION

Roots of grasses excavated from the field have coherent layers of aggregated soil encasing their younger regions while the older regions are bare and devoid of soil. Neither persistent shaking, nor rinsing with water will remove the layers of soil adhered to the younger roots. These coherent encasements of soil are called soil sheaths, or rhizosheaths. Rhizosheaths are a feature of mesophytic grasses and were first described on wheat by Sachs in 1865, and then were re-discovered on corn by Vermeer and McCully in 1982 (see Table 1.1). They are also found on xerophytic grasses (Price, 1912; see Troughton, 1957). The rhizosheaths, or sand sheaths, of xerophytic grasses are strikingly-large, perhaps explaining why they have been reported more often than those of mesic grasses, in addition to the fact that many studies of mesophytic grasses (i.e., the cereal crops), are done on roots scrubbed free of soil, or those grown in nutrient solution. As yet, there are no reports in the literature of rhizosheaths forming on dicotyledonous plants, in mesic or xeric conditions (Table 1.1).

Rhizosheaths have been most extensively characterized for field-grown corn (Vermeer and McCully, 1982; McCully and Canny, 1988). They start to form at approximately 1-2 cm from the root tip (see Abeysekara and McCully, 1993, for a study of this special tip region), and extend 20-40 cm towards the base. They are composed of soil particles, root hairs, mucilages and a native microflora (St. Aubin *et al.*, 1986; Gochnauer *et al.*, 1989). The mucilages are produced by the root-cap cells, which are left in the sheath, along with their mucilage, as the root grows through the soil (Vermeer and McCully, 1982), and by specific mucilage-producing bacteria. Root-cap and bacterial mucilage (i.e. from a *Cytophaga* sp) can be very effective soil-binding agents, as shown in a model system (Watt *et al.*, 1993). Other bacteria native to the sheath stimulate the formation of bends, or distortion points, along root hairs (unpublished study by H. Wang, McCully and Canny Laboratory). These distorted regions of hairs coincide with places where soil is particularly tightly-bound, and it is thought that these bacteria may participate

in the solubilization of the hair walls at places where soil becomes permanently lodged (M. Canny, per. comm.).

The sheathed regions of roots have large late metaxylem (LMX) vessel elements that are still alive and not yet conducting the transpiration stream. At the point where these large vessels become open (i.e., the protoplast of the elements die and the crosswalls are lost), the root loses the sheath and becomes bare (St. Aubin *et al.*, 1986; McCully and Canny, 1988; Wenzel *et al.*, 1989). The opening of the LMX vessels has important implications for axial flow in the root because, according to the Hagen-Poiseuille relation (flow varies with radius to the fourth power), each LMX (average diameter 100 μm) would carry, on average, 625 times the volume of fluid of an early metaxylem vessel (average diameter 20 μm) (see McCully and Canny, 1988, where this sample calculation is presented and discussed in detail). As a result, there is much less flow in the sheathed region of the root compared to the bare. Accordingly, Wang *et al.* (1991) have shown conclusively that the sheathed regions are relatively wet, while the bare regions are dry. In turn, this difference in water status between sheathed and bare roots accounts, in large part (along with changes in the surface features of the roots, M. McCully, per. comm.), for the differences in associated microflora (Gochmayer *et al.*, 1989). Plus, as shown in this thesis, a high water content (that changes little from day to night, Wang *et al.*, 1991) in the rhizosheath plays a role in soil adhesion by both the mucilage of the native bacteria, and that of the root cap.

Although sheathed roots are wet, and feel fleshy to the touch, casual observations in the field, over the course of several summers, have indicated that rhizosheaths formed in drier soil conditions are larger, and more tightly bound to the root, than those formed in wetter soils (M. McCully, per. comm.). This apparent contradiction, i.e., sheathed roots are wet, yet seem larger (have more adherent soil) in drier soil, combined with numerous reports of large rhizosheaths on the roots of xerophytic grasses (Table 1.1), indicates that the soil water content plays an important role in rhizosheath formation and stabilization,

perhaps in conjunction with the water status of the root. Therefore, the study presented here concentrates on the role of the water content of the soil adjacent to where the rhizosheath develops in the formation and stabilization of the sheath.

First, in Chapter 2, the relationship between the soil water content and the size and stabilization of the sheath is quantified. To do this, I used a model system whereby the primary roots of maize were grown through three-layered profiles of soil with dry or wet middle layers. Following, in Chapter 3, the same model system is employed to study the structural features of the portions of the corn roots that had developed rhizosheaths in dry or wet soils. The structural features were related to rhizosheath development, and to the water relations of the plant. In Chapter 4, I have conducted a field-survey of a range of plants, including dicotyledons, for rhizosheath formation. The structural features of the roots, and the architectures of the root systems, were studied to find commonalities among plants that form rhizosheaths. This study also includes preliminary evidence that rhizosheaths may function as nutrient-acquiring zones for the plant. Then in Chapter 5, the work of the preceding three chapters has been incorporated into a general discussion of how, and why, rhizosheaths form in the field, particularly under dry conditions.

Table 1.1. Rhizosheath formation reported on plants of mesic and xeric habitats

<u>Habitat</u>	<u>Species</u>	<u>Source</u>
mesic	<i>Triticum aestivum</i>	Sachs, 1865
xeric	<i>Aristida pungens</i>	Price, 1912
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mesic	C4 grasses	Wenzel <i>et al.</i> , 1989
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xeric	<i>Ferocactus acanthodes</i> , <i>Opuntia ficus-indica</i>	North and Nobel, 1992

CHAPTER 2

EFFECT OF SOIL WATER CONTENT ON RHIZOSHEATH FORMATION AND STABILIZATION

INTRODUCTION

Large rhizosheaths are a recognized feature of desert grasses (see Table 1, Chapter 1). In addition, very substantial rhizosheaths appear to form on mesophytic grasses particularly in the drier soils of mid-summer, while smaller, more-easily removed sheaths can be found on roots in the wetter soils of early spring. These findings suggest that the water status of the surrounding soil plays an important role in rhizosheath formation and stabilization. In this chapter, I report the use of a model system to verify and quantify this relationship between the soil water content and the formation and stabilization of soil sheaths.

The experimental system was adapted from one used by Nambiar (1976) to study Zn^{65} uptake by roots growing in dry soil. He grew the roots of small seedlings through a three-layered profile of soil that had a dry, Zn^{65} -labeled middle layer, isolated from the top and bottom layers by hydrophobic partitions. From this study he obtained two interesting results. First, he discovered that the regions of the root in the dry soil were able to take up more labeled zinc than expected, despite exposure to water-stressed conditions. Second, the root portions from the dry-soil layer were surrounded by a tightly-bound "annulus" of soil that was difficult to rinse from the root, while the rest of the root was relatively soil-free. Although Nambiar does not refer to these annuli of soil as rhizosheaths, they appear as such in the figures in his paper. Therefore, not only do these results indicate a correlation between nutrient uptake and sheath formation, but they also show that soil water content influences soil adhesion to roots.

Here, I have preset the water content of a layer of soil to either "dry" or "wet" (for the sandy-loam soil used: dry = just wetter than the wilting coefficient; wet = just drier than field-capacity), isolated it from the rest of a soil profile by a wax-paraffin partition, and quantified sheath size and tightness of soil binding between root samples and along individual roots.

MATERIALS AND METHODS

Cultivation of plant material

Corn (*Zea mays* L. cv. Seneca Chief) seeds were imbibed 3 to 5 h and germinated on moist filter paper in sealed Petri dishes for 40 to 48 h at 26°C. Uniform seedlings with radicles 2 to 3 cm in length were each carefully transferred to 2 cm below the surface of the top layer of a prepared soil profile in a growth tube (see below). The plants were left to grow for 5 days in a greenhouse (ca. 23°C, 14h light/10h dark) by which time their primary roots had grown through the soil profile. A minimum of 10 plants were grown for each treatment. They were watered with tap water every 1.5 d from the top and on day three from the bottom.

Preparation of soil profiles

Soil profiles were prepared from sandy loam top soil obtained from a field plot at the Central Experimental Farm of Agriculture Canada, Ottawa. Similar to Nambiar's (1976) method, each profile contained three layers of soil, isolated from each other by hydrophobic partitions (Fig. 2.1A). These partitions allowed the layers to be maintained at different water contents but did not hinder penetration of the roots through the profile.

Soil profiles were prepared in 5 cm-diameter black plumbing pipes (ABS/DWV T162, Canadian Tire, Ottawa, Canada), cut to 30 cm lengths and slit open down one side so the tubes could be hinged open at the time of harvest of the roots. The tubes were held together in the radial direction and sealed along the vertical slit with electrical tape during soil profile preparation and plant growth. Snug-fitting plumbing caps with five holes (1 cm diameter each) for drainage and aeration of soil were used as pot bottoms.

The first layer of soil (L1) was gently packed (and its surface flattened) into each tube to a height of 10 cm from the bottom. The water content of the soil was unaltered and similar to that found under normal field conditions [15-17% (w/w) water]. Then 15 ml of a molten mixture (M.P. 37-39°C) of 1 part bees-wax (Benson Bee Supplies, Metcalfe, Ontario, Canada) and 2.5 parts paraffin oil (viscosity 345-455, S894, J.T. Baker Chemical Co., Phillipsburg, NJ, USA 08865) was poured over the entire surface of L1 and allowed to solidify.

The second, 4 cm deep, middle layer of soil (L2) was gently packed and flattened over the wax:paraffin partition. In contrast to L1, this soil was initially prepared to a desired water content [either 9% (w/w) water for "dry" samples or 20% (w/w) water for "wet" samples] by thoroughly mixing a predetermined weight of air-dried soil with the appropriate amount of tap water. The dry soil was just above the wilting coefficient (approximately -10 bars) and the wet soil was just below field capacity (-0.6 bars) (Clarke Topp, Agriculture Canada, per. comm., Brady, 1990). Another 15 ml of molten wax and paraffin oil was poured over L2, sandwiching the layer of soil of controlled water content between two hydrophobic barriers. Finally, the remainder of each tube (16 cm to top) was filled with unprepared soil similar to that used for L1. This was the seed-bed layer (L3).

Plant harvest

Plants were easily recovered with their root systems intact from hinged-open tubes provided that the wax partitions were first carefully cut away from the contact with the root. To maintain the water status of the excavated roots and their adhered soil, the three regions of the primary roots were immediately covered with soil from the corresponding layer (L1, L2 or L3). Quantitative observations were started within 15 to 30 min of uprooting the plants and qualitative observations of root structure were also performed on fresh plants or plants preserved in formaldehyde (3.7% in tap water) or in ethanol (30-50% in tap water).

Quantitative measurements

Soil water content

The soil water content (SWC) of L2 was determined at soil profile preparation and at plant harvest for each sample. During initial experimental trials, SWC was determined for L1 and L3. A small aliquot of soil (~1-2 g) was quickly scooped into a tared, 10 ml glass vial, capped and its fresh weight (W1) determined. Dry weight (W2) of the soil was determined after 4 to 5 d drying at 105°C. Then SWC was calculated from:

$$\text{SWC} = \% \text{ water (w/w)} = [(W1 - W2) \text{ g} / W2 \text{ g}] \times 100$$

Relative volume of sheath to volume of root

The extent of sheath formation around the portion of root from L2 was quantified by measuring the ratio between sheath and root volumes, assuming cylindrical shapes for the piece of root and its surrounding sheath of soil. The root portion from L2 was cut from the rest of the primary root and, with minimum agitation, measured length-wise (l) to give the mid-point. From there, three thin (<200 µm) transverse sections were taken (the root portion lay on soft dental wax and a fresh, wet, surgical steel razor blade was carefully drawn through sheath plus root), mounted in a drop of immersion oil (to prevent dispersion of the sheath) on a microscope slide, and a coverslip was gently placed on top. To prevent squashing and thus distorting the soft root sections, the coverslip was supported by two shims made from a broken coverslip. Using ocular and stage micrometers in a microscope, radii of the root (r₁) and the sheath plus root (r₂) were obtained from their diameters measured at three points (120° apart) on each cross section circumference. From these radii, the ratio of sheath to root volume (V) was calculated from the derived formula:

$$V_{\text{sheath}}/V_{\text{root}} = \frac{(r_2)^2 - (r_1)^2}{(r_1)^2}$$

Strength of soil adhesion

After removal of the transverse sections for relative volume calculations, the remaining halves of the root portion were immediately used to determine how tightly the soil of the sheath was bound to the root. Fractions of the sheath soil were removed by three successive treatments worked out during previous studies of maize rhizosheaths in our laboratory (unpublished data).

For fraction 1 (F1), the root halves were placed in a tared 10 ml glass vial filled with distilled water, capped and put in a sonicator bath (Bransonic 1200 Ultrasonic Cleaner, Bransonic Ultrasonic Co., Danbury CT, USA) at room temperature for 10 min. The vial was removed from the bath and the loosened soil allowed to settle to the bottom. The two root halves were then transferred to another 10 ml vial filled with distilled water and left at 65°C for 48 h (lid on vial). The root halves were then gently shaken free of heat-loosened soil (F2) and transferred to a watch-glass with ~5 ml of distilled water. There, a small brush was used to gently scrape off any soil remaining bound to the root surface. The water with the mechanically removed soil (F3) was carefully poured (with a funnel) into a third 10 ml vial, adding distilled water to clean off watch-glass and brush.

All vials with their fractions of loosened soil were dried for 48 h at 105°C (drying curves indicated weight stabilization of vials after 24h and fractions after 30h) (lids off). Then the vials plus soil were left to cool to room temperature for 3 h (lids off), weighed (lids on), and dry weights of soil determined by subtracting the dry weights of the vials from those of the vials and soil.

Microscopy and photography

Plants in tubes hinged open to reveal profiles of soil were photographed with a Nikon camera mounted on a tripod, under the natural lighting of a glasshouse. A lavender-coloured filter, held in front of the camera lens, ultimately produced the best contrast between dry and wet soil. To photograph the regions of roots from dry and wet zones, roots of excavated plants were placed on a piece of glass, supported 15 cm above the base of a copystand, and illuminated by 4 x 60 watt bulbs.

Mounts of tangential sections were observed and photographed through an Olympus SZH stereo microscope in the dark-field mode. Some whole root pieces (cut to length 0.4 cm for cross-sections and 1 cm for observation of the tangential surface) were frozen in liquid nitrogen slush, coated with Al and observed with a JEOL JSM 6400 cryo-scanning electron microscope at 7 kV. An electrical bias of ca 300 V was placed across the microscope stage to reduce charging of the soil particles.

All photographs and micrographs were recorded on Kodak TMAX 100 film.

RESULTS

Experimental design

Plants excavated from the tubes were uniform in size and appeared healthy. The primary roots easily penetrated the wax-paraffin partitions to L1 of the soil profile with no signs of "buckling" at the root tips. The tips reached the tube bottom, plus or minus approximately 1 cm (Fig. 2.1A). On occasion, the root grew along the edge of the pot in L2 in which case the root was not used.

Figure 2.1

Figure 2.1A. Pipes have been hinged-open to reveal the three-layered profiles of soil through which the primary roots of 7-day old corn (*Zea mays*) plants have grown. Wax-paraffin partitions (small white arrowheads) keep the soil of the middle layers (L2) "dry" (9% SWC) (pot on left hand side), or "wet" (20% SWC) (pot on right hand side), but allow penetration of the roots (large arrowhead). Open arrows indicate shoots. (X0.3)

Figures 2.1B-D show primary roots of corn which have grown through a soil profile with a dry or wet middle layer, similar to those shown in Figure 1.

Figure 2.1B. Roots have been removed intact from the soil profiles and parts of the wax-paraffin partitions remain attached to the roots at the L2 boundaries (arrowheads). Root on left is from a soil profile with a dry L2 layer: a thick, evenly-distributed layer of soil covers the root portion from the dry L2 layer, while less soil adheres to the root portions from the wetter L1 and L3 layers (above and below the partitions). Root on right is from a soil profile with a wet L2 layer: uneven patches of soil adhere along the exposed epidermal surface that was in the wet L2 layer, and more soil is bound to root portions from the relatively drier L1 and L3 layers. (X1.5)

Figure 2.1C. Transverse sections of root portions from L2 layers as in Fig. 2A. These sections are similar to those used to measure sheath and root volumes. The upper section, through a root portion from a dry L2 layer, is surrounded by a thick rhizosheath. The lower section, through a root portion from a wet L2 layer, has little attached soil which does not form a complete sheath. (X28)

Figures 2.1D. Portion of root from a dry L2 layer, sectioned by hand, and immediately frozen in liquid nitrogen and viewed in cross-section with a cryo-scanning electron microscope. An extensive rhizosheath of tightly-packed soil is closely associated with the epidermis proper (arrows). Root hairs (arrowheads) extend into the sheath, where they are intertwined with soil particles. A small amount of liquid (likely from the living late metaxylem vessels) has collected on the stele (S) during preparation (asterisk). (C) indicates cortex. (X60)

The SWC of L2 was approximately the same at soil profile preparation and plant harvest indicating that the wax-paraffin partitions were hydrophobic yet allowed root penetration. The L2 of "dry" samples stayed dry, at 9% ($\pm 1\%$) SWC, and the L2 of "wet" samples stayed wet, at 20% ($\pm 1\%$) SWC.

Size and stability of sheaths

Both the qualitative observations and the quantitative measurements show clearly that soil sheaths formed in dry soil were larger, more coherent and more tightly adhered to the root than those formed in wet soil (Tables 2.1, 2.2; Figs. 2.1B and C, 2.2A and B). If a primary root grown through a profile of soil with a dry L2 was briefly washed with running tap water immediately after excavation, a wide band of sheathing remained in the L2 portion (9% SWC) of the root that was greater than the sheathing on the L1 and L3 (15-17% SWC) portions. However, after a similar brief washing, a primary root that had grown through a layer of wet soil had little soil adhered in the L2 portion (20% SWC) and that part of the root appeared almost bare, while the L1 and L3 (15-17% SWC) portions had sheathing similar to that found in the L1 and L3 portion of a dry L2 sample.

Quantitative results

In dry soil (9% SWC), the volume of the sheath was five times the volume of the subtending root, while in wet soil (20% SWC), the volume of the sheath was only one and a half times the volume of its underlying root (Table 2.1). Similarly, Table 2.1 shows that the actual width of the sheath subtending a dry sample was approximately three times that of the sheath of a wet sample. The root diameter was the same for dry and wet samples (Table 2.1). The means of the measurements taken from the wet samples have greater standard deviations because the little soil that adhered to the root was clumped in patches (Figs. 2.1B

and 2.2B) and therefore was not always a complete cylinder around the root. In contrast, the surface of the sheathing formed in dry soil was relatively uniform (Figs. 2.1B and 2.2A).

The soil of the sheaths from the dry samples was not removed as easily as the soil of the sheaths from the wet samples. After 10 min sonication, only 50% of the soil of the dry-sample sheath became loosened compared with 90% of the sheath of a wet sample. In addition, after the F2 fraction had been removed, 35% of the soil sheath was left to be mechanically disrupted while only 2% of the sheath remained on the wet sample (Table 2.2).

Table 2.1. Relative measurements of underlying portions of primary corn roots and their subtending rhizosheaths when the rhizosheath is formed in dry or wet soil conditions (means $\pm$ S.D.; n = 8)

	<u>Volume sheath</u> <u>Volume root</u>	Diameter of Root (mm)	Thickness of Sheath(mm)
Dry (9%)	5.0 $\pm$ 0.8	0.8 $\pm$ 0.1	0.6 $\pm$ 0.2
Wet (20%)	1.7 $\pm$ 0.7	0.8 $\pm$ 0.1	0.2 $\pm$ 0.2

Figure 2.2

Figures 2.2A,B. Portions (B) of primary roots of corn developed in dry (9%SWC) or wet (20% SWC) L2 layers of soil profiles shown in Figure 1A, Chapter 2. Samples quick frozen, viewed frozen with a cryo-scanning electron microscope.

Figure 2.2A. From dry L2 soil. Rhizosheath completely obscures underlying root; some of the root hairs (white arrowheads) are visible protruding from between soil particles (S). (X43)

Figure 2.2B. From wet L2 soil. Same magnification as Figure 1A. Minimal rhizosheath formation allows observation of the epidermis proper of the root surface (R) and root hairs (white arrowheads). Some soil particles (S) are stabilized by root hairs, but others are bound directly to epidermis proper. (X43)

Table 2.2. Percentage of soil of the sheath removed after successive disruptive treatments from portions of corn primary roots grown in isolated zones of dry or wet soil (means $\pm$ S.D.)

<u>Treatment</u>	<u>Fraction</u>	<u>% of sheath removed</u>	
		<u>Dry (9% SWC)^a</u>	<u>Wet (20% SWC)^b</u>
sonication	F1	47 $\pm$ 11	91 $\pm$ 7
heat	F2	18 $\pm$ 11	7 $\pm$ 5
abrasion	F3	35 $\pm$ 5	2 $\pm$ 2

^a.n=6

^b.n=7

DISCUSSION

The results of these experiments confirm observations of corn plants and other mesophytic grasses growing in the field: rhizosheaths are larger, and adhered to the root more tightly, when formed in dry soils, while rhizosheaths formed under wetter soil conditions are not as coherent nor as tightly bound to the root surface (Figs. 2.1C and 2.2; Tables 2.1 and 2.2). The results also correspond with long-standing observations that highly developed rhizosheaths are a feature of grasses growing in dry, sandy habitats (Price, 1912; Arber, 1934; see Buckley, 1982 for additional references; Huang *et al.*, 1993).

No previous study has quantified the relation between larger, more tightly-bound rhizosheaths, and a low water content in the surrounding soil. However, a study of soybean

plants growing under water stress in sand culture reported similarly increased adhesion of soil to the roots in drier conditions (Sprent 1975). In addition, Peterson and Stasovski (1990), in a study of drought tolerance in corn (cv Seneca Chief growing in vermiculite or Turface) observed that more "soil" adhered to the roots as the soil water content dropped. More recent work with the desert plants, *Ferocactus acanthodes* and *Opuntia ficus-indica* shows that rhizosheaths on both intact and excised roots become more tightly bound when the surrounding soil is dried out. Their sheaths are easily slipped from the root when in wet soil (North and Nobel, 1992; Huang *et al.*, 1993).

In contrast, Fyson and Oaks (1987), working with very young seedlings of corn (60 to 72 hours old), report that the most substantial sheaths were formed in *wetter* soils (21% SWC), at lower temperatures (15°C). These puzzling results go against the findings of the study presented in this chapter, our observations in the field (i.e., that the wet, cool soils of spring result in smaller sheaths), and the observations reported in the literature cited above. I think that Fyson and Oaks may have reported something that I initially suspected could obscure measurements of sheath size relative to soil water content. That is, wetter soils with a clay and organic component (such as the soils Fyson and Oaks used), alone tend to be "stickier", and clump together in larger aggregates than those found in a similar drier soil. Roots grown in wet soils have these clumps attached to them, not forming a coherent sheath, but rather weakly stuck in patches along the root. If the roots are placed in water, as was done by Fyson and Oaks (1987) to determine the dry weight of the entire sheath, all the soil immediately washes from the root, and thus can be misinterpreted for the soil of a coherent, firmly-adhered rhizosheath. My study avoids this problem by first measuring the thickness and then removing the sheath in fractions. This effect would be intensified by the use of very young seedlings with recently developed root hairs that start approximately 2 cm from the tip. Figures presented in a subsequent cryogenic scanning electron microscope study of these "sheathed" roots (Fyson *et al.*, 1988) show clearly soil trapped between very young, straight, turgid root hairs, but raised above the surface of the epidermis (cp. Figs. 1 and 5 of

Fyson *et al.*, 1988). This observation prompted these authors to report that the root hairs had "pushed" the soil away from the root surface as they grew, creating a space between the "sheath" and the root. Unfortunately, this has led to misrepresentations in the literature of root-rhizosheath associations (e.g., see Figure 1 of Clarkson, 1991, which shows a wide gap between the root and the sheath). In fact, as shown in my Figs. 2.1C and D, the root surfaces and a developed rhizosheath maintain tight, intimate contact.

In the studies reported here, by exposing only a small part of the entire root (~13%) to the dry or wet soils of the L2 layer, I have ensured that the overall water status of the root was not changed. Thus the soil water content, acting on a localized portion of the root, caused changes in rhizosheath development. Clearly, it has influenced the components and mechanisms involved in rhizosheath formation and stabilization. These include adhesion of soil to the root by plant and bacterial mucilages, stabilization by root hairs, and presence of water, likely of plant origin, especially when the water content, or potential, outside the root is low.

CHAPTER 3

STRUCTURAL FEATURES OF PORTIONS OF ROOTS WITH RHIZOSHEATHS FORMED IN DRY OR WET SOILS

INTRODUCTION

A portion of a young primary root of corn will form a large or a small rhizosheath if it has grown and developed in a layer of dry or wet soil, respectively. This relation between rhizosheath size and stability, and soil water content, was quantified in the proceeding chapter for rhizosheaths formed on portions of the primary roots of corn which were grown through soil profiles with preset dry or wet middle layers. I showed that a portion of primary corn root produced a substantially different rhizosheath when grown and developed in a layer of dry versus wet soil. However, I was interested to see if structural features of the root itself were altered by exposure to such dry or wet soil, which represent moisture conditions that normally occur in the field (i.e., the dry soil: almost wilting coefficient; approximately -10 bars, the wet soil: just drier than field capacity: -0.6 bars, Brady, 1990; Clarke Topp, Agriculture Canada, per. comm.). In the chapter presented here, I have grown the primary roots of corn through the soil profiles of Chapter 2 to observe, on root portions and along individual roots, changes in the root structural features related to rhizosheath development and to growth and development in soils of differing water contents. These structural features include maturity of the late metaxylem vessels, density, morphology and surface features of root hairs, suberization of the hypodermal and endodermal layers, and formation of branch roots.

Late metaxylem vessels

In maize, closed (next page) late metaxylem vessels are a feature of sheathed roots. The transition from sheathed to bare root coincides with the opening of the underlying late metaxylem (LMX) vessels, and thus their joining of the transpiration stream (St. Aubin *et al.*, 1986). St. Aubin *et al.* (1986) showed that the LMX vessel elements retain their crosswalls anywhere from 20 to 40 cm from the tip, depending on the type of root. Based

on these findings, rhizosheath measurements in the preceding chapter were made on portions of the primary root of corn approximately 12-17 cm from the tip (see Fig. 3.2), assuming the LMX vessel elements were closed, similar to sheathed roots in the field. However, since the roots were grown through soil profiles with wax barriers, and exposed to soils of different water content, I have examined such roots to confirm that sheath measurements were made on portions of root with closed LMX vessels.

Root hairs

Root hairs are a recognized component of rhizosheaths (Sachs, 1865; Goossens and Stapelberg, 1933; Vermeer and McCully, 1982; Wullstein and Pratt, 1981; Huang *et al.*, 1993). Further, the presence of adhered soil is often reported in conjunction with root hairs (e.g., Weaver *et al.*, 1924; Dittmer, 1937; Sprent, 1975). The function(s) of root hairs is still not fully understood (Hofer, 1991), however it is contentiously believed that root hairs increase the area of root-soil (solution) contact, and thus increase access to water and ions. Root hairs are also thought to enhance root anchorage. Despite their likely importance to the plant, relatively little research has been conducted on the effects of environment, such as soil water content, on the density, length, shape, longevity and surface histochemistry of root hairs. The soil profiles used in the studies presented here are ideal for such observations because field-soil is used, each root is exposed to a transition in water content as it grows, and, finally, portions of different roots of similar age have developed in either dry or wet conditions, and thus can be compared.

¹ "Closed" LMX vessel means that the individual elements that make-up the vessel still have living protoplasts with primary walls (crosswalls) that traverse and thus close the vessel to transpirational flow. In an "open" vessel, the elements (cells) have lignified walls and have lost their protoplast and crosswalls.

Suberization of the endodermis and hypodermis

The endodermis is the layer of cortical cells that surrounds the stele, and the hypodermis is the outer layer of the cortex, immediately underlying the epidermis. In roots of all vascular plants (except the Lycopodiaceae, Priestley and North, 1922), the cells of the endodermis progress through three stages of increased suberization (and lignification) as they get older. These are presented in Fig. 3.1 and consist of Stage 1; deposition of suberin within the existing anticlinal (radial and transverse) primary walls to form a Casparian band, Stage 2; formation of a continuous suberized lamella (layer) between the plasmalemma and the primary wall on all sides of the cell, and Stage 3; formation of a secondary wall (cellulosic but impregnated with lignin and suberin) inside the suberized lamella- in grasses, this wall is often thicker on the inner tangential side (these Stages are described in Priestley and North, 1922, and later in Clarkson and Robards, 1975). The hypodermal cells of many plants, including corn, have also been shown to go through a similar, three-stage sequence of suberization, starting with the formation of a Casparian band in the anticlinal primary walls (Van Fleet, 1950; Peterson *et al.*, 1982; see Permulla, 1990, for a recent survey of plants for formation of Casparian bands). A suberized lamella then forms around the primary wall, followed by some secondary thickening (Ferguson and Clarkson, 1976) (refer to Fig. 3.1 for a diagram of these three stages and how they appear in transverse section).

Many researchers consider the suberized¹(next page) endodermis, and now the suberized hypodermis (occasionally referred to as an "exodermis", Peterson, 1988), to act as barriers to radial movement of solutes and/or water in the apoplast, and even the symplast, if plasmadesmata are blocked (Peterson, 1988; Peterson *et al.*, 1993). However, this is still very much in question (Kochian and Lucas, 1983; Clarkson *et al.*, 1987; see Clarkson, 1993, for a review), as is, still, the pathway followed by water and solutes in the root (Ginsburg and Ginzburg, 1970; Canny and Huang, 1994).

Stage 1

Suberized Casparian band deposited in primary radial and transverse walls

Stage 2

Suberized lamella deposited between plasma membrane and primary cell walls

Stage 3

Secondary suberized wall deposited inside lamella and primary wall of entire cell

Figure 3.1. Three stages of suberization of endodermis and hypodermis

In attempts to elucidate the function of the suberized endodermis and hypodermis, researchers have grown roots in an environment of low water status to check for enhanced suberization and thus reduced ion movement and hydraulic conductance (e.g., Clarkson *et al.*, 1987; Jupp and Newman, 1987; Stasovski and Peterson, 1991; Cruz *et al.*, 1992). Results from these studies are inconclusive, primarily because the studies were conducted under conditions of long term exposure of roots to extreme drought (well below the wilting coefficient of most plants) and this hindered proper root growth. My model system, with only the middle layer at a low or a high soil water content, does not change the overall water status of the root, and roots grow to similar lengths regardless of the water status of the middle layer. In addition, there is a space and time separation between the root tip and the positions of final stages of suberization of the endodermis and the hypodermis along the root, meaning that rates of growth at the root tips have been uncoupled from suberization. Therefore, the experimental system is ideal to study whether localized changes in the soil water content (and osmotic concentration) resulted in changes in the development, i.e., extent of suberization, of the endodermal and hypodermal layers of portions of roots from those controlled layers.

¹ The term "suberized" will be used to generally describe endodermal and hypodermal cells that have initiated and are progressing through the formation of Casparian bands, suberized lamellae and secondary walls.

MATERIAL AND METHODS

Plants

Seeds of corn (*Zea mays* cv. Seneca Chief) were germinated for two days on moist filter paper. Then they were transferred to three-layered profiles of sandy-loam field soil. The roots grew through the soil layers for five additional days. Details of plant growth and preparation of soil profiles are given in Chapter 2.

In one study of branch root formation, plants were left to grow in the profiles for six days instead of five. After six days, the portion of the root in the L2 zone was approximately 19-23 cm from the tip, and thus older, and branch roots had just started to emerge from the root surface and could be counted. In another study, seeds were not germinated for two days on filter paper, instead they were soaked 5 h in distilled water, and then transferred to soil for five days.

Sampling

Results were obtained from at least 50 plants (25 dry / 25 wet treatment) over the course of five experiments. Plants were observed on the day of harvest, or preserved until use in 3.7% formaldehyde, or in 30-50% ethanol. To check for root hair vitality, tissue was prepared and observed immediately upon harvest of the plants. For observations of tissue suberization, plants were used within two days of harvest, maintained in 50% ethanol on ice. Ethanol-preserved material was firmer and therefore easier to section than that kept in formaldehyde, and had less overall autofluorescence, which interfered with observation of suberized regions (see p. 34).

A minimum of five hand-cut sections were observed from each of the three portions (designated A, B, and C; see Fig 3.2) of each root, which had developed within the three zones of the soil profile. Care was taken to sample consistently in each portion at the same distances from the tip of the root for each plant: in portion A (from L1 soil layer), sections were taken at approximately 5-7 and 10-11 cm from the tip; in portion B (from L2), at 15-16 cm; in portion C (L3 layer), at 22 cm and at 28-32 cm (see Fig. 2). Sampling at similar points along the root, notably for the survey of hypodermal and endodermal suberization, ensured that variation between the samples was due to changes in soil environment, and not to age of the tissue. Each root was sampled individually and results from sections subsequently combined, for studies of root hair densities and for the first samples used for observations of suberization. For other studies, sections of root pieces from each portion were immediately pooled with those of similar portions of other roots and observed together.

Sectioning

A piece of tissue (1-3 cm long) was excised from portion A, B or C of a root using a single-edged razor blade, placed in tap water or 70% ethanol and then hand sectioned, using constantly-sharp, double-edged, surgical steel razor blades (to minimize damage by those dulled by soil particles), in the transverse or longitudinal direction. Most sections were 15-30 μm thick. Root hairs were studied on grazing, tangential sections or peels (100-500 μm thick) of the cell layers of the root surface. In most cases, sections were cut under a dissecting microscope in a drop of tap water or 70% ethanol, on soft dental wax. Occasionally the tissue, especially if fresh, was firm enough to allow transverse sections to be made directly between thumb and forefinger without the use of the dissecting microscope (see O'Brien and McCully, 1981).

All prepared sections were mounted on glass slides and topped with coverslips supported by shims (pieces of broken coverslips) to prevent tissue distortion by the weight of the unsupported coverslip.

Figure 2. Diagram of primary root of corn excavated from soil profiles with three layers: L1, L2, L3. Portions of root A, B, C correspond with these layers respectively.

Structural observations

Developmental stage of the late metaxylem (LMX)

Transverse and longitudinal sections (~30 μm thick) through the stele were stained for 30-60 s in 0.05% (w/v) toluidine blue O (Polysciences Inc., Warrington PA, C.I. 52040, Lot # 86864) in sodium benzoate buffer (pH 4.4) (O'Brien and McCully, 1981), rinsed in tap water (3 x 1-2 min), and mounted in tap water. Sections were observed with bright-field optics.

Properties of root hairs

Density and length

Tangential sections of the epidermal surface were either stained with toluidine blue (as described above), or left unstained, mounted root-hair side up in tap water, and viewed using a bright-field microscope. For unstained sections, the iris diaphragm was closed down approximately 75% to enhance contrast. The root hairs were counted through the microscope on an area (square) of epidermis, which was subsequently measured with a calibrated eyepiece. Lengths of root hairs were measured on the same tangential sections, or on transverse sections. Only five to ten of the longest hairs per square were measured, because of the great variation in lengths even on a single section.

Vitality

Tissue was handled quickly and gently to preserve and observe any vitality since root hairs are susceptible to damage when removed from their surrounding soil. Tangential sections or peels of the root surface were taken directly from portion A, B or C of the intact, harvested root, placed briefly in a drop of tap water and:

- i. Mounted in a drop of tap water and immediately observed with Nomarski optics.
- ii. Stained for 5-10 min in 1% neutral red (Chromagesellschaft, Schmid and Co., Stuttgart, Unterturkheim, 1B 469) in 0.025 M phosphate buffer (pH 7.8), (freshly made and filtered every 2 h) (Wenzel and McCully, 1991), then rinsed briefly and mounted in 0.025 potassium phosphate buffer. Root hairs and other epidermal and hypodermal cells were immediately observed with bright-field and Nomarski optics.

Morphology and hydrophobicity

Transverse and tangential sections were observed to determine the shapes of the root hairs, especially their points of attachment with soil. Also, differences in hydrophobicity of the hairs and the epidermis proper between root portions from dry and wet L2 layers were observed. Sections were:

- i. Stained with rhodamine B (1:10,000 in aqueous solution) for 8 min then mounted in either tap water, aniline blue (Polysciences Inc., Warrington, PA, Lot# 1532) (0.05% w/v in phosphate buffer, pH 8.6), or 0.025M dibasic potassium phosphate buffer (pH 8.0) and observed with fluorescence optics.

ii. Left unstained, mounted in tap water and observed with fluorescence optics (to check autofluorescence), or with dark field optics.

iii. Stained with Sudan Black B (described in section below).

Suberization of hypodermis and endodermis

The stages of suberization of the hypodermal and endodermal cell layers (see Fig. 1 showing the three typical stages in their development) were studied using transverse sections either by fluorescence or a bright-field staining procedure.

For the fluorescence method, all sections were first treated with the periodic-acid Schiff's (PAS) reaction (Pearse, 1968) to quench autofluorescence of non-suberized tissue. The procedure was: 10 min in 1% periodic acid (di-hydroxyl groups are oxidized to dialdehydes), 2 x 1 min in tap water, 20 s in Schiff's reagent, and 2 x 1 min in tap water. (Any tissue that had been preserved in formaldehyde was initially soaked for 5 min in 1% sodium borohydride to reduce any interfering aldehyde groups of the fixative.) Most PAS-treated sections were immediately mounted in tap water and viewed. Others were stained for an additional 1-2 h in 0.01% fluorol yellow (Sigma Co., C.I. 26050 lot 40H0556) in polyethylene glycol (400 M.W.): glycerol: water (50:45:5 v/v/v), rinsed briefly in water and then observed with the fluorescence optics (Brundrette *et al.*, 1991).

For bright-field observations, sections were stained 1-1.5 h in 1% Sudan Black B (Mochrome, Edward Gurr Ltd., London) in 70% ethanol, rinsed (3 x 15 s) in 70% ethanol, mounted in 75% glycerol and observed.

Branch root formation

In the first experiment, plants grown for six days in the soil profiles were used. The extra day ensured that branch roots had emerged from the root portions in the dry or wet soils of the L2 layers. Upon excavation of the plants, the number of emerged (i.e., visible on the root surface) branch roots per surface area of main root were counted under a dissecting scope. Also, the lengths of the five longest branch roots were measured.

In the second experiment, the basal branches on roots from seeds that were not initially germinated on filter paper for two days, but planted directly into the soil, were compared with those on roots that grew from seedlings germinated on filter paper (i.e., those roots used for studies in Chapter 2 and all other structural studies of the chapter presented here).

Microscopy and photomicrography

Bright-field observations were made using an Olympus CH or Vanox microscope. Nomarski observations were made with a Vanox Olympus microscope or a Zeiss Axiophot. Fluorescence studies were carried out on a Vanox Olympus microscope using a UV exciter filter (UG-1, 350 nm) and a "U" barrier filter (L-420 with built-in dichroic mirror, DM400) for hypodermal and endodermal studies, and UV exciter and "V" barrier filter (Y-455 with built-in dichroic mirror, DM455) for rhodamine B. Dark-field mounts were observed with an Olympus SZH stereo microscope.

Micrographs were recorded on Kodak TMAX 100 film, Fuji 100 or Kodak Elite colour slide film. Negatives and then prints were made from slides at Glebe Photo Centre. Micrographs of suberized regions were taken using similar exposure conditions (10s, 32s, 60s) for all sections.

RESULTS

Late metaxylem vessels

The presence of primary cross-walls, which stained pink with toluidine blue, between the LMX vessel elements signaled that the elements were still immature (i.e., had intact protoplasts with cellular contents such as nuclei), and that the vessels were closed. Absence of such walls, and the presence of a rim of lignified wall, which stained green, where the broken-down cross walls had been attached, indicated that the LMX vessels were mature (i.e., had lost their protoplasts) and were open. All roots examined (5 dry; 5 wet) had closed LMX vessels, with intact protoplasts and nuclei (Fig. 3.3A and C), all along their length until approximately 27-30 cm distal from the tip. Thus, root portions from both dry or wet L2 zones had immature LMX elements. In the basal regions of the roots (> 27-30 cm from the tip) the LMX vessels were open, lacked cross walls and had rims of lignified wall where the primary cross walls had been anchored (Fig. 3.3E). The number of LMX vessels increased from the tip to the base of all roots from soil profiles with either dry or wet L2 layers. Typically, at 10 cm from the tip, there were five large vessel elements, while seven were developed by 25 cm from the tip.

Root hairs

Density, length and morphology

Root hairs were four and a half times more dense on root portions from dry L2 layers compared to those from wet L2 layers (cp Figs. 3.3B and D, Table 3.1). The density of root hairs also varied along a single root, as seen with the wet samples, which had fewer hairs per unit surface in the wet L2 zone than in the drier L3 and L1 layers (Table

3.1). There was no significant difference in the mean lengths of the root hairs on root portions from dry and wet L2 layers (Table 1). However, the hairs on the dry samples were less straight and tended to be distorted around tightly-held soil particles, compared to the straighter, soil-free root hairs on wet samples (cp Figs. 3B and 3D). This difference in morphology may have resulted in an underestimation of the lengths of the hairs of the dry soil portions. In addition, "branched" hairs appeared to be more frequent on dry samples, and they too may have affected length measurements. Soil was tightly held at points where the hairs branched (Fig. 3B). Bacteria, seen with Nomarski optics, were often associated with the places where soil adhered to bent or branched regions of hairs.

Table 3.1. Density and length of root hairs on portions of primary roots of corn developed in soil of varying soil water content (SWC)

<u>Root portion and soil layer^a</u>	<u>Density^b</u> (# root hairs / mm ² of epidermal surface)	<u>Length^b</u> (nm)
B: Dry L2 (9% SWC)	140 ± 53 ^{c,f,h}	0.78 ± 0.2 ^{c,i}
B: Wet L2 (20% SWC)	31 ± 15 ^{c,f,g}	0.56 ± 0.2 ^{c,i}
A or C: L3 or L1 of Dry or Wet (15-17% SWC)	85 ± 35 ^{d,g,h}	nm ^c

^a Refer to Figure 1

^b Mean ± standard deviation

^c n = 15 sections / 4 roots

^d n = 26 sections / 8 roots

^e nm = not measured

^f significantly different at 2% according to Student t test

^g significantly different at 5%

^{h, i} not significantly different at 10%

Figure 3.3

Figures 3.3A-F. Primary roots of corn grown through soil profiles shown in Figure 1A, Chapter 2. Hand-cut sections of fresh or preserved (formaldehyde or ethanol) tissue. All figures except 3.3E show sections of B portions of root (see Fig. 1, this chapter) developed in dry (9% SWC) or wet (20% SWC) L2 soil.

Figure 3.3A. Wet L2 soil. All the late metaxylem (LMX) vessels are closed. In transverse section, the cross walls and cytoplasmic contents (black arrowheads; white on black arrowhead points to a nucleus) of five immature LMX elements can be seen. The contents of the other two xylem elements in the section which are also immature, have fallen out during processing. The innermost layer of the cortex (C) is the endodermis which surrounds the stele (red arrows), the outermost layer of the cortex is the hypodermis (blue arrows). Black arrowheads indicate epidermis; (e) is early metaxylem vessel. Section stained with the PAS reaction, viewed with bright-field optics (X140)

Figure 3.3B. Dry L2 soil. Tangential section of the root surface stained with rhodamine B, mounted in aniline blue and viewed with fluorescence optics. Numerous root hairs (white arrowheads) extend from the epidermis proper (E). Soil particles (S) are often held in places where the root hairs are bent. (X115)

Figure 3.3C. Dry L2 soil. Longitudinal section through a closed LMX vessel, similar to those shown in Fig. 4A. White arrowheads point to crosswall between two living vessel elements. Red arrowheads show secondary walls of mature early metaxylem vessel. Section stained with toluidine blue, viewed with bright-field optics. (X500)

Figure 3.3D. Wet L2 soil. Tangential section of the root surface. After preparing section, little soil remains adhered to the root. Root hairs (white arrowheads) are less numerous and straighter compared to those in Fig. 4B. (E) epidermis proper. Section unstained, viewed with Normarski optics. (X90)

Figure 3.3E. Longitudinal section through an open LMX vessel of a C portion of root (30cm from the tip) developed in L3 soil (15-17% SWC). The crosswall is gone, leaving rims of lignified secondary wall (black arrowheads). Toluidine blue stain, bright-field optics. (X480)

Figure 3.3F. Dry L2 soil. Root hair stained with Sudan Black B and illuminated with bright-field optics. Note spotted staining pattern, bends in hair (white arrowheads) and embedded soil particles. (X675)

Vitality

Tangential sections of root portions from dry or wet L2 layers, observed for vitality of root hairs in the rhizosheath, were obtained from 10 plants (5 dry and 5 wet) over the course of two experiments. On all sections observed, only two root hairs (observed with Nomarski optics) showed cytoplasmic streaming. Both were on sections from dry-soil L2 layers: one was a very young hair on a newly emerged branch root and the other was also young, but on the main axis of the root. However, all other sections, from dry and wet soil, had root hairs that were not streaming, but were either traumatized when the section was cut, or were recently dead. Their vacuoles were still appressed against the edges of the hairs, and nuclei had not yet rounded-up. In other words, none of these root hairs had been dead for a long time prior to observation; none had aggregated, distorted cytoplasm and/or collapsed cell walls. Young root hairs from root portions from L1 layers (~5 cm from the tip) were streaming.

The neutral red dye was not taken up by any of the root hairs, or cells of the epidermis proper of L1 root portions from of dry or wet soil. However, it was sequestered by the vacuoles of cortical cells of L2 root portions, and by a few of the very young root hairs of L1 root tissue. It is possible that the suberization of the older root hairs (see section below) inhibited penetration of the dye molecules (shown to occur with suberized hypodermal cells; Wenzel and McCully, 1991), or that the vacuoles of the root hairs do not contain the aromatic compounds necessary to trap the dye molecules (Stadelmann and Kinzel, 1972).

Figure 3.4

Figures 3.4A-C. Portions of primary root of corn grown through soil profiles shown in Figure 1A, Chapter 2. All are hand-cut transverse sections stained with rhodamine B, mounted in high pH buffer and observed with fluorescence optics.

Figure 3.4A. Dry L2 soil, B-portion of root. Outer periclinal walls of epidermal cells (ep) are bright yellow-white. The periclinal and radial, anticlinal walls of the hypodermis (h) are more orangey. The cortical cells (c) fluorescence a pale blue. (X900)

Figure 3.4B. Wet L2 soil, B-portion of root. Walls of epidermal cells (ep) are pale orange-red while the walls of the hypodermis (especially the outer periclinal ones) are reddish. The cortical cells (c) fluorescence pale blue. (X900).

Figure 3.4C. L3 soil (15-17% SWC), C portion of root. Walls of epidermal cells (ep) are pale orange (cp Fig. 6B) hypodermal cells (h) pale pink, and the cortical cells (asterisk) just inside the hypodermis also have a pale pink (suberized), thickened wall. (X600).

Hydrophobicity

Root hairs and cells of the epidermis proper of portions from both dry and wet L2 layers autofluoresced, and stained positively with the hydrophobic dyes, rhodamine B and Sudan Black B (Table 3.2). The only consistent, but subtle, difference between dry and wet samples occurred in the epidermis proper. When mounted in tap water and observed with fluorescence optics, the epidermal cells of dry-soil samples autofluoresced a pale yellow colour, compared to those of the wet-soil samples which had a darker, orange-yellow hue. When observed after staining with rhodamine B, the epidermis proper of dry L2 roots had whiter periclinal, outer walls, while the epidermal cells of root from wet L2 layers had a less-bright outer walls, and more reddish periclinal, inner walls, along with reddish hypodermal anticlinal walls (Table 3.2; cp Figs.3.4A and B). Therefore, it is unclear which sample type is more hydrophobic and, overall, the qualitative observations made by eye indicated that a transition to growth in dry or wet soil did not produce a striking change in the hydrophobicity of the surface cells of the root portions.

A difference in the hydrophobicity of the root surface (and outer-most cortical cells) was also seen along each individual root. Compared to portions from the L2 layers, regardless of their SWC, root from L1 layers (~ 7 cm from the tip) showed only weak signs of surface hydrophobicity, while the basal region of the roots (sections ~ 30 cm from the tip, from L3 layers) had much more hydrophobicity in the hypodermal and cortical cells. These changes, related to the age of the tissue, were most evident after rhodamine B staining: the epidermis of the wet soil portion (Fig. 3.4B) was very similar to that of the L3, older portion (Fig. 3.4C).

Noteworthy is the spotted staining pattern produced by Sudan Black B on the root hairs of root portions from dry and wet soil (Figs. 3.3F). Views at higher magnification showed the Sudan Black B had stained specific areas on the plasma membrane, which had

plasmolysed away from the unstained wall. Although Sudan Black B appeared to stain the epidermis proper only weakly, sections were viewed in cross section, and thus only a thin portion of their walls was seen. Tangential observations (not made) are necessary to see if the outer periclinal surface of the plasma membrane or walls of cells of the epidermis proper (i.e., those that did not form hairs) produce a spotted staining pattern similar to that of the root hairs.

Table 3.2. Hydrophobicity of the root hairs and epidermis proper of transverse sections through portions (B) of corn primary root, taken from a dry or wet L2 layer of soil

<u>Stain / Optics</u>	<u>Staining Intensities^a and Colour Differences</u> (root hairs / epidermis proper)	
	Dry (9% SWC) ^b	Wet (20% SWC) ^b
none (tap water) / autofluorescence	(+ / +) pale yellow / yellow	(+ / ++) pale yellow / orange yellow
rhodamine B / fluorescence	(++ / ++) orange / orange white	(++ / +) orange / orange red
Sudan Black B / bright field	(+++ / ND) ^c	(+++ / ND)

^a "+++" = very intense / bright positive fluorescence or staining; "+" = weak positive fluorescence or staining; "-" = no staining

^b n > 25 sections from 5 plants

^c ND = Not determined. Since transverse sections were observed, I was unable to determine if the outer periclinal wall of the epidermis proper cells (i.e., those not forming hairs) were stained in a spotted pattern (see text).

Suberization of the hypodermis and endodermis

The hypodermal and endodermal cells were at similar stages of suberization in root portions from both dry and wet soil L2 layers (sections from 10 dry and 10 wet) (cp Figs. 3.5A and C, with 3.5B and D). The hypodermal cells were in stage one, with Casparian bands, but no strong evidence of suberized lamellae, and the endodermal cells were between stages one and two of suberization, with fully suberized Casparian bands and the occasional suberized lamella (refer to Fig. 3.1 showing these stages and to Fig. 3.3C for orientation of cells layers shown in Fig. 3.5).

Although root portions from dry or wet L2 layers showed no discernible difference in suberization, a characteristic increase, from tip to base, was seen consistently along single roots, regardless of the soil water content of the L2 layer. At ~7 cm from the tip hypodermal cells had not started obvious suberization, and only at ~ 10 cm from the tip were Casparian bands evident in transverse sections. The endodermal cells showed suberization of Casparian bands earlier than the hypodermal cells, at ~5 cm from the tip. In the older, L3 portions, at ~ 30 cm from the tip, where the LMX vessels were lignified and open, the hypodermal and epidermal cells had completed all three stages of suberization, with Casparian bands, suberized lamellae and a thickened, suberized secondary wall. The cortical cells inside the hypodermis also had thickened, hydrophobic walls (Fig. 3.4C).

Branch root formation

The number of emerged branch roots, and their lengths, did not differ significantly between root portions taken from dry and wet L2 layers. Root portions from dry L2 layers had 0.5 ± 0.1 emerged branches / mm^2 of root surface (not including root hairs) (mean $\pm$ standard deviation; $n = 5$ plants), while those from wet layers had 0.4 ± 0.1 branches / mm^2 root surface. Similarly, the five longest branches on portions from dry L2 layers had

Figure 3.5

Figures 3.5A-D. Primary roots of corn grown through soil profiles shown in Fig. 1A, Chapter 2. All figures are of transverse sections through B-portions of root (all 15-16 cm from the tip) (see Fig. 1, this Chapter) developed in dry (9% SWC) or wet (20% SWC) treated with the PAS reaction followed by staining in fluorol yellow, and viewed with fluorescence optics.

Figure 3.5A. Dry L2 soil. Suberized Casparian bands (white-on-black arrowheads) can be seen in the radial, anticlinal walls of the hypodermal cells (h). Suberized lamellae are just starting to form. (rh) root hair, (ep) epidermal cell, (C) cortical cell. (X600)

Figure 3.5B. Wet L2 soil. Section showing similar development to that in 5A. Same labels as Fig. 5A. (X900)

Figure 3.5C. Dry L2 soil. Suberized Casparians bands (white-on-black arrowheads) can be seen in the radial, anticlinal walls of the endodermal cells (e). Suberized lamellae are more developed than in the hypodermal cells of Figs. 5A and B. Late metaxylem (LMX) vessels are immature and not lignified, while early metaxylem (E) vessels have lignified secondary walls. Asterisks mark sieve tubes of phloem poles. (X300)

Figure 3.5D. Wet L2 soil. Section showing similar development to that in 5C. Labels as for Fig. 3.5C. (X225)

a mean length of 5 ± 2 mm ($n = 5$ plants), and those from wet L2 layers had a mean length of 5 ± 1 mm.

Although the different soil water contents of L2 (9% and 20%), did not alter the density and length of branch roots ~20 cm from the tip, germination on filter paper did dramatically change the density of branches at the base of the root. Roots used in rhizosheath measurements of Chapter 2 and in this structural study were germinated on moist filter paper before transfer to soil and consistently produced a few branches in the basal zone (2-3 cm), which contrasted sharply with the numerous branches produced just distal to this zone. However, when the seeds ($n = 3$) were not germinated on filter paper, but soaked for 5 h in distilled water and planted directly into L3 soil, there were many more branches in the basal 2 to 3 cm and their density did not contrast sharply with that of those formed just distal to this area. However, these basal branches, whether on roots initially germinated on filter paper, or on roots planted directly into the soil, were always thicker in diameter and longer than the more distal branches which were thinner and shorter. The LMX was open in the basal region of all roots.

DISCUSSION

Late metaxylem vessels

St Aubin *et al.* (1986), working with cv. Seneca Chief report that the LMX vessels remain closed until 20-40 cm from the tip of all axile roots of the root system, although they do not specifically mention the primary root. Studies with the primary root of maize of other cultivars (e.g., Higinbotham *et al.*, 1972 and others mentioned therein) also show that the LMX vessels are still living, and thus closed to axial water flow, up to 28 cm from the tip. The results here show closed late metaxylem vessels extending approximately 27-

30 cm behind the tips of the primary roots grown through the dry or wet soil profiles. They coincide with previous findings, and show that growth through the wax barriers, and through a dry or wet layer of soil, did not promote or retard the development of the large vessels. This latter observation regarding growth in dry soil contradicts that of Cruz *et al* (1992). Working with sorghum grown in vermiculite, these authors report that the LMX vessels of drought-exposed roots (the first 18 days with water; the next 15 without plus drying air blown through the pots) remained closed longer than in the well-watered controls, and thus reduced axial conductivity. However, their stressed roots grew almost ten times slower, and thus were much shorter than the controls, and only reached maximum lengths of 7-12 cm; 14 cm short of the length at which the LMX vessels matured in the normal controls. This slow growth rate is hardly surprising since the water potential of the dry vermiculite was reported to be -35 bars, far below the point at which most mesic plants will permanently wilt (approximately at -15 bars, Brady, 1990). These researchers could not separate the effect of slow growth at the tip from the rate of xylem maturation as changed by the soil water content, as I have (see discussion of *Suberization of the endodermis and hypodermis*).

Root hairs

No previous study has quantified the density or lengths of root hairs on corn roots growing in field soil. However, Dittmer (1937) reports a density of 25 hairs/mm length of rye root (does not specify type of root or age) when grown in the field, and hair lengths ranging from 0.3 to 1.5 mm on a variety of field-grown grasses not including corn (Dittmer, 1949). Nye and Tinker (1977), generalizing from values in the literature, say that densities on grasses fall between 50-100 hairs/mm length of root (the number of hairs is counted on the entire surface of a length of root; from Dittmer's work, 1937). Although none of these measurements account for the diameter, and therefore actual surface area of

the root, and the studies do not mention the water content of the environment, the values appear to fall within the range of my density (approximately 100 hairs / mm²) and length (0.7 mm) measurements (see Table 1).

In accordance with my findings of root hair density (Table 3.1; Figs. 3.3B and D), Weaver and Himmel (1930) (working with hydrophytes such as *Typha latifolia*), and North and Nobel (1992) (working with the xerophytes *Ferocactus acanthodes* and *Opuntia ficus-indica*), observed increased hair production as the soil water content decreased. On the other hand, Meisner and Karnock (1991), working with soil-grown peanut roots, did not find a significant difference in root hair density in wetter and drier soils, and Vartanian (1981) reports absence of hairs on *Sinapis alba* in "drought" conditions (does not give water content, but says that tip elongation had practically ceased). Kramer (1983) mentions that root hairs (does not specify types of plants) are inhibited in "very dry" conditions. Finally, Dittmer (1938) reports 10 times more root hairs on field-grown rye compared with that grown in the greenhouse in soil, but does not relate the difference to soil water content.

All other studies I encountered on root hair production were with plants growing in nutrient solution or humid air (e.g., Tanaka and Woods, 1972; Moore, 1974; Filippenko, 1980; Miller, 1981; Jaunin and Hofer, 1986). Unfortunately, observations made in these studies (as with the soil observations) were qualitative only, similar to the observations provided in the few reviews of root hair development in the literature (e.g., Cormack, 1962; Hofer, 1991). However, the culture studies all report that drier conditions stimulate root hair production. Specific to maize, Filippenko (1980) and Jaunin and Hofer (1986), working with the tips of primary roots, show that no hairs will form in water, only in humid air. These results correspond with my findings, that is, the wet soils suppressed hair production (or dry soils promoted hair development) and the number of hairs changed as the root tip progressed through the soil profiles (Table 3.1). Filippenko (1980) state that the determination of epidermal cells to become hairs occurs in the elongation zone, just behind the meristematic cells of the tip. The spatial and temporal separation between the

meristematic tip and the elongation zone is too short (approximately 2-3 mm; the width of my wax barriers) for me to determine which place receives and sends the signal of the wet or dry soil. Jaunin and Hofer (1986) believe that the destiny of epidermal cells to become hairs is determined only in the tip region (they do not specify which part), and that new hairs cannot be produced past the elongation zone. However, others have reported the initiation of new hairs far back from the tip (see Cormack, 1962). The results presented in this chapter do not prove new hairs were formed subsequent to passage of the tip through the dry zone (although some hairs were shorter than others); to show this, a specific portion of the root would need to be observed (hair density calculated) immediately upon growth through the soil, carefully replanted, and then excavated and re-examined after specific time intervals.

Distorted root hairs are recorded in the literature (Sachs, 1865; Goossens and Stapelberg, 1933; Dittmer, 1949; Hofer, 1991). Dittmer (1949), in a survey of many field grown plants, points out that soil has little to do with such distortion since the roots of certain species had crooked hairs, while others of different species had uniformly straight hairs, despite all having grown in the soil. He did not change the soil water content for any of the plants, thus did not notice an increase in distortion on similar plants when grown in drier conditions compared to wet, as I did (cp Figs. 3B and 3D). Unpublished studies in the McCully and Canny laboratory have shown that bacteria from the sheath can cause deformation of root hairs in the absence of soil. It might be expected that the soil water content would influence the microflora associated with the root, thus indirectly altering the shapes of root hairs.

A hydrophobic, fatty layer, similar to cutin and suberin, which increases in thickness as the hairs get older, has also been reported in previous studies on root hairs (Cormack, 1962; Scott *et al.*, 1963; Scott, 1963; Kramer, 1983; Hofer, 1991). This agrees with my observation of a hydrophobic layer on the hairs from both dry and wet L2 layers. Although both dry and wet samples stained with the hydrophobic dye rhodamine B, the

staining was more intense with the dry samples, indicating that the drier environment stimulated the hydrophobicity (see Figs. 4A and 4B). The Sudan Black B produced an interesting spotted pattern on the plasma membrane of the hairs. Scott *et al.* (1963) report pits or pores on root hair *cell walls* that have been isolated from the protoplast by sonication. They propose that these pores are places where lipids are delivered to, and deposited on, the outside of the wall. Perhaps the hydrophobic regions I observed on the plasma membrane were originally attached, via periplasmic strands, to the wall pores seen by Scott *et al.*

There was no sign that the soil of the dry L2 layer promoted death of root hairs. Again I could find no careful studies of root hair vitality of root hairs growing in field soil (although Jupp and Newman (1987) report live root hairs at -10 MPa), and I agree with Newman (1974) who says that there is no evidence to confirm that water stress kills root hairs, although the assumption is often made (e.g., Kramer, 1983). Under severe drought, when the entire root shows definite signs of desiccation (i.e., Stasovski and Peterson, 1991 where vermiculite-grown plants were not watered for 34 days and had zero water for the last 8), of course the root hairs are killed, together with the cells of the epidermis and cortex. But, under the extremes of normal field conditions (similar to the 9% and 20% SWC's used in my study), the hairs of corn, and likely of many of the grasses (Dittmer, 1937), can remain alive. This would imply that the numerous root hairs of the larger sheaths formed in drier soils are streaming and metabolically active, and thus involved in nutrient and water absorption (Cailloux, 1972; Newman, 1974).

Although the drier soil resulted in more numerous, longer, and more hydrophobic hairs, it is not clear that the actual water content *per se* caused the observed changes. It is possible that a corresponding change in ion concentration in the soil solution occurred and thus affected hair development (as seen with calcium and root hair density when grown in nutrient solution, Tanaka and Woods, 1972). Perhaps a shift in pH (note that this in turn would alter nutrient availability) changed the hairs (Moore, 1974, reports longer hairs in

solutions with higher pH). Differences in density of hairs have also been attributed to changes in concentration of oxygen or carbon dioxide in soil (Weaver and Himmel, 1930; Cormack, 1962; Hofer, 1991). For example, Weaver and Himmel (1930) speculate that wetter conditions resulted in decreased aeration, which in turn limited the oxygen for the cells and interfered with their respiration. In addition, Scott (1963), believed that increased exposure to "air" increases the lipid (or hydrophobic) content of the walls of root hairs. This corresponds with my observations where the drier soils (with more air-filled pores) resulted in more hydrophobic hairs. Interestingly, Snow (1905, reported in Dittmer, 1938) felt that decreased growth rate of epidermal cells meant an increase in the likelihood of forming hairs. However, the roots used in my study, whether grown through a dry or wet middle layer, had no significant difference in total length (data not shown), and differences in other developmental features such as endodermal and hypodermal suberization or maturity of LMX vessels, were not observed. In addition, root hairs from dry or wet soil appeared equally healthy. These points would indicate that the rate of extension in the dry or wet layer was not different, or too subtle to detect and was masked by measuring only the total length (perhaps roots that grew slowly in the mid-portion, sped-up proportionally in the lower portion).

Suberization of the endodermis and hypodermis

Roots from both dry and wet samples were starting to form endodermal Casparian bands at approximately 5 cm from the tip, and hypodermal Casparian bands were evident at approximately 10 cm from the tip. The position of the development of the endodermal Casparian bands was farther back from the tip than distances reported in the literature for corn (cv. Seneca Chief) primary root; for example, Peterson and Permulla (1984), working with solution-grown roots, report it at 2-3 cm from the tip; Permulla and Peterson (1985), studying vermiculite-grown roots, at 1.2-2 cm from the tip. However, Sutherland (1976),

also working with solution-grown Seneca Chief, reports Casparian bands at 4-5 cm from the tips of rapidly-growing roots. The hypodermal Casparian bands appeared later in my roots compared to those in the study of Peterson and Permutt (1984), where the bands were reported at 4-5 cm from the tip, but corresponded with the distance of 12 cm from the tip observed by Permutt and Peterson (1985) in cv. Seneca Chief. In the latter paper, the authors point out that the position at which the hypodermis becomes suberized is highly variable, and can depend on plant species, age and growth rate. The same has been said for the development of the endodermis which has also been shown to be dependent on growth rate; the slower the rate, the closer the development to the tip (Wilcox, 1962). Unfortunately, very few studies of endodermal and hypodermal development are done with field-grown roots, and those performed on roots grown in other media often do not state from where the tissue has been sampled. Therefore, until I perform controls with roots grown through soil profiles without wax barriers, or through soil profiles with wax barriers but with middle layers of normal water content (15-17% SWC), I am not able to say that the roots studied here had delayed endodermal and hypodermal suberization compared to roots grown in normal field conditions.

Exposure to dry versus wet soil in the L2 layers did not change the development, i.e., the extent of suberization and rate of progression through Stages 1, 2 and 3, of the endodermis and hypodermis of portions of primary root of similar age (Fig.3.5). This contradicts the findings of Jupp and Newman (1987), Stasovski and Peterson (1991) and Cruz *et al.* (1992). However, Jupp and Newman (1987) grew the plants in clay loam soil; the other researchers use vermiculite (Cruz *et al.*, 1992) and/or Turface (Stasovski and Peterson, 1991), and this, coupled with the long periods of withholding water (i.e., their soils got drier and drier for 20, 15 and 34 days, respectively), makes it very difficult to compare their soil water conditions with those of my experiments. I can only say that Jupp and Newman (1987) observed plants that had grown in soil in the range of -20 to -50 bars; Cruz *et al.* (1992), around -30 bars; Stasovski and Peterson (1991) -30 kPa until zero

water content, depending on the stage of the experiment. They were all drier than my dry conditions (approximately -10 bars), which approximates dry field conditions. In these studies, as noted in the Introduction, especially in the case of the study by Cruz *et al.* (1992), the growth rates of the water-stressed plants were extremely slow compared to the controls, and this caused maturation of the cells to occur closer to the tip. When the tip is very close to the differentiating endodermal and hypodermal cells, these researchers are unable to distinguish whether the internal conditions of the tip, i.e., slow growth, result in observation of enhanced suberization, or the external conditions of the surface cells produce the signal to suberize. In my experiments, the cells in the tip are rapidly dividing and are at least 5 cm from the suberizing endodermis, and at least 10 cm from the suberizing hypodermis; the tip cells are uncoupled from the surface in contact with dry or wet soil. Therefore, I am able to conclude that the endodermal and hypodermal cells did not differentiate differently due to local differences in soil water content, as these other studies have reported. I did not change the overall water status and growth rate of the root, and the soil water conditions were comparable to those found in the field.

A complete discussion of the evidence surrounding the role of the endodermis and hypodermis in the pathway of water and nutrients in the root is beyond the scope of this chapter. However, it is important to emphasize that the results presented here show that the suberization of either layer is not changed in response to the soil water content, but rather in response to age and position along the root. In other words, the root does not adapt to the local water status of the soil by modifying the suberization of these apoplastic barriers. This is in contrast to the changes seen in the outer layer of cells where the root hairs and epidermis proper showed modifications in dry versus wet soil (Table 1 and 2, Figs. 4A and 4B). Interestingly, Varney *et al.* (1993), working with the roots of full-grown maize plants, conclude that water enters the roots at the outer-most cell membranes. Canny and Huang (1994) also conclude that solutes enter this outer surface. My results support the view that water flow is symplastic and does not follow the walls of the apoplast (Canny

and Huang, 1994). It seems likely that water flow is through the cells where it can be controlled actively, while it seems unlikely that roots, especially in drier conditions, would make the pathway, if apoplastic, more suberized, thus limiting uptake of what little water is available (although preventing desiccation), and limiting uptake of water that arrives post drought. It is difficult to say how the observed suberization of the root surface functions in water and ion uptake. It would be interesting to conduct a study on root portions from the dry and wet soil layers of my soil profile, similar to that of Canny and Huang (1994), employing the technique of O'Dowd and Canny (1993) to see if and where the rates of diffusion of dye molecules are altered from exposure to different soil water contents.

The role of the suberized endodermis and hypodermis in water and ion uptake is still very much in question. In fact, studies have shown that the *highest* rates of water and ion uptake along roots correspond with the *heavily* suberized regions (e.g., Clarkson *et al.*, 1968; Clarkson *et al.*, 1987; Varney *et al.*, 1993), and that the hypodermis or exodermis may not act as a barrier to solutes (Kochian and Lucas, 1983), although Varney *et al.*, (1993) state that it limits movement in the wall apoplast, which has been shown to be a very slow path for diffusion of ions anyway (Canny and Huang, 1994). These findings are further confused by observations of ions in the intercellular spaces of the root cortex of corn (Canny and Huang, 1993), and of "breaks" in the suberized layers of the endodermal and hypodermal cells (Canny and Huang, 1994; X.L. Wang, Ph.D. Thesis, Carleton University, 1994).

Branching

Although not related to rhizosheath development, worth mentioning is the change in density of basal branching observed between seedlings in which germination was on filter before transfer to the normal soil profiles (with wax barriers), and those in which germination was directly in the soil profiles (but without wax barriers). Either germination

in the Petri dish caused less branching, or contact with the wax barrier by the extending tip altered the branching pattern. If the wax barrier had caused the reduced branching, a second region of reduced branching as the tip had encountered the second barrier would be expected. Although it is unlikely that the wax barrier was the causal agent, the control to clarify the validity of the latter explanation must be performed, that is, plants must be planted directly in soil profiles which include the hydrophobic barriers. It is difficult to guess what factors in the Petri dish environment *per se* caused less basal branching: the availability of water (the filter paper is wet, however the air in the dish is relatively dry compared to the soil of the seed bed) or the lack of contact with, for example, soil, are both possibilities. This observation should be followed up with definitive experiments.

Although the basal branching was visibly altered by germination on filter paper versus soil, the distal branch root emergence (at ~20 cm from the tip) was not significantly different in the dry and wet soil of the L2 layers (see first paragraph of *Branch root formation*). Again this is only preliminary evidence for the effect of soil water content on branching, and more samples must be observed; perhaps also counting root primordia that have not yet emerged from the root surface. However, these distal branches appeared shorter, and smaller in diameter, than the basal branches, and thus may not be affected to the extent seen with the basal branches by changes in the environment (recently studied by L. Enns, Honours Project, 1994, McCully and Canny Laboratory). Interestingly, Cannon (1949), in studying the architecture of root systems, noticed that the basal branches (first order) of the maize primary root were longest, and says that they may be important for obtaining rainfall from the surface layers of soil. This would certainly be true when the seedling is young, and the nodal roots have not started to dominate the maize root system.

CHAPTER 4

SURVEY FOR RHIZOSHEATH FORMATION IN FIELD-GROWN ROOTS

INTRODUCTION

At the beginning of this Master's study, I grew a range of plants in the field to compare the extent of rhizosheath formation on several grasses, and to check for evidence of sheathing on some dicotyledenous plants. Upon their excavation and transport to the lab, I decided to check the overall architecture of the root systems for similarities between those plants that do, and those that do not, form sheaths. In addition, I was interested to find specific features common to roots that formed sheaths, i.e., root hair formation in younger regions and root tips that produced a soil-sticking mucilage. In order to determine if rhizosheath formation affects mineral uptake into the root, some roots with rhizosheaths were quick-frozen in liquid nitrogen soon after excavation, and then analysed for ion concentrations, held *in situ*, in the root.

This chapter presents most of the data collected in the above-mentioned survey. Although it is somewhat unrelated to the studies of Chapter 2 and 3, which apply an experimental system to relate soil water content to rhizosheath and root development, the chapter here is still very much concerned with sheathed roots. The data (although much is preliminary) allows for discussion of the factors involved in rhizosheath development and their possible function in the field. It also points to areas in the study of roots still awaiting research.

MATERIALS AND METHODS

Plants

Field-grown

Seeds of corn (*Zea mays* L. cv. Seneca Chief), barley (*Hordeum vulgare* L. cv. Bruce), wheat (*Triticum aestivum* L. cv. Katepwa), rye (*Secale cereale* L. cv. Gazelle), annual ryegrass (*Lolium multiflorum* L.), flax (*Linum usitatissimum* L.), sweet clover (*Melilotus alba* Mill.), red clover (*Trifolium pratense* L.) soybean (*Glycine max* L.), radish (*Raphanus sativus* L. cv. Cherry Belle) and hairy vetch (*Vicia villosa* L.) were planted in August, 1992 in a plot of sandy loam soil at the Central Experimental Farm in Ottawa. At 25 and 35 days the seedlings were excavated, observed and then transported back to the laboratory in a bag of field soil. Once in the laboratory plants were:

- i. Immediately photographed and then used for macro and microscopic observations, or
- ii. Preserved in 3.7% formaldehyde or 30% ethanol (both aqueous) for future light microscopic observations, or
- iii. Pressed (1 year between newspaper) in a herbarium press, glued onto herbarium paper, and then photographed, or
- iv. Sectioned in portions (~3 cm in length) taken various distances from the root tips, quick frozen and stored in liquid N₂ (-196°C) in a Thermolyne Locator 8 Cryobiological Storage System until used for scanning electron microscope studies.

Axenicallly-grown

Seeds of corn, wheat, rye, hairy vetch, sweet clover and flax were surface sterilized for 6 min in 6% sodium hypochlorite (commercial Javex), washed three times in sterile distilled water, soaked at room temperature in sterile water for 4h, then transferred to sterile, sealed Petri dishes (lined with Whatman filter paper moistened with 12 ml of sterile distilled water) for 5 d at 26°C. Germinated seedlings were used to test the soil binding ability of the root cap mucilage (see *Soil binding*, p.63).

Preparation of tissue for microscopy

Light microscopy

Entire roots, pieces of root (1-3 cm long), and transverse and longitudinal hand cut sections (30-100 μm thick) of roots were observed with a stereo microscope using both dark and bright-field optics. Thin sections were also observed with a bright-field compound microscope. Occasionally the transverse and longitudinal sections were stained (30-45 s in 0.05% (w/v) toluidine blue O in sodium benzoate buffer (pH 4.4) [O'Brien and McCully, 1981] followed by 3 x 1-2 m rinses in tap water), otherwise the tissue was observed unstained. Both stained and unstained tissue were mounted in water, in shallow Petri dishes for larger tissue which was observed under the dissecting microscope, and on microscope slides under coverslips supported by shims for sections under the microscope.

Cryo-scanning electron microscopy

Pieces (~3 cm) of frozen root were taken from the Locator, trimmed under liquid N₂ and were mounted, either vertically for cross-sectional observation, or horizontally for

tangential, surface observation, with Tissue Tek (Miles Inc., Elkhart, IN, USA) on aluminum stubs and immersed in liquid N₂.

For pieces which were to be observed in surface view, the stub and frozen tissue were immediately transferred under liquid N₂ to the sample stage (-170°C) of the cryo-scanning electron microscope, coated (~50 nm thick) with evaporated Al, and observed at a voltage of 7 kV. For observation of root pieces in cross section, each stub and tissue was transferred under liquid N₂ to the chuck of a cryomicrotome (CR2000, Research and Manufacturing, Inc., Tucson, AZ, USA) and, at -80°C, planed with a pre-cooled glass knife to a smooth surface. Planed samples were transferred to the sample stage of the scanning electron microscope and observed uncoated at 1 kV as the stage warmed to ~ -90°C. This warming caused slight etching of the sample surfaces, and was stopped when outlines and internal striations of the cells became visible. Then samples were cooled again, coated with Al and returned into the sample stage (-170°C) for observation.

Qualitative and quantitative studies

Architecture of root systems and surface features of roots

The architecture of the root systems was observed and the degree of rhizosheath development noted immediately upon excavation of the plants. The surface features, including presence of root hairs, regions of adhered soil and spatial arrangements of rhizosheaths, if present, and nature of root caps and their mucilage, were also noted.

For a few species, diameters of roots, lengths of root hairs and thicknesses of rhizosheaths were measured. To do this, stained (toluidine blue; described above), hand-cut, transverse sections (30-100 µm) of formaldehyde or ethanol-preserved material were measured through a bright-field microscope with eyepiece and stage micrometers.

*Soil-binding by root-cap mucilages (method of Watt *et al.*, 1993)*

Roots of the axenically-grown seedlings were excised from the seed and their tips placed on moist pieces (0.5 cm x 0.5 cm) of sterile nylon transfer membrane (Hybond N⁺, Amersham Canada Ltd., Ontario). The roots and nylon were left overnight in sterile, moist, sealed Petri dishes to allow any mucilage from the root caps to exude and bind onto the membrane surface. Then, the roots plus nylon were left to dry 2h, briefly re-wetted, and the roots removed from the nylon membrane.

Sterile field soil (sieved to particles $\leq 74 \mu\text{m}$ and autoclaved 1 h) was sprinkled onto pre-wetted (with sterile distilled water) pieces of nylon membrane where root tips had lain. Nylon membranes plus soil were left to air-dry, then rinsed under gently running tap water. Depending on the root sample, different amounts of soil remained adhered to root treated nylon membrane. The amount of soil was quantified by placing the nylon membrane with mucilage plus soil under an optical densitometer (MacBeth TD 5011.B), and reading the optical density (O.D.) of a standard surface area for each sample. The O.D. of the nylon alone was subtracted from this value, giving the O.D. of the soil. (Previous studies (data collected for Watt *et al.*, 1993) showed the difference between the O.D. for a piece of nylon membrane alone, and nylon membrane plus mucilage is negligible.)

Root and soil-treated nylon membrane were stained with toluidine blue, Coomassie blue or by the periodic acid Schiff's reaction to determine presence of mucilage on the nylon membrane.

Ion concentrations in root and rhizosheath

The microanalytical capability of the cryo-scanning electron microscope was used to locate and quantify concentrations of ions in and on the root surface, and in the

rhizosheath. Root portions were mounted, etched, planed and coated with Al for transverse observation (described p. 61).

Energy dispersive x-ray microanalysis (EDX) was done with a LINK eXL LZ-4 system using a Be-window at 15 kV with probe current set at 1.00 nA. A standard area scan raster covered the portion of the root analysed. Concentrations (mM) of elements were calculated from formulae derived from analyses of frozen standard solutions (Canny and Huang, 1993). These concentrations were mapped onto drawings from SEM micrographs of the analysed regions of root plus sheath to see any patterns of ion accumulations.

Microscopy and photography

Light microscopy was done with an Olympus CH or Vanox microscope, or an Olympus SZH Stereomicroscope. Cryo-scanning analytical microscopy was done with a JOEL 6400. All pictures were taken on TMAX 100 film.

RESULTS

Architecture of root systems and rhizosheath formation

The architectures of the excavated root systems of the grasses were similar to each other. The younger nodal roots tended to arch over the older, longer nodal and seminal roots, which hung down in the vertical axis (Fig. 4.1). Depending on the species of grass, however, the difference in length between the younger nodal roots and the older (usually seminal) roots, varied (cp. Figs. 4.1A and B with 4.1C and D).

Table 4.1. Qualitative observations of field-grown roots related to rhizosheath development

<u>Plant</u>	<u>Rhizosheath developmental sequence^a</u>	<u>Extent of sheathing^b</u>	<u>Root hairs^c</u> younger region	<u>Root hairs^c</u> older region	<u>Root tips^d</u> adherent soil?	<u>Root tips^d</u> expanded in water ^e
Wheat (n=10)	yes	++++	VLD	+ then -	yes	yes
Barley (n=10)	yes	++++	VLD	+ then -	yes	yes
Rye (n=8)	yes	+++	VLD	+ then -	yes	yes
Rye grass (n=8)	yes	+++	VLD	+ then -	yes	yes
Corn (n=5)	yes	+++	LD	+ then -	yes	yes
Hairy Vetch (n=20)	yes	+++	VLLD	+ then -	yes	yes
Radish (n=16)	yes	++	VLLD	+ then -	nd	nd
Sweet Clover (n=10)	maybe	+	SS	+ then -	no	no
Red Clover (n=5)	maybe	+	SS	+ then -	no	no
Soybean (n=6)	maybe	+	SS	+ then -	no	no
Flax (n=20)	no	-	VS	VS	no	no

^a Youngest portions of the roots are covered in a coherent layer of soil resistant to removal by shaking; oldest portions of the roots are relatively bare and soil-free.

^b "++++" = large rhizosheath relative to the diameter of the underlying root; "++" = rhizosheath; "+" = soil adhered in patches; questionable whether it constitutes a coherent rhizosheath; "-" = few particles of randomly adhered soil; "0" = virtually no adhered soil

^c "VLD" = very long (relative to diameter of root), dense root hairs; "LD" = long and dense; "VLLD" = very long but less dense than hairs of grasses; "SS" = short and sparse; "VS" = very sparse; "+ then -" = number of root hairs present decreases as the root gets older

^d Root tips that still retained their root caps after removal of the root system from the soil

^e Some root tips expanded when placed in water indicating that their root cap cells produced, and were surrounded by, mucilage that swells in water

Figure 4.1

Figures 4.1A-D. Grasses grown in the field (plot of sandy-loam soil, Experimental Farm, Ottawa) late summer, 1992.

Figure 4.1A. Corn (*Zea mays* L. cv. Seneca Chief) (27 d old) Primary root or seminal root (black arrowheads), coming from seed (red arrow) has lost its sheath and only has soil (hollow arrow) caught between basal branches. The tier 1 nodal roots are branched and have little sheath (e.g., white on black arrowhead). Tier 2 nodal roots are sheathed (blue arrowheads) starting approximately 2cm from the tips which have soil adhered to their root caps (blue arrows). (X0.5)

Figure 4.1B. Barley (*Hordeum vulgare* L. cv. Bruce) (27 d) Both plants have seminal roots (emerging from seeds: red arrows) that are in the process of losing their sheaths (black arrowheads). The nodal roots coming from the base of the stems are heavily sheathed (blue arrowheads) to approximately 1 cm from the tips which have soil adhered to their root caps (blue arrows). (X0.57)

Figure 4.1C. Wheat (*Triticum aestivum* L. cv. Katepwa) (34 d) Seminal roots (black arrowheads) are completely bare while nodal roots are heavily sheathed (blue arrows) to approximately 1cm from the tips which also have soil adhered to their root caps (blue arrowheads). Red arrow: seed. (X0.5)

Inset: Transverse section through a sheathed nodal root of wheat (r). A dense mat of root hairs (white arrowheads) protrude from soil of sheath(s). There is still much soil present after sectioning and mounting in water (X18)

Figure 4.1D. Rye grass (*Lolium multiflorum* L.) (27 d) Seminal roots (e.g. black arrowhead) are bare, and roots from the mesocotyl (green arrow) are also bare, or are in the process of losing their sheaths. Nodal roots from stem base are heavily sheathed starting approximately 1.5 cm from the tips which have soil adhered to their root caps. (X0.5)

The shapes of the grass root systems contrasted with those of the dicotyledons, which consisted of a main taproot (thicker at the base) with laterals extending from it. Radish, however, was an exception with numerous branches at the top of a long tap root and this shape is analogous to that of buckwheat- a dicotyledon that appears to form rhizosheaths, which was shown to me by M. McCully, but not studied in this survey.

In the grasses, as was expected, large coherent rhizosheaths formed on the youngest portions of their root systems, while the oldest portions were bare (Table 4.1; Figs. 4.1A-D). Generally, the younger nodal roots were each completely covered in a rhizosheath, while the older nodal roots were either completely sheathed, in the process of losing their rhizosheaths, or completely bare with no sheathing, even behind their tips, after excavation (Fig. 4.1). The seminal roots, which were the first to emerge from the seed (and thus are the oldest), were completely bare (Figs. 4.1B-D), with the exception of the primary root of corn (the only seminal root in cv. Seneca Chief), which had soil clumped between the numerous branches at its base (Fig. 4.1A). Wheat and barley had the most substantial rhizosheaths, closely followed by rye, ryegrass and corn (Fig. 4.1, inset Fig. 4.1C). The rhizosheaths on these plants were resistant to removal by shaking, and were tightly-bound to the root (Fig. 3C) and remained attached to the roots even after rinsing, sectioning and staining (inset Fig. 1C). In water, under the dissecting microscope, these rhizosheaths could be gently pried apart. Much of the soil held fast at distorted points along root hairs, and on the epidermis proper. The rest of the soil, instead of diffusing away, stayed in the vicinity of the sheath, held by what appeared to be mucilage expanding into the surrounding water.

The only dicotyledon that showed substantial rhizosheath formation was hairy vetch (Table 4.1, 4.2; Fig. 4.2B). The roots had a coherent layer of soil that covered the entire root system. However these rhizosheaths did not appear as tightly-bound, nor were the component soil particles as closely-packed within the sheath as those of the grasses (cp insets of Figs. 4.1C and 4.2B). However, expanding mucilage retaining loosened soil was

also noted when hairy vetch roots were placed under water. The other dicotyledon with signs of rhizosheath development was radish (Fig. 4.2A). Its basal branches had rhizosheaths, and were strikingly shorter than the bare primary (tap) root. However, the radish rhizosheaths were less coherent and less tightly-bound to the root than those of the hairy vetch and the grasses. Sweet and red clover, and soybean had soil bound in patches, generally in the youngest portions of the roots. However, this "sheathing" was very patchy and did not form a coherent covering over the root, and was easily dislodged. Flax root systems were completely devoid of bound soil (Fig. 4.2C).

The formation of rhizosheaths coincided with the presence of long and/or dense, deformed root hairs (Table 4.1 and 4.2). In the roots measured, hairs extended at least the width of the rhizosheath (Table 4.2), and in the case of hairy vetch were twice as long. Older regions of the grass roots that had recently lost their sheath, still had hairs, although they appeared collapsed and deteriorating. The bare seminal roots of wheat, barley and ryegrass had completely lost their epidermis and most of their cortex, and were composed only of a stele surrounded by a heavily suberized endodermis.

Rhizosheath formation also coincided with the presence of root tips that, when excavated from the field had adherent soil (Figs. 4.1 and 4.2B), and when in water, had a bulbous shapes and were larger in diameter than the root proper. Therefore, they had root cap cells that produced a mucilage that expanded in water. These root tips had adherent soil that seemed hold the mucilage around the tip and remained bound to the tip, during at least the time of observation (up to 30 min). In contrast, the root tips (when present, as many appeared broken off) of roots with little (patchy), or no sheathing appeared small and pointy. These never had bound soil. Therefore, these root tips did not have a mucilage that expanded in water, nor bound soil, as was seen on the grasses and the hairy vetch. This was confirmed by the soil-binding studies with immobilized, root-cap mucilage of axenic seedlings. The wheat, rye and hairy vetch root-cap mucilage bound similar amounts of soil, while clover and flax bound much less (Table 4.3; Figs. 4.2d-h). Rye observed

with the scanning electron microscope showed soil particles and root hairs coated with mucilage (Fig. 4.3B). In summary, plants that formed rhizosheaths in the field had numerous root hairs in the youngest portions of their roots, had root tips that expanded in water and produced a mucilage able to bind soil. This contrast was most evident in the dicotyledons, specifically between hairy vetch and flax.

Table 4.2. Ratio of lengths of root hairs to thickness of sheath

Species ^a	<u>lengths of root hairs</u> ^{b, c} thickness of sheath
barley	1.2
corn	1.7
rye	1.3
hairy vetch	2.13

^a All plants used had been preserved in ethanol or formaldehyde; therefore some soil of sheath had rinsed away; sections from a minimum of 5 roots from 5 plants measured

^b Ten longest root hairs measured on each section

^c Ratio of means

Table 4.3. Optical densities of soil adhered to immobilized root-cap mucilages of axenically-grown seedlings

Plant source of mucilage	Optical density of soil ^a
Wheat (n=5) ^b	0.4 ± 0.06
Rye (n=3)	0.2 ± 0.07
Hairy vetch (n=4)	0.2 ± 0.1
Sweet clover (n=5)	0.08 ± 0.03
Flax (n=5)	0.04 ± 0.03

^a Mean ± standard deviation

^b n = number of pieces of nylon membrane with immobilized mucilage

Figure 4.2

Figures 4.2A-C. Dicotyledonous plants grown in the field (plot of sandy loam soil, Experimental Farm, Ottawa), late Summer, 1992.

Figure 4.2d-h. Pieces of nylon membrane with immobilized axenic root-cap mucilage. This has been hydrated, treated with soil, dried with the soil and rinsed.

Figure 4.2A. Radish (*Raphanus sativus* L. cv. Cherry Belle) (34 d) Taproot (white arrowhead) is relatively free of branches except for those at the base which have much adhered soil (black on white arrowheads). (X2)

Figure 4.2B. Hairy vetch (*Vicia villosa* L.) (27 d) All roots are sheathed (blue arrowheads) including main root which has nodules (small red arrows). Sheathing begins approximately 1 cm from the tip. As in the grasses, these tips have bound soil on their root caps (blue arrows).

Inset: Transverse section (fallen on its side) of hairy vetch root (r= root proper). After sectioning and mounting in water some soil particles (s) are anchored tightly to places along the hairs (white arrowhead). (Compare with inset, Fig. 1C) (X1)

Figure 4.2C. Flax (*Linum usitatissimum* L.) (27 d) Root system devoid of soil except for a small region at the base (black on white arrowhead). Root tips (inverted white Vs) have easily broken off and/or show no adhered soil. (X1.5)

Figure 4.2d. Wheat. (X20)

Figure 4.2e. Rye. (X20)

Figure 4.2f. Sweet clover. (X20)

Figure 4.2g. Hairy vetch. (X20)

Figure 4.2h. Flax. (X20)

Ion concentrations in and around roots with rhizosheaths

Analyses were done to measure any differences between the concentrations of ions found in epidermal cells with tightly bound soil of the sheath, and epidermal cells with no bound soil (i.e. up against a space in the sheath) (Fig. 4.2D). In addition, concentrations of elements found in the grooves of the radial walls of the epidermal cells were compared to concentrations on the tangential surfaces of these cells (Fig. 4.3E). Very few samples were analyzed due to the time needed to obtain an ideal sample for analysis. It is particularly difficult to plane the root plus soil without breaking off pieces of rhizosheath and thus disturbing the *in situ* arrangement of sheath around the root (see Fig. 4.3A). There were a variety of elements analysed for, however, since K is the most abundant nutrient element in plants, and an element likely to benefit from the high surface area and close spatial arrangement of the sheath (it has a low diffusion coefficient), I have chosen it for comparisons of concentrations in different places in the sheath and root. Although the analysis does not distinguish ionic forms, most K is expected to be in its cationic form, so will be referred to as such.

The few results provide preliminary evidence for the accumulation of higher concentrations of K^+ ions inside rhizosheath epidermal cells that tightly adjoin soil particles, than in rhizosheath epidermal cells that are surrounded by a space and have no soil immediately touching them (Fig. 4.3D and 4.4). In addition, concentrations of ions such as K^+ appeared to have accumulated in the grooves between the epidermal cells (data not shown) and in the grooves above the radial walls of hypodermal cells in older parts of the roots (when the epidermis is no longer living) (see Figs. 4.3E and 4.5).

Figure 4.3

2 of/de 2

MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS
STANDARD REFERENCE MATERIAL 1010a
(ANSI and ISO TEST CHART No. 2)

Figures 4.3A-E. Scanning electron micrographs of sheathed nodal roots of rye grown in the field late August, 1992, quick frozen in liquid nitrogen and observed frozen on a cryo stage. Figures A and C-E were planed prior to observation.

Figure 4.3A. Transverse view of root plus surrounding sheath (S). Some of cortex has broken off (red arrow) during planing. White arrowheads indicate living late metaxylem elements (X55).

Figure 4.3B. A view inside the sheath. Root hairs (white arrowheads) are intertwined among soil particles (S). A shiny mucilage (absent from top left corner of micrograph) coats soil and hairs. (X230)

Figure 4.3C. The epidermis (E), including root hairs (RH), forms a tight junction with the soil of the sheath (white arrowheads). Transverse section 3 cm from the root tip. (H)hypodermis (X1000)

Figure 4.3D. Transverse section 2 cm from the root tip. Some epidermal cells (E) are closely associated with soil of sheath (S), while others are surrounded by a space (SP) in the sheath. (see Figure 4 showing higher K⁺ concentration in soil-associated versus space surrounded epidermal cells). (H)hypodermis; arrowhead points to a root hair. (X255)

Figure 4.3E. Transverse section approximately 6cm from the tip. Epidermis (E) has died and a higher concentration of potassium ions are in the radial, anticlinal grooves (white arrowheads) between the living hypodermal (H) cells compared to over the outer periclinal wall (see Figure 5). (X1020)

Figure 4.4

Figure 4.4 Diagram showing concentrations of potassium ions in epidermal cells, with or without adhered soil (n= 4 cells for each type), of a sheathed rye root. Diagram made from a sample that was viewed and analyzed with a cryo-scanning analytical electron microscope.

K⁺ Concentration (mM)

Kye nodal root (2cm from tip)

Figure 4.5

Figure 4.5 Diagrammatic representation of a rye, sheathed root viewed and analysed with a cryo-scanning electron microscope. There are higher concentrations of potassium ions in the radial grooves between the hypodermal cells than on the surfaces of these cells. (n=5 for each of groove and surface locations)

K⁺ Concentration (mM) in Hypodermal Grooves

Rye nodal root
(4 cm from tip)

Discussion

There are very few studies of the architectures of soil-grown root systems. Perhaps the most famous, and still most informative, are those done by Weaver (see Weaver, 1919; Weaver, 1926) where he, if necessary, dug trenches, as deep as his height, alongside developed root systems for *in situ* observations. He focused on full-grown grass plants, emphasizing the importance of the lateral branches (this is particularly true for the corn he observed: *Zea mays* cv. *indentata*) . However, he does mention, on wheat, oats and rye, the presence of "glistening white tips on deep main roots (nodal) that were turgid, covered in root hairs (to which soil clung tenaciously), and had no branching until approximately 10 -15 cm towards the base". Surely these are sheathed regions, and in fact he speculates that they are "functioning vigorously as absorbing organs" (Weaver, 1926). Cannon (1949) attempted to classify different types of root system, but discusses the limitation of this since changes caused by the environment can override genetic architecture. In addition, he does not discuss the architecture of young grass root systems, and only refers to Weaver's diagram of a fibrous system of a full-grown maize plant. More recently, Fitter (1987) and Fitter *et al.* (1991) have tried to relate architecture of root systems to habitat and function. They conclude that a "herring bone system" (where branching is predominantly along the main axis) is most effective in nutrient poor soils, but it is not the best for transport to the shoot and is expensive in terms of tissue production. However, they use simulation models to draw their conclusions primarily because there is little field data available (it is very difficult to collect), and the data available is very variable owing to changes in root systems even among similar species growing in what appear to be similar environments.

I originally had hoped to find common architectures in root systems with rhizosheaths, and also to relate the architectures and the position of the rhizosheaths to the function of rhizosheaths. However, I am limited in what can be discussed for the following reasons. First, I excavated the plants before examining them. This means that I

do not know the position (i.e., the angle of extension at the base) of the roots in the soil; once excavated all roots appear to hang down, and I have re-positioned them (artificially) for preservation on film or herbarium paper. Also by excavating the plants (although carefully), I have perhaps lost some of the youngest regions. This is particularly true for the oldest nodal roots of the grasses, which are branched, have started to lose their sheath, but did not show a sheathed region at the tip. For barley, in particular, I cannot say if I have lost the youngest portions of these older roots, or if they have stopped growing, awaiting the younger sheathed roots to catch-up in length, and matured (become bare) acropetally (this was observed for Seneca Chief corn, Wenzel *et al.*, 1989). The same can be said for the seminal roots of all the grasses, although these roots appeared very mature and likely had stopped growing. Also, it must be remembered that I looked at plants of only one age, in all cases, 27-35 days, and that the architectures of the root systems likely change over time. This means that different types of root likely take over from others and become important for the changing demands of the shoot during the life of the plant, and that I can only relate the importance of sheaths to plants of this age.

Despite the limitations of the study, I can say for the grasses that they form large, stable rhizosheaths on the young nodal roots that initially grow through the upper layers of soil, and later mine the lower depths. Often sheathed roots are in the surface as well as in the lower layers of soil (Fig. 4.1B, D). At the age of about 30 days, these grasses had sheathed roots covering at least 50% of their root systems. It may be assumed that the late metaxylem (LMX) vessels were closed in the sheathed portions of all grass roots (shown for corn, St. Aubin *et al.*, 1986; for three other C4 grasses, Wenzel *et al.*, 1989; for barley: I found LMX closed in the sheath at 15 cm from the tip (data not presented here), Sanderson *et al.*, 1988, report LMX closed to 20 cm from the tips of seminal roots; for wheat: Huang *et al.*, 1991, found closed LMX at up to 30 cm from the tips of primary roots). These sheathed roots have a low axial, hydraulic conductance, and therefore cannot be expected to transport large amounts of water to the shoot. When the plant is older, the

sheathed roots can be expected to provide the shoot with only 10% of the plant's daily requirement of potassium (McCully and Canny, 1988), despite the ability of the LMX elements to sequester ions (McCully *et al.*, 1987). However, what is the case for younger plants? Young grasses with few leaves do not necessarily need large amounts of water, especially if they are effective at generating root pressure which would create a reservoir of water for the next day in the surrounding soil (Schwenke and Wagner, 1992). Also young grasses have very short shoots, in which their nodes and internodes are prepared and packaged, until they rapidly elongate when ready to flower. It can be speculated that these plants, when young, require many nutrients, perhaps special ones, which are effectively acquired by sheathed roots, to prepare the shoot (i.e., for cell division and initial specialization). Later, when the shoot is rapidly elongating (here the cells require water to expand), the sheathed roots are not so important compared to the numerous lateral roots that have developed and have started to contribute most of nutrient solution going to the shoot by virtue of their high, overall surface area (Varney *et al.*, 1993).

Passioura (1972), working with wheat plants, suggests that root systems with a high hydraulic resistance when young will conserve water during early (vegetative) growth, leaving more water available in the soil for grain-filling time. At present there is no data to support either of these ideas, however, referring to Passioura's suggestion, it seems likely that any water left in the soil by the plant in early stages might easily be lost through evaporation from the soil surface. This would indicate that, in grasses with roots with low axial movement of water, it is nutritional capabilities of the rhizosheaths to enhance mineral nutrition that are important for the plant. More studies in the field are necessary. Perhaps growing the root system along a panel of clear plastic, in combination with indicators of pH or specific ions in the soil (see the elegant technique of Marschner *et al.*, 1986) may provide some data regarding the functions of rhizosheaths at different stages of the plant's life.

Nutrients may be acquired in the sheath (its high water content (Wang *et al.*, 1991) promotes their uptake) very effectively by cells in contact with soil particles (Figs. 3D and 4). These nutrients may also accumulate in the grooves between the epidermal cells or when the epidermis is dead they may accumulate in the corners between the hypodermal cells (Figs 3E and 5). These grooves are places where bacteria (M. McCully, per. comm.), and soil particles accumulate. Therefore, they are likely sites, not only for water influx (as shown by Varney *et al.*, 1993), but of efflux of exudates from the plant, perhaps present to attract specific bacteria that in turn aid the plant. However, more studies with the cryo-scanning electron microscope are needed to confirm the results presented here.

The dicotyledonous plants had tap root systems, although that of hairy vetch approximated a "herring bone" shape. Hairy vetch had a root cap mucilage that bound soil and numerous, long root hairs along the youngest portions of the roots (Tables 1-3). However, it is not possible to conclude that this plant forms rhizosheaths similar to those of grasses because I have not checked the LMX (i.e., where it opens), and I have not compared how tightly the soil is bound to these roots compared with grasses grown in similar conditions (especially of soil water content). It is necessary, when confirming rhizosheath formation, to carry out a series of procedures (such as those performed in Chapter 2) to remove the sheath and quantify rhizosheath stabilization. However, it is possible that various plants may form some kind of sheath, especially when very young. Grasses with root-cap mucilage that is very adherent to soil and the root surface, and with numerous roots hairs, and with a native microflora which helps to stabilize the sheath, represent the extreme in a continuum. However, of interest here is the radish root system. The lateral roots appear sheathed and are placed well to mine the surface soils for ions, while the main root taps the lower soil for water. In this way, such a root system represents the ideal hydraulic lifter (Caldwell and Richards, 1987, discussed in the next chapter).

CHAPTER 5

GENERAL DISCUSSION AND CONCLUSIONS

Introduction

The experimental system with maize primary roots grown through soil profiles with dry or wet middle layers of soil showed that dry soil in the region of rhizosheath formation resulted in large sheaths that were very difficult to remove once formed. Conversely, wet soil resulted in weak, easily dislodged sheaths. Root hairs; their density, morphology and surface histochemistry, were affected by the water content of the surrounding soil. However, the maturation of the large water-conducting elements of the roots in the upper L3 layer was neither promoted nor retarded by the soil water content of the L2 layer. Similarly, the extent of suberization of the hypodermal and endodermal apoplast was unchanged, contradicting reports in the literature. The latter finding, convincing in the experimental system used, puts into question the importance of the suberized endodermis and hypodermis in the control of water and ion uptake. Finally, a field-survey of different plants showed that sticky root-cap mucilages, and numerous root hairs, are at least two of the features common to roots that form rhizosheaths.

In this final chapter, I present a discussion of the effect of soil water content on root hairs and mucilages, two important components of developed rhizosheaths identified in this study and by earlier workers (e.g., Price, 1911; Goossens and Stapelberg, 1933; Wullsten and Pratt, 1981; Vermeer and McCully, 1982; Huang *et al.*, 1993). The necessity for water exudation from the root for sheath development, especially in dry soils, is presented, followed by evidence in the literature for such exudation. Finally, the function of rhizosheaths is speculated upon, emphasizing their possible role as nutrient-acquisition zones.

The effect of soil water content on root hairs and mucilages in rhizosheath formation and stabilization

Root hairs

Root hairs act as a physical framework for the sheath, retaining larger aggregates of soil within the sheath, and very tightly anchoring soil particles at specific places along hairs. The studies in Chapters 2 and 3 showed that the dry soil conditions that resulted in large, stable sheaths, also stimulated root hair production on the underlying portion of the root. Alternatively, the wet soil conditions, where small, weak sheaths formed, suppressed hair development (see Discussions of Chapter 3 regarding possible mechanisms). Clearly, the numerous hairs produced in the dry soil helped to develop a large sheath. However, numerous hairs alone are not responsible for sheath formation (as suggested by some researchers, i.e., Buckley, 1982; Goodchild and Myers, 1987). They must be distorted (recall hairs developed in dry soil were more distorted than those in wet, Chapter 3). Bends in sheathed-root hairs are caused by bacteria that are present in the sheath in high numbers (unpublished studies in the McCully and Canny lab) and are places that soil particles are very tightly held. Mucilages, the primary gluing agents in the sheath, become trapped in these bent regions, and firmly attached the soil to the root. This trapped glue was then in accessible to degradation. It is possible that the bacteria may loosen the wall in the place of the bend, and the soil particles in these regions become embedded in the wall (Sachs, 1865)).

Mucilages

Contrary to a misconception that exists in the literature, these adherent mucilages are not produced by the root hairs, nor by the cells of the epidermis proper, as speculated in

older studies (see Price, 1911; Goossens and Stapelberg, 1933), and erroneously stated in more recent literature (e.g., Foster and Rovira, 1976; Clarkson, 1991). The cells of the epidermis, in the region of the sheath, show no signs of polysaccharide synthesis, such as active golgi bodies or large amounts of mucilage retained in the periplasmic space. However, it is important to note that the epidermal cells near the root tip where the sheath has not yet started to form, and where root hairs have not yet developed, are covered in a wall-like pellicle (shown in an extensive fine structural and histochemical study of the surface of young corn roots, Abeysekara and McCully, 1993). Neither root-cap mucilage nor soil adhere to this pellicle and it is only after the pellicle disintegrates in the region of root hair development that isolated patches of root-cap mucilage begin to adhere to the root surface.

There is ample evidence to show that the mucilages stabilizing soil particles and root surfaces in the sheath are formed by the root-cap cells and certain native bacteria. Unlike the epidermal cells in the sheath, the root-cap cells are equipped to produce and excrete large amounts of mucilage composed of complex, highly branched polysaccharide molecules (Bacic *et al.*, 1986). Numerous, large golgi can be seen in these cells, located near pockets of condensed mucilage retained in the periplasmic space (see Figs. 6 and 7 in Guinel and McCully, 1986). In fact, these root-cap cells are often used as model systems for polysaccharide synthesis and packaging. Microscopic studies, using, for example, lectins specific for fucose (an uncommon sugar yet a component of root-cap mucilage), have shown that the mucilage in the sheath has similar histochemical properties to that produced by root-cap cells (Vermeer and McCully, 1982). Root-cap mucilage is either released from the cells while they are still attached at the root tip and left *in situ* as the root grows through the soil, or the mucilage is released from the root-cap cells once they are sloughed off and are in the sheath (Vermeer and McCully, 1982). It is likely that mucilage is accumulated in the sheath by a combination of these two events. Studies with isolated root-cap mucilage of corn have shown that it can bind soil very effectively (Morel *et al.*, 1991; Watt *et al.*, 1993). Floyd and Ohlrooge (1971) report a soil-binding gel coating the surface of maize nodal roots that had a

molecular weight similar to that calculated for root-cap mucilage of soil-grown corn roots (Sealey *et al.*, in press). However, perhaps the most convincing evidence that root-cap mucilage is certainly binding soil within the sheath is the fact that soil always adheres to the surface of root caps of grasses (and perhaps hairy vetch) (McCully and Canny, 1988; see Figs. 4.1 A-D, 4.2, Chapter 4 of this thesis).

In addition to root-cap mucilage, mucilages from bacteria are present in the sheath (see micrographs of Vermeer and McCully, 1982; McCully and Canny, 1988, Gochnauer *et al.*, 1989), and they contribute to the adhesion of soil (shown with a *Cytophaga* sp. from the maize rhizosheath, Watt *et al.*, 1993). Such mucilage-producing bacteria are common to other grass roots that form sheaths, for example, Webley *et al.* (1965), report high numbers of bacterial isolates, which produce a capsule or slime material, associated with the soil adhered to the roots of ryegrass.

General studies of soil aggregation have shown that mucilages of both plant and microbial origin stabilize or bind soil strongly (Cheshire, 1979), especially under a state of dehydration, such as that produced around growing roots (Reid and Goss, 1981; Reid and Goss, 1982). Specific experiments on the water-related properties of maize root-cap mucilage showed that it dries easily (Guinel and McCully, 1986). In the model system of Watt *et al.* (1993), both root-cap mucilage and bacterial mucilages acted as strong adhesives when they were initially wetted and then allowed to dry together with soil particles. The mucilage-soil adhesion was irreversible even after soaking 48 h in water (Watt *et al.*, 1993). This coincides with what was observed with the rhizosheaths formed on roots growing in dry soil (Chapter 2): 35% of the soil of the rhizosheaths was still bound to the root after sonication and 48 h in hot water. It is likely that the sonication and hot water steps loosened the soil that was retained by the easily-accessed mucilages (which would have hydrated and diffused away) while the mucilages that become bound to soil and root surfaces were protected from loosening and retained the soil *in situ*. Interestingly, there is a low pectic component in the root-cap mucilage (3% of total polysaccharides, from Bacic *et al.*, 1986)

and as a result it is not hydrophilic (Watt *et al.*, 1993). Therefore, dried, hydrophobic mucilage, trapped and binding soil particles in, for example, distortion points on root-hairs or grooves in the epidermal surface, would actually repel water and such soil could be removed only by persistent abrasion. Such protected mucilages are also inaccessible to degradation by microorganisms and such degradation is a cause of aggregate destabilization in the field (Cheshire, 1979).

If nylon-immobilized root-cap mucilage and soil are not given the opportunity to dry together, soil particles can easily be washed away (Watt *et al.*, 1993). This was also seen with rhizosheaths formed in wet soil where 90% of the soil from the sheath was dislodged by sonication for 10 minutes in water (Chapter 2; Table 2.2). In the wet soil conditions (20% SWC in my experiments), it is likely that the mucilages were not given the opportunity to gel in contact with the root surfaces and soil. Recall that the large late metaxylem vessels are still living and thus not conducting water in the sheathed portion of the root (St. Aubin *et al.*, 1986), and tensions from the smaller early metaxylem vessels during transpiration do not dry the root which remains at a high relative water content (Wang *et al.*, 1991). Thus transpiration would not be expected to have an impact on drying the wet soil around the root in this region.

However, in both dry and wet soil mucilages must first be present in the sheath as an expanding gel, coating soil and root surfaces, before they are dried and can act as an adherent. It seems likely at first glance that hydration in the "wet" (20% SWC) soil would be enough to cause the mucilages (especially that produced by the root-cap) to expand. However, Guinel and McCully (1986) showed that root-cap mucilage will lose water in an environment with a water potential more negative than -0.07 bars. It is estimated that the sandy-loam soil I used has a soil water potential of -10 bars and -0.6 bars for dry (9% SWC) and wet (20% SWC) soils, respectively (C. Topp per. comm., Brady, 1990). Therefore, based on these values, the root cap mucilage should lose water, and never become hydrated in either dry or wet soil. Guinel and McCully (1986) were working with three-day-old maize

seedlings grown on 1% agar under sterile conditions. Here, the gel is partially expanded. These conditions are not the heterogeneous environment of the soil, where, for example, mucilages from bacteria also interact (I do not know their water potentials), and the pores in the soil have different water potentials, some either higher or lower than others. However, although the Guinel and McCully study is limited, the fact also remains that root caps never have expanded mucilage when taken out of even quite wet soil (M. McCully, per. comm.). This mucilage at the tip will expand, though, when put in water (Scaley *et al.*, 1994). Thus root-cap mucilage in soil, at least, needs a source of water for it to expand, and enough water is not present in "wet" (20% SWC) soil. This water must, therefore, come from the root. The necessity for this becomes obvious in the dry soil conditions where there is at least a 100-fold difference between the water potential calculated for root-cap mucilage in a model system, and the water potential of the soil (approximately -10 bars). Yet it is in such "dry" soil that rhizosheaths are large, coherent, moist structures.

The root as a source of water for mucilage expansion in dry soils

There are now a considerable number of reports in the literature of water leaving the surface of roots of both mesophytic and xerophytic plants. Referring to Table 5.1, it is clear that exudation of water from roots is common to many plants, and there seems little doubt that roots that form large, stable rhizosheaths in dry mesic or xeric conditions have the ability to leak water to their surrounding soil; Wang *et al.* (1991) have already shown that the sheathed portions of maize roots have a higher relative water content than the bare regions, and the rhizosheaths of *Oryzopsis hymenoides* have more water than the surrounding soil (Bristow *et al.*, 1985). In addition, Huang *et al.* (1993) report that the sheaths of *Opuntia ficus-indica* have a water potential slightly lower than the roots, but higher than the bulk soil.

Table 5.1. Reports of water exudation from roots of a variety of species

<u>Reference</u>	<u>Species</u>
Hunter and Kelley (1946)	corn
Breazeale and McGeorge (1952)	tomato
Head (1964)	apple
Schippers <i>et al.</i> (1967)	broadbean
Cailloux (1971)	oats
Molz and Peterson (1976)	cotton
Mooney <i>et al.</i> (1980)	<i>Prosopis tamarugo</i> (tree native to Chile)
Shone and Flood (1980)	barley
van Bavel and Baker (1985)	bermuda grass
Baker and van Bavel (1986)	bermuda grass
Richards and Caldwell (1987)	sagebrush (<i>Artemesia tridentata</i>)
Caldwell and Richards (1989)	sagebrush (<i>Artemesia tridentata</i>)
Blum and Johnson (1992)	wheat
Schwenke and Wagner (1992)	corn
Dawson (1993)	maple
Wan <i>et al.</i> (1993)	room snakeweed (<i>Gutierrezia sarothrae</i>)
Vetterlein and Marschner (1993)	millet
Williams <i>et al.</i> (1993)	sagebrush and bunchgrass
Phillips and Riha (1994)	<i>Eucalyptus</i>

However, the driving force behind such water exudation is unclear. Some researchers, for example, Mooney *et al.*, 1980, van Bavel and Baker, 1985, Richards and Caldwell, 1987, and Dawson, 1993, believe that the water is pulled out, passively, when the water potential outside the root is lower than that inside. It was Richards and Caldwell

(1987) who coined the term "hydraulic lift" to describe the transfer of water, via roots (acting as bridges), from wet, deep soil layers, to dry, upper layers, during the night. However, other workers have suggested that the exudation involves energy (i.e., Cailloux, 1972; Schwenke and Wagner, 1992). Whether passive or active, both mechanisms require that transpiration, and thus tension from the top of the plant, cease for a period of time (overnight), and that some part of the root, for example, the tip, has access to water.

Based on my experiments, it seems likely that active exudation predominates, but that water can also leave roots passively. Supporting active exudation is my observation that I noticed the leaves had drops of fluid at their tips after I had removed the plants from the light of the glasshouse in the morning and had let them sit for a few minutes in the darker laboratory. These guttated drops were a consequence of positive pressures built up inside the plant in the absence of transpiration. This is called "root pressure" and is a well known (yet poorly understood) phenomenon that involves energy and the active expulsion of water from cells (Steward, 1968). Generally, xylem exudation from the base of an excised root, or the stump remaining after part of the shoot has been removed, is attributed to root pressure. H. Schwenke (per. comm., and references in Schwenke and Wagner, 1992) says that his rhizodermal exudation is also a consequence of root pressure; it is the release of water by the surface, epidermal cells (via stretch receptors and activation channels) in response to turgor build-up when the root tip is in water.

Also indicating that active exudation was taking place in my experiments was the fact that I observed changes in the epidermal cells of root portions grown in dry versus wet soil (not only were there more root hairs formed in the dry soil, but the surfaces appeared more hydrophobic, Fig. 3.4, Chapter 3), but not in the suberized layers of the apoplast, around the endodermis and hypodermis. The surface cells responded to a change in the soil water content, indicating that they are directly functioning in soil solution uptake, and thus likely also in water output. This corresponds to findings of Cailloux (1972), who, by

making direct measurements on the hairs of oat roots, found the hairs and cells of the epidermis proper actively involved in water uptake, and water exudation.

If exudation is active, as is likely, based on the evidence presented above, water will have leaked from the root into the wet soil, as well as into the dry. This conclusion is supported by the measurements of sheaths developed in wet soil (Chapter 2). With additional hydration from the root, and little or no subsequent drying from the wet soil (it was almost at field capacity), it is likely that much of the mucilage expanded by the exudation fluid keeps expanding, slowly diffusing away from the sheath. Guinel and McCully (1986), and Sealey *et al.* (1994) have shown that root-cap mucilage of maize, when in water, will keep expanding, lose its integrity as a gel, and eventually diffuse away. The root-cap mucilage may have diffused away even when at the tip, not lasting long enough *in situ* to become part of the sheath. Cailloux (1972) found that exudation from root hairs was actually enhanced under conditions of poor aeration, such as those that might be expected in the wet soil of my experiments. Vetterlein and Marschner (1993) found water exudation into soils with a water potential was less negative than that of my wet soil.

Therefore, I can conclude from the guttation, and the responses of the surface layers to the soil water content, that root pressure was operating in my plants, and that root exudate can leave roots actively into both wet and dry soil, and that it is not solely transferred from wet to dry soil by differences in water potential. It is possible, however that roots in locally-dry soil, that are part of a root system well-supplied elsewhere with water, are structured to allow for some passive leakage from the apoplast to hydrate the sheath, and do not compensate by developing heavy suberization. In this way, the tension from the drier soil may also be able to pull out water from the roots, adding to water that is pumped out. I am unable to say from the experiments presented here whether more water leaked from root portions in dry versus wet soil, as would be expected if passive leakage (i.e., hydraulic lift) predominated. Actually, my experimental design with dry versus wet

layers of soil is ideal to study this, especially if combined with a means of measuring ¹ changes in SWC around the root.

Not all workers agree that roots lose water through their surface, especially in dry soil. For example, Shone and Clarkson (1988) report that roots can act as rectifiers via the endodermal and hypodermal layers. When the surrounding soil is very dry these apoplastic barriers become very suberized preventing loss of water from the root, even though when large amounts of water become available, these same endodermis and hypodermis layers do not impede rapid uptake of water (Nobel and Sanderson, 1984; North and Nobel, 1991). However, all these studies used entire roots grown under dry conditions and are confused by differences in rates of root growth in dry (very dry; in the case of Nobel and Sanderson, roots were subjected to -936 bars water potential) versus wet soil (see Discussion in Chapter 3). Furthermore, evidence from my thesis work and other studies (see Chapter 3) indicates that the endodermal and hypodermal apoplastic barriers are not involved in the control of water uptake, and, by implication also control of water loss. The limited evidence for rectification referenced above also indicates that it most certainly involves the symplast, and not the suberized layers of the apoplast. Roots logically must be able to control both the uptake and loss of water and ions. By assisting in the formation of rhizosheaths, such water loss may play a major role in the growth of the plant.

How much water leaks from roots in the sheathed zones? And, is the amount enough to hydrate the mucilage? Despite the limited information regarding the gelling properties of mucilages, both root-cap and bacterial, found in the rhizosheath, I have done a sample calculation of how much water, exuded from the root mucilage must encounter to

¹ The amount of water leaving the root could be quantified using a method called Time Domain Reflectometry (TDR), developed by Clarke Topp of Agriculture Canada. Changes in the dielectric constant due to small changes in soil water are sensed with cables (thin enough to be inserted in soil layer of tube) and recorded.

expand and diffuse in a sheath developing in dry soil. Take for example, the volume of sheath surrounding one millimeter (0.1 cm) length of root, in the region where the root hairs have emerged, the epidermal surface pellicle is being shed, and the root-cap mucilage is known to start to stick to the root and thus bind soil in the sheath (Abeysekara and McCully, 1993). In the dry soil, the thickness of the sheath was approximately 0.06 cm (Chapter 2), so the volume of sheath overlying 0.1 cm of root would be 0.003 cm^3 , or 0.003 ml of sheath. The SWC around the sheath was 9%; volumetrically, this is 0.12 ml of water per 1 ml of soil. According to Guinel and McCully (1986) the mucilage is a slightly hydrated gel at -0.1 bars. To be in this state, the mucilage must be in soil that has a water content of 0.4 ml water per 1 ml of soil (C. Topp, per. comm., from a curve correlating water content and water potential for the sandy loam soil I used). The difference between the dry soil and the soil of the sheath for hydrated mucilage is 0.28 ml water per 1 ml of soil. The sheath volume has 0.003 ml, therefore approximately 0.8 μL of water would have to be supplied by the surface cells to the 0.1 cm length of developing region of the sheath. If water exuded at this rate will hydrate root-cap mucilage in dry soil, where the hydrated mucilage is subsequently stabilized during the day by water loss to the surrounding soil and the root(minor importance), it would likely "over-hydrate" mucilage in the wet soil, in the sense that the surrounding soil (and root) cannot subsequently pull water from the mucilage to effect the tight binding between the soil and the root.

Unfortunately there are no data for amounts of the water exuded per root per unit time into soil. However H. Schwenke (per. comm.) says that a portion (~5cm) of maize root in a sealed, glass tube, with its tip in water, will exude a total of 100 μL after three days. This would be about 0.7 μL per day per 0.1 cm of root. Thus, my calculated amount of exudation and that measured by Schwenke correspond nicely, however, experimental measurements with a less artificial system than his are needed. Upon observing his experiments (H. Schwenke visited the McCully and Canny laboratory recently), I feel some of the epidermal exudation into the tube that Schwenke obtained in Germany (Schwenke

and Wagner, 1992) may have been artifactual, i.e., the roots, especially the epidermal cells, were possibly dead after three days. Also, he used excised roots and these may have very different hydraulic and osmotic properties compared to those attached to shoots (Zimmermann *et al.*, 1992, 1993 recently found gradients in the osmotic concentrations of cortical cells of roots that were collapsed by excision of the shoot).

Part of the sheath that has formed and stabilized against the root (this theoretically could occur after one night, or a very rainy day, followed by a sunny day) cannot subsequently come away from the root and then reform. Studies (in Chapter 2) to remove sheaths developed in dry soil showed 35% of the sheath was still bound to the root after 48 hours in hot water, indicating clearly that some soil particles are permanently cemented to the root. Experiments with root-cap and bacterial mucilages immobilized on nylon membrane also showed that once dried together, mucilage and soil are very difficult to separate and in a sense are "fixed-dry". Therefore it is expected that the sheath becomes progressively more stable behind the tip. As the sheath gets older, it becomes more and more stabilized by slight drying tensions from the small functioning metaxylem inside the plant (minimal but present during the day, Wang *et al.*, 1991) and from outside in the bulk soil. Microorganisms may also aid in the stabilization by producing additional mucilages, or by distorting root hairs thus providing anchor points for soil. Conversely, contributions by some microbes may be counteracted by degradation of the mucilages by others.

Possible functions of the sheath

At present in the literature, the functions specifically attributed to rhizosheaths are related to water uptake or loss. For example, Price (1911), suggests that they aid in water absorption, or conversely, protect the root from water loss through the water-holding capacity of the mucilages. Buckley (1982) considers that they may protect the stele from water loss. Bristow *et al.* (1985) also state that rhizosheaths may have a role in water

uptake, but decide they do not hold water as suggested by Price. North and Nobel (1992) state that sheaths may protect root from water loss, but do not suggest a mechanism. Huang *et al.* (1993), using excised sheathed roots *Opuntia ficus-indica* with air blowing through them, conclude that sheaths prevent water loss from the root to the soil (they do not state which soil they are referring to) by shrinking and creating "gaps" that are less permeable to water. These researchers do not specify where the gaps are, but imply there may be a gap between the sheath and the bulk soil. In cryo-scanning electron micrographs (Figs. of Chapters 2 and 4), it is clear that the root surface and sheath soil are tightly associated, a condition that would reduce the resistance to water movement into (or out of) the root (Oertli, 1991). However, I have not looked for a space between the sheath and the outside bulk soil. It would be interesting to try to observe the junction between sheath and soil during the night and during the day, *in situ*, looking for no gap and a gap, respectively. This could be done by freezing the sheaths *in situ* in the field and examining the sheath/bulk soil interface in the cryo-scanning electron microscope.

There is no direct evidence in the literature showing that rhizosheaths function as nutrient acquiring zones. However, there is a substantial amount of indirect evidence from other root studies, not specifically with sheaths, suggesting that the rhizosheath may represent a special region for enhanced nutrient uptake. Although it is generally accepted that the older regions, with numerous branch roots, take up the highest concentrations of ions (branch roots have a high surface area, and they are connected to the main axial root through which the transpiration stream flows) (Brady *et al.*, 1993; Varney *et al.*, 1993), these ions are likely transported to the shoot. The growing tip, however, may receive its ions, in addition to specific microbial products, from the sheathed region of the root, which can act as an efficient ion accumulator in the absence of much water flow (McCully and Canny, 1988). In addition, as discussed in Chapter 4, rhizosheaths may play a greater role in plant nutrition at different times in the plant's life. It would be interesting to conduct a

study of field-grown plants measuring the proportion of the root system covered in sheath at different times in the plants' lives.

The fact that the sheaths come away with the excavated roots indicate that they form a very close association with the root. The root surfaces in the sheath, hairs and epidermis proper are alive, and can absorb both water and ions (Läuchli, 1967; Cailloux, 1972; Varney *et al.*, 1993). Since the sheath is continually being made, the root is continually placing a young, large surface area of root (the numerous root hairs) in contact with new soils. Large surface areas are important for obtaining ions that move slowly by diffusion, such as phosphorous and potassium (Jungk, 1991). Further measurements of ions in surface cells in contact with soil of the sheath versus those surrounded by a space, using the cryo-scanning analytical microscope, may provide more evidence regarding the importance of soil-contact in nutrient acquisition.

Mucilages may act in mobilizing nutrients, such as magnesium and iron, from the surfaces of soil particles to the root (Uren, 1993). In addition, exudates (such as phytosiderophores which sequester iron) from the root surface would be expected to influence and enhance nutrient uptake from the rhizosheath, since this has been shown for maize "rhizospheres" (Marschner *et al.*, 1986; Baylock and James, 1994). The close spatial arrangement of the sheath would allow the root to re-absorb root exudates. This has been shown to beneficially occur in maize (Jones and Darrah, 1992, 1993). In addition, root exudates must influence the microflora, as shown by Gochnauer *et al.* (1990) with the interactions between detached root-cap cells and corn rhizosheath bacteria. In turn, these specific sheath bacteria likely provide beneficial compounds to the roots. Therefore, formation of a sheath results in the solubilization of ions from newly-contacted soil particles via its high water content (relative to older portions of roots), and results in enhanced acquisition of (specialty) compounds mobilized by specific exudates (both root and bacterial). These ions and compounds can then diffuse the short distances to the root.

Inside the root, ions, etc. may be immediately used by the growing tip (McCully and Canny, 1988).

In fact, there is some quantitative data which suggest that rhizosheaths acquire relatively high amounts of nutrients, especially in drying soils. Nambiar (1976, 1977), from whom I adapted my experimental system of Chapters 2 and 3, showed that root portions grown in dry soil bind much more soil than portions in contact with wet soil, and these sheathed regions take up surprisingly-high amounts of labeled zinc and other micronutrients, provided the roots are receiving water from another part of the soil. There is also ecological evidence that roots with regions of high surface area and adhered soil serve plants living successfully in nutrient-poor, dry soils (Lamont, 1993). An example of such a plant is lupin. It can form proteoid roots, small clusters of very-closely spaced branch roots (which in turn may have root hairs) that show enhanced phosphate mobilization. In fact these proteoid roots are sheathed and it is the microflora of bacteria within the sheath that solubilize the phosphates (Wenzel *et al.*, 1994). Therefore, proteoid roots, with high surface area of root in close contact with adhered soil, and a specific microflora, are an example of the sheathed environment benefitting the plant.

Conclusions

In summary, a delicate balance between hydration and dehydration produces a large, stable rhizosheath in dry soil conditions. Sheath formation is initially dependent upon hydration of mucilage (from root-cap cells and bacteria) by water exuded from the root surface. In dry soil, the hydrated mucilages in the sheath are then stabilized between soil particles and root surfaces by the drying potential of the bulk-soil. In wet soil, these

mucilages likely diffuse away, especially during hydration in the night, and many are never stabilized during the day. The bulk-soil water status also influences the formation and stability of soil sheaths by its effect on the number of root hairs that develop. Root hairs are more numerous, and more distorted when the soil is dry, and mucilages bind the soil particles of the sheath preferentially to the distorted regions if they are allowed to dry together. Rhizosheaths, with their close contact between soil and root, likely function as nutrient acquiring structures, especially in dry soil.

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FIN

Figure 4.4 Diagram showing concentrations of potassium ions in epidermal cells, with or without adhered soil (n= 4 cells for each type), of a sheathed rye root. Diagram made from a sample that was viewed and analyzed with a cryo-scanning analytical electron microscope.