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HISTOCHEMICAL, HISTOLOGICAL AND STRUCTURAL
STUDY OF FIELD-GROWN CORN ROOTS

by

© JANET VERMEER MACLEOD, B.Sc.

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

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ABSTRACT

Roots of field grown plants of Zea mays have a distinct root cap with associated mucilage and sloughed cells, and an epidermal mucilage which is continuous over the entire root surface where epidermal cells are intact. Both mucilages demonstrate the presence of terminal fucose residues as determined by their reaction with a fucose specific lectin.

Rhizosphere mucilage binds the soil of the adherent soil sheath to the root surface except at the apical 25 mm and over old basal regions of roots. This mucilage contains numerous sloughed cells and shows histochemical, structural and lectin labelling characteristics similar to those of root cap mucilage. The histochemical and ultrastructural features of the epidermal cell walls, epidermal mucilage and root hair walls are distinct from those of the sheath mucilage and sloughed cells walls.

Root cap origin of the free living sloughed cells and the rhizosphere (sheath) mucilage is postulated.

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LIST OF ABBREVIATIONS

CW	-	cell wall
D	-	dictyosome
EM	-	electron microscope, electron microscopy
Ep	-	epidermis, epidermal
ER	-	endoplasmic reticulum
GMA	-	glycol methacrylate
LS	-	longitudinal section
M	-	mitochondrion
MLS	-	median longitudinal section
Mu	-	mucilage
N	-	nucleus
PAS	-	periodic acid Schiff's
PBS	-	phosphate buffered saline
Pl	-	plastid
RC	-	root cap
SC	-	sloughed cell
TBO	-	toluidine blue O
TS	-	cross section
V	-	vacuole

GENERAL INTRODUCTION

The statement by Brown (1975), "Plants as we see them growing in the soil are products of their environment; the microorganisms of the root region are part of the environment and by their activities for good or bad affect the product," is perhaps one of the best ways to explain much of the variation in the growth and physiology of plants in the field. However, there is evidence that the plant is more than a docile participant in this process. Indeed, every living root in the soil is surrounded by a zone with a distinct microbial gradient extending out from the root an average of 2mm (Rovira 1969a). This zone, the rhizosphere, is a poorly defined region which comprises three components, namely the soil matrix, the microflora and the plant roots. Hiltner (1904, quoted in Timonin 1965) first described the rhizosphere as that region of the soil under root influence but it is now generally accepted that the portion of soil where the microbial population is altered, both quantitatively and qualitatively, by the presence of plant roots is the rhizosphere (Rovira 1969a). The root surface, where microbial colonization is particularly pronounced, is termed the rhizoplane (Timonin 1965; Hale et al. 1971). The influence of plants on the components of the rhizosphere has been the subject of numerous reviews (eg. Timonin 1965; Rovira 1969a, 1969b, 1979; Rovira and Davey 1971; Hale et al. 1971;

Darbyshire and Greaves 1973; Bowen and Rovira 1976; Rovira and McDougall 1976; Newman 1978; Oakes 1978; Hale and Moore 1978).

The establishment of the rhizosphere is dependent on the root and its physiological activities. Regardless of growth conditions observed, but generally determined in nutrient culture, plant roots have been shown to exude a large variety of simple molecular species including monosaccharides, amino acids, organic acids and phenolics. Soluble exudates vary both qualitatively and quantitatively but at least some compounds of each class are consistently present (Vancura and Hovadik 1965; Smith 1969; Papavizas and Kovacs 1972; Bokhari et al. 1979). Maximum rates of exudation coincide with the period of maximal vegetative growth (Rovira 1959; Kobayashi and Suzuki 1979b) and young plants exude more than old mature plants (André et al. 1978). Factors such as nutrient availability (Kalachova and Vancura 1975), plant species [corn, for example, shows the least pronounced effect (Darbyshire and Greaves 1973)], either absence of microbes (Rempe and Grunberg 1970) or their presence (Barber and Martin 1976), water stress (Katznelson et al. 1954; Barber and Martin 1976), immersion in distilled water (McDougall and Rovira 1965), mild abrasive damage (McDougall 1968; Ayers and Thornton 1968), lowered pH (McDougall 1970), temperature

increase (Rovira 1959; Vancura 1967), physical contact with particles such as glass beads (Kobayashi and Suzuki 1979b), and increased light intensity (Rovira 1969a) have all been shown to have pronounced effects on both the quantity and type of materials exuded. Applications of extraneous hormones such as the herbicide 2,4-dinitrophenol also affect exudation, either by increasing losses (McDougall 1970), or by decreasing short term losses of amino acids (Richter et al. 1968). Plant growth hormones present in the rhizosphere also have some affect (Anilkumar and Chakravarti 1970).

Loss of water soluble compounds from the root system account for on average 0.1 - 0.4% of assimilated photosynthates (Rovira 1979) and for some grasses, values as high as 5 to 10% (Barber and Martin 1976) and even 14 - 23% (Martin 1977) have been reported. The percentage of photosynthate exuded by roots is actually lowest during the stage of maximal growth, although exudation rates are highest at this time (André et al. 1978). In addition to soluble exudates which account for 60 - 70% of materials released by mature portions of the root (Rovira 1969b), most roots release nondiffusible exudates such as insoluble mucilages and sloughed root cap cells which account for 60 - 70% of the exudates released from the tip of the root (McDougall and Rovira 1970). Peanut roots release

between 0.26 and 0.73 mg/plant/wk (0.15% of the total root carbon) as nondiffusible exudates (Griffin et al. 1976).

While the concentration of free soluble carbon substances around the root is never high, with a maximum of 10 ppm for only a few hours near the root surface (Newman and Watson 1977), it is generally assumed that materials exuded by the roots are used as substrate by microorganisms (Rovira and Davey 1971; Newman 1978; Rovira and McDougall 1978) particularly since microbial colonization has been observed to increase as roots begin to secrete amino acids and carbohydrates. Although the evidence is circumstantial, a recent report (Billès 1978) has shown that the simple materials exuded by plant roots can be recovered in more stable products formed by the rhizosphere microorganisms (approximately 40% of the C^{14} labelled material released by the roots was shown to be present in microbial polymers). Microbes have also been demonstrated to be capable of rapidly breaking down sugars and amino acids supplied by the root, and simple phenolics present in the root zone (Figure 2, Haider and Martin 1975).

The root and its lysates, exuded mucilaginous material, and diffusible exudates represent a virgin ecological niche for soil microbes. In the presence of an inoculum such as organic matter or decomposing root

material (Bowen and Rovira 1976) the establishment of a rhizosphere can be observed as early as two days following seed germination. Initially only rod-shaped bacteria are present but these are soon replaced by a greater variety of forms (Rovira 1956; Bowen and Rovira 1976). Microbial growth is logarithmic until the substrate supply is only sufficient for population maintenance at which point the growth rate levels off (Rovira 1956). Roots are not completely covered by microorganisms and areas of both high and low density, several mm in extent, have been observed (Newman and Bowen 1974). Sloughed root cap cells are quickly colonized (Bowen and Rovira 1976). Variation in the pattern of distribution of the microorganisms suggests the presence of distinct zones or sites of exudation (Polonenko and Mayfield 1979). Root cap mucilage itself may provide one of these sites (Kirby and Roberts 1971).

Collectively, the soluble exudates, mucigel (non-soluble exudate) and sloughed root cap cells provide organic materials close to the root while it is active in nutrient uptake from the soil and their influence on the rhizosphere is important for this reason (Newman 1978). The acid groups of the root cap slime may alone trap cations (Kirby and Roberts 1971). Microbeam analysis has shown that plant nutrients are actually concentrated in the rhizosphere and it was

suggested that mucilagenous materials on the root chelate nutrients and concentrate them about the root (Tan and Nopamornbodi 1979). Although cations are bound to the root surface in a typical lyotropic series ($H^+ > Ba^{+2} > Ca^{+2} > Mg^{+2} > Co^{+2} > Rb^+ > NH_4^+ > K^+ > Na^+$), they are not necessarily taken up into the root in this order (Williams and Coleman 1950). Root exudates of forest trees may also have some role in nutrient cycling (Smith 1976). Populations of microorganisms in the rhizosphere are potential competitors for nutrients and they can decrease nutrient availability either by chelating mineral nutrients to their polysaccharide coats (such as the molybdenum chelation by gram negative bacteria described by Tan and Loutit [1977]) or by sequestering minerals for their own metabolic cycles.

Root cation exchange capacity, when observed, is always associated with the root tip and elongation zone (Crooke et al. 1960; Brans 1969; Esterman and McClaren 1961). Microscopically, the surface of this area of the root is characterized either as a granular matrix material bound by an outer layer (Jenny and Grossenbacher 1963) or as an aggregation of fibrils extending freely away from the root surface and making intimate contact with the soil deposits (Leppard and Ramamoorthy 1975). Although not proven, it is thought that the acidic carboxyl groups of the polygalacturonic

acid side chains of the mucilage attract the ions localized on the surface of the soil mineral particles (Jenny and Grossenbacher 1963; Leppard and Ramamoorthy 1975; Oades 1978).

With respect to root-environment interactions, the outer boundary of the root has traditionally been visualized as a clearly defined static entity composed of the peripheral epidermal cell walls and the extended root hairs. According to the few structural studies that have been done of soil grown roots, the boundary is at best ill-defined, and the published micrographs confirm this. The definition of the "boundary" is confused by the presence of lysed tissues, degenerate cellular debris, sloughed cells of undetermined origin, and by the presence of extracellular mucilages and microbes (eg. see Jenny and Grossenbacher 1963; Old and Nicolson 1975; Foster and Rovira 1976; Umali-Garcia et al. 1978, 1980; Foster 1981; and reviews by Mosse 1975; Balandreau and Knowles 1978; Oades 1978). Problems of interpretation are increased by the lack in most of these studies, of information on developmental aspects of the structures concerned, absence of chemical analysis of all or part of the mucilages or knowledge of their biochemical characteristics, and by lack of knowledge of the characteristics of axenically-grown roots of the same species.

It was the purpose of this study to consider the

structure of field roots and to establish the nature of associated mucigels and microorganisms in order to elucidate some aspects of the interaction of rhizosphere components. Corn was chosen because, almost certainly, it is the plant for which the surface features and development of axially-grown primary roots have been best characterized. Not only is there a considerable amount of information on structural and biochemical aspects of root cap mucilage and its secretion (eg. Kirby and Roberts 1971; Wright and Northcote 1974; Paull and Jones 1975a, 1975b), but the development and histochemistry of the root cap, epidermis and their mucilages has been thoroughly studied (Clarke et al. 1979; Miki et al. 1980). In addition, Flyod and Ohlrogge (1970, 1971) have described the presence of a gel on nodal roots of field-grown corn which is chemically similar to, but not identical to, corn root cap mucilage. Thus, corn was felt to be the best plant to use in an attempt to define the structural contribution of the root to the rhizosphere and to more accurately define the root boundary.

GENERAL MATERIALS AND METHODS

1. Growth of Plant Materials. Seeds of Zea mays L. var. Seneca Chief (supplied by Robson Seeds Farm Corporation, Hall, New York, purchased from Ritchie Feed and Seed, Ottawa) were used.

1.1 Axenic roots. Seeds were surface sterilized for five minutes in full strength commercial Javex as previously described (Clarke et al. 1979). Disinfested seeds were rinsed thoroughly in at least three changes of sterile distilled water then imbibed in sterile distilled water for 3 h. Twenty to thirty seeds were germinated on either sterile 1% agar or on two layers of sterilized filter paper (Whatman, #1, 12.5 cm diameter) moistened with approximately 12 ml of sterile distilled water, in a 15 cm Petri dish. Dishes were sealed with Parafilm, wrapped in foil and incubated for 3d at 24 - 26 C in a growth chamber. Axenic roots grown in this manner were used for collection of epidermal strips and root cap mucilage, and for histochemical and lectin experiments.

1.2 Greenhouse-grown corn roots. Corn was grown in 155 mm azalea pots with five seeds per pot. The following soil mixes were used. 1) Greenhouse potting soil containing peatmoss, perlite, clay loam, and sand (1:1:1:1) 2) Field soil collected from the garden plot, Environmental Labs, Carleton 3) Vermiculite 4) A peat-lite mix (Promix BX). Plants were grown in the

greenhouse or in a growth chamber and were used after the first set of nodal roots had elongated to at least 2 - 5 cm, some of the roots were much longer.

Some of the pots were soaked with a soil extract immediately after sowing to provide an inoculum of soil microorganisms. All pots were subsequently watered daily with tap water and fed once a week.

1.3 Field grown-corn roots. Corn was seeded on June 2, 1981, in the garden plot, Environmental Labs, Carleton University. The soil was rototilled immediately before seeding but was otherwise not amended. Seeds were placed 4 - 5 per hole, 2 - 3 cm deep with 4 ft separating plants between and within rows. Plants were thinned to one plant per space before the end of June. Plants were collected as required for lectin experiments and fixation. The spacing used allowed for adequate sampling without damaging the root systems of neighboring plants.

2. Collection and Preparation of Plant Material.

2.1 Preparation of epidermal peels. Peels were collected by Jan Clarke and Sheila Forsyth. Selected axenic roots approximately 3 cm long from 3 d seedlings were wiped free of excess root cap mucilage with a Kimwipe and placed under a dissecting microscope. The root surface was nicked just above the tip-cap junction and again at about 1.5 - 3.0 cm from the apex. Starting from the upper nick a small piece of tissue was

separated with fine forceps and torn free of the root by pulling it up and forward towards the tip. The thin strip thus obtained includes only the epidermis and its associated mucilage (see Clarke et al. 1979). Strips from a large number of roots were collected in a vial, freeze-dried and sent to Dr. G. Reid, Stirling University for biochemical analysis.

2.2 Collection of root cap mucilage. Whole root caps of axenic primary roots were removed from tips of 3 d seedlings, collected in a vial, freeze-dried and sent to Dr. G. Reid for biochemical analysis.

Root cap mucilage used for the inhibition control for lectin incubations was obtained from the caps of axenic primary root tips which had been soaked in groups of 10 - 15 in 1 ml drops of distilled water in a Petri dish. The hydrated mucilage was collected by sucking it up with a pipet. This mucilage, and the drops of water in which the roots had been soaked, were collected in a test tube, mixed with an equal volume of distilled water and sonicated for 30 min. to reduce the viscosity of the slime (Rougier et al. 1979). Approximately 80 - 100 roots were required to generate a total volume of between 7 and 10 ml of sonicated mucilage. Mucilage was either used immediately or frozen and thawed as required.

2.3 Collection of roots for hand sectioning and fixation. Axenic roots 3 - 4 cm long were chilled in

the refrigerator for approximately 1 h before hand sectioning (chilled roots are firmer and more easily sectioned); axenic roots for fixation were used directly after 3 d germination.

Field-grown roots were collected at two week intervals during the growing season. Selected plants were carefully dug up by loosening the soil around the plant and severing the root system at 1 - 2 ft. below soil level. Roots were either cut from the root system immediately and placed in fixative or distilled water, or were selected from isolated root systems transferred to the laboratory in plastic bags on ice.

Material to be hand sectioned was selected from nodal roots 4 - 10 cm long. None of these roots possessed emerged lateral roots. Roots to be fixed were generally of variable lengths from 1 cm to at least 1 ft. (the tips of longer roots were usually severed during collection). Most roots selected for fixation were between 1 and 20 cm long and without emerged lateral roots, but for one experiment, mature (ie. bearing lateral roots) root segments were selected.

Greenhouse-grown roots were removed from the pots and rinsed free of larger clumps of soil. Selected regions of young roots (up to 7 cm long) as well as lateral-bearing regions of older roots were excised and fixed immediately.

3. Preparation of Sections for Microscopy.

3.1 Hand Sections. Hand sections were cut from chilled roots ranging in length from 3 - 10 cm. Longitudinal sections (LS) were cut by placing the root on the index finger, anchored with the thumb, and drawing a moistened surgical steel razor blade from the tip or cap upwards to give thin (10 μ m) median sections approximately 0.5 - 1.0 cm long. Sections were collected in distilled water in a watch glass. Cross sections (TS) were prepared by placing the root between thumb and forefinger and removing slices of root tissue, approximately 10 μ m thick, from the top of the organ segment with a moistened surgical steel razor blade. Thin LS and TS sections were selected under a binocular microscope.

3.2 Fixation. Small blocks approximately 1 mm x 2 mm of appropriate sections of sampled roots were excised within 1 h after collection and placed into vials of 3% (v/v) glutaraldehyde on ice. Roots collected in glutaraldehyde were excised and placed in fresh fixative. The glutaraldehyde was prepared immediately prior to use (10 ml of purified 8% stock glutaraldehyde; JB EM Services, Montreal; 6.7 ml of 0.1 M phosphate buffer [equal parts of 0.1M K_2HPO_4 and 0.1 M KH_2PO_4]; 10 ml of distilled water) to give a final solution of 3% glutaraldehyde in 0.025 M phosphate buffer, pH 6.8. Tissues were fixed in this

solution for 12 - 16 h on ice.

Following glutaraldehyde fixation, tissues to be embedded in glycol methacrylate (GMA) for routine light microscopy and histochemistry were rinsed three times with phosphate buffer (0.05 M), and dehydrated in a methyl cellosolve, propanol, ethanol, butanol series (Feder and O'Brien 1968) with at least one hour between changes, 2 changes per solvent. Following an overnight infiltration in 50:50 butanol: GMA monomer mixture (95 ml GMA, Hartung Associates, Camden, N.J., USA; 5 ml propylene glycol 200; 0.15 g 2,2' azobis, Eastman Chemical Co., Rochester, N.Y., USA) tissues were infiltrated for at least 3 w with pure monomer mixture in small capped vials on a rotator. The GMA monomer was changed at weekly intervals. After infiltration, the samples were transferred to fresh GMA monomer in aluminum weighing dishes, capped with a second dish to exclude all air and polymerized overnight at 60 C (Feder and O'Brien 1968; O'Brien and McCully 1981).

Glutaraldehyde fixed tissues to be embedded in Spurr's resin (Spurr 1969) for electron microscopy, were rinsed 5 X with phosphate buffer (0.05M), and post-fixed in 2% osmium tetroxide in the same buffer (equal parts of 4% osmium tetroxide, JB EM Services, Montreal; and 0.1 M phosphate buffer) for 1 - 2 h on ice. Tissues were then rinsed 3X in phosphate buffer (0.05M), dehydrated on ice with an acetone series (10%,

30%, 50%, 70%, 90%, 100%, 100%) with 0.5 h per solvent.

Samples were brought to room temperature and gradually infiltrated with Spurr's resin using a graded series (100% acetone, 3:1 acetone:resin mix, 1:1 acetone:resin mix, 1:3 acetone:resin mix) with at least 2 h between changes, on a rotator in small capped vials. The 1:3 acetone:resin mix solution was replaced with 100% resin mixture overnight. Resin mixture was then replaced daily with a minimum infiltration period of 3 d. Infiltrated tissues were transferred to fresh resin mixture in flat rubber embedding molds (JB EM Services, Montreal) and polymerized overnight at 60° C - 70° C.

3.3 Sections of fixed and embedded material.

Sections, 1 - 2 μ m thick, of GMA embedded material were cut on either a dry glass knife or diamond knife on a Porter Blum (Ultramicrotome MT 1). Sections were transferred to drops of water on glass slides and dried down onto the slide on a slide warmer at 37° C overnight.

Sections of Spurr's resin embedded material (0.5 - 1.0 μ m thick for optical microscopy or showing purple-gold to gold interference colours for electron microscopy) were cut with glass knife or a diamond knife (the latter was always used for "dirty" roots) on an LKB ultramicrotome III. The sections for optical microscopy were heated onto glass slides overnight on a slide warmer (50° C). The thin sections showing

purple-gold or gold interference colours were expanded to a silver interference colour with xylene vapour and then collected on precleaned, 75 X 300 mesh copper grids. Grids were cleaned by dipping several times in turn into each of: 10% nitric acid, 2 distilled water rinses, chloroform, 2 distilled water rinses. Only sections showing purple-gold and gold interference colours could be used for electron microscopy because of infiltration difficulties resulting in instability under the electron beam which was much more pronounced with very thin sections.

4. Staining Techniques.

4.1 Histochemistry. GMA embedded sections previously dried onto glass slides were either equilibrated in 0.05 M K_2HPO_4 , pH 8.6, for autofluorescence and birefringence determinations or stained by one of the following methods.

(a) Toluidine blue. (C.I. # 52040, Fisher Scientific Co.) Sections were stained for 30 s to 1 m in 0.05% (w/v) toluidine blue O in benzoate buffer pH 4.4 (0.5 g benzoic acid; 0.29 g sodium benzoate in 200 ml distilled water) washed briefly with distilled water and air dried. To maximize the metachromatic reaction of some of the root constituents, sections were breathed on and mounted in oil as the water vapour evaporated. This provided adequate moisture for the development of the metachromatic colour reaction of

certain structures without excessive logging of the sections. As a stain for phenols, 0.05% (w/v) toluidine blue O in KCl/HCl buffer, pH 1.5 (50 ml 0.2M KCl; 26.8 ml 0.2M HCl; made up to 200 ml with distilled water) was used in the same manner.

(b) Alcian Blue 8GS. (Lot # 71123, JB EM Services, Montreal) Sections were stained at room temperature for 45 m, in 0.3% (w/v) alcian blue 8GS in 0.1N sodium acetate buffer pH 5.6 (Clarke et al. 1979). Methanol purified alcian blue 8GS solution was prepared and filtered just before use. This procedure gives a stronger staining reaction, but comparable staining pattern, to that obtained with the lower pH alcian blue technique given by Pearse (1968) by which sections were stained in freshly filtered alcian blue (1% [w/v] in 3% [v/v] acetic acid, pH 2.5) for 30 m.

(c) Periodic acid-Schiff's (PAS) reaction. Sections were treated in filtered 2,2-dinitrophenolhydrazine solution (saturated in 15 ml glacial acetic acid; made up to 100 ml with distilled water; filtered) for 10 m to block inherent aldehyde groups. After rinsing for 20 m in running water, sections were oxidized in 1% (w/v) periodic acid for 10 m at room temperature, rinsed quickly in water, and stained with modified Schiff's reagent (Lot # 701581-12 or 773517-12, Fisher Scientific Company, Chemical Manufacturing Division, Fair Lawn, N.J., USA). Stained

sections were rinsed quickly in three changes of 0.5% (w/v) sodium metabisulphite, rinsed well with running water for 3 m, air dried and mounted in immersion oil (Feder and O'Brien 1968).

(d) Tannic acid-uranyl acetate. Sections were stained for 10 m with freshly prepared, filtered 5% (w/v) aqueous tannic acid (Lot #721797, Fisher Scientific), rinsed in distilled water, poststained for 10 m with filtered aqueous solution of 2% uranyl acetate and rinsed well with water (Sannes et al. 1978). Sections were mounted in immersion oil. For a control 1.0M urea was added to the tannic acid solution before use.

(e) Nile blue. (C.I.# 51180, BDH Stains, BDH Chemicals Ltd., Poole, England). Sections affixed to glass slides were stained for 5 m at 60 C with 1% (w/v) aqueous nile blue and differentiated with 1% (v/v) acetic acid for 30 s at 60 C (Jensen 1962; Considine and Knox 1979). Sections were rinsed, air dried and mounted in immersion oil.

(f) Calcofluor White. (Cyanamid of Canada Ltd., Toronto) Sections were stained for 2 m with 0.01% (w/v) aqueous Calcofluor White M2R New (Hughes and McCully 1975), rinsed, air dried and mounted in immersion oil.

(g) Aniline blue. (C.I.# 42755, Lot # 1532, Polysciences Inc., Warrington, PA, USA) Sections affixed to slides were mounted directly in 0.05% (w/v)

aniline blue in 0.67 M K_2HPO_4 buffer pH 8.6 (Smith and McCully 1978). Slides were made permanent by ringing the coverslip with nail polish.

Note: for additional details and references for the foregoing techniques, see O'Brien and McCully (1981).

4.2 Staining of thin sections for electron microscopy.

(a) Uranyl acetate. Sections on copper grids were stained in drops of 2% aqueous uranyl acetate (Lot # 721797, Fisher Scientific) on small pieces of dental wax, in the dark, for 30 m. Grids were subsequently rinsed with 20 - 30 drops of filtered distilled water and air dried. Uranyl acetate was prepared by adding 0.2 g uranyl acetate to 10 ml distilled water. The solution was sonicated until the uranyl acetate was dissolved (30 m), filtered, and stored for a maximum of two weeks in a foil-wrapped flask in a refrigerator. Immediately before use, a small amount of this stock solution was filtered through a Millipore filter (0.22 μ m pore size).

(b) Lead citrate. Sections on copper grids were post-stained in drops of lead citrate on small pieces of dental wax in a covered Petri dish, which also contained a small watch glass of NaOH pellets. Following a 30 m staining period, grids were washed with 30 drops of sterile distilled water and air dried. Lead citrate was prepared in small amounts

immediately before use by adding 0.5 ml sodium citrate solution (3.52 g/100 ml water) to 0.5 ml lead nitrate solution (2.66 g/100 ml water) in a small test tube. The resultant precipitate was dissolved by adding 0.2 ml of 1 N NaOH (prepared with CO₂ free [boiled] water and stored over CaCO₃ pellets in a desiccator). This method minimizes the problem of lead citrate contamination frequently encountered with other techniques.

4.3 Staining of Spurr's resin embedded sections for optical microscopy.

(a) Toluidine blue O. Sections 0.5 - 1 μ m thick were stained with 1% (w/v) toluidine blue O in 1% (w/v) sodium borate with gentle heating over a flame or on a slide warmer at 60 C. Slides were then rinsed thoroughly with water, air dried, and mounted in immersion oil.

(b) Aniline blue. Sections were treated for 2 - 5 m with a solution of Sodium methoxide (excess NaOH pellets in methanol) to remove the resin (Sutherland et al. 1976), rinsed well with water, mounted in aniline blue solution (see 4.1[h]) and observed with fluorescence optics to demonstrate the presence of callose in certain structures associated with the roots.

4.4 Observation of microorganisms on the root surface. Selected greenhouse-grown whole roots were

surface stained for 1 h in the following solution (Jones and Mollison 1948): 15 ml 5% (w/v) aqueous phenol (0.75 g in 15 ml distilled water); 1 ml 1% (w/v) aqueous water soluble aniline blue (C.I.# 42755, Fisher Scientific); 4 ml glacial acetic acid. Stained roots were quickly rinsed in 95% ethanol and mounted in water on a glass slide.

5. Microscopy and Micrography:

5.1 Optical microscopy. Whole mounts of roots, hand sections and thick sections of fixed and embedded materials were examined with a Carl Zeiss microscope equipped with bright field, phase contrast, and interference contrast optics. When necessary, contrast was enhanced with coloured filters. Simple polaroid filters were used for the observation of birefringence. For fluorescence microscopy, the same microscope was equipped with epifluorescence optics and an HBO-200 DC lamp. Both autofluorescence and aniline blue-induced fluorescence were observed with excitor filter BG 12, beamsplitter F1 and barrier filter No. 44; Calcofluor-induced fluorescence was observed with UG 1 excitor filter, barrier filter No. 41, and the F1 beamsplitter.

For black and white photomicrography, fluorescent images and low contrast bright field images were recorded on Kodak Plus X 35 mm film, other black and white photomicrographs were made on either 35 mm Kodak

Panatomic X film or 4 in X 5 in Kodak Ektapan sheet film. The 35 mm films were developed in full strength Microphen (Ilford). The sheet film was developed in DK-50 (Kodak) diluted 1:2 with water. Photomicrographs in colour were taken on Kodachrome 64 film using appropriate colour correction filters, and processed commercially.

5.2 Electron microscopy. Thin sections mounted on copper grids were observed with a Seimens Elmiskop I transmission electron microscope at an accelerating voltage of 80 kV. Images were recorded on either Kodak electron image file (#4463) or Kodak electron microscope file (#4489). Both types of 2.5 in X 3.0 in plate film were developed in Kodak D-19 developer diluted 1:2 with water.

6. Miscellaneous Methods.

6.1 Preparation of soil extract as a source of inoculum for greenhouse grown roots. Soil from the plow layer was collected in April from the garden plot in the region where corn had been grown the previous season. Three hundred g of soil was added to 500 ml of tap water (pH 5-6) and left at room temperature for 24 h. After this time, clay was flocculated out of the suspension by addition of 100 ml 0.1 M CaCl_2 . The remaining suspension was filtered through Whatman #1 filter paper and the filtrate was used to "water" a few of the greenhouse pots sown with corn. Pots were

flooded only once immediately after seeding.

6.2 Analysis of freeze-dried root samples.

(Analysis provided by Dr. Grant Reid, Department of Biochemistry, University of Stirling, Stirling, Scotland). Samples provided were hydrolyzed with 72% and 4% H_2SO_4 for total hydrolysis of all polysaccharides including cellulose. Neutralized hydrolysates were analyzed qualitatively (paper chromatography with ethyl acetate 8: pyridine 2: water, 1 as the solvent) and quantitatively by GLC. "Samples were prepared for GLC analysis by borohydride reduction of the aldoses to alditols and peracetylation of the alditols to alditol acetates. This gives a measure of only neutral sugars but qualitative analysis did not indicate high levels of uronic acids." (Reid, personal communication.)

Histochemical, histological and structural study of field-grown corn roots. Part I: Comparative study of the localization of fucose residues in the surface deposits of axenic and field grown corn roots.

I.1 Introduction.

The sugar fucose is not a usual component of the polysaccharides of higher plants. Biochemical analyses, however, have shown fucose to be present in relatively high concentrations in mucilage associated with the root tips of corn; the peripheral root cap cells of corn are particularly active in the synthesis and export of a mucilagenous polymer containing a high percentage of fucose (Harris and Northcote 1970; Bowles and Northcote 1974; Wright and Northcote 1974, 1975; Wright 1975; Green and Northcote 1978; James and Jones 1979a, 1979b). Segments of the root axis of corn have been shown to contain a relatively small proportion of fucose which is thought to be a minor component of the galacturonorhamnan of the pectin (Harris and Northcote 1970).

Autoradiography, using ^3H -fucose, has indicated that fucose containing polymers are synthesized and secreted by the outer region of the root cap (Kirby and Roberts 1971; Paull and Jones 1975b; Rougier 1976; Forsyth 1982) as well as by epidermal cells on the flanks of the root pole (Kirby and Roberts 1971; Rougier 1976; Forsyth 1982). Rougier et al. (1979)

demonstrated the use of fluorescein isothiocyanate-labelled lectins to study the monosaccharide constituents of the root cap mucilage. In particular, fucose-specific lectins were found to bind strongly to the root cap mucilage and sloughed cells of intact root tips.

To date, however, published studies involving localization of the fucose containing polymer of corn roots have dealt only with the root tip region of laboratory-grown seedlings. This report presents results of a histological study of the binding pattern of the fucose-specific lectin (see Allen *et al.* 1977) from Lotus purpureus Moench (Lotus tetragonolobus L.) to sections hand cut from both young and mature regions of axenic and field grown roots.

1.2 Materials and Methods.

Roots from 3 d old seedlings germinated on either 1% agar or moist filter paper (see General Materials and Methods, p. 9) were excised and median longitudinal sections cut by hand. Nodal roots were excised from field-grown plants 7, 10, 12, and 13 weeks after seeding. These roots were transferred to the laboratory in distilled water on ice and hand sectioned immediately (excised roots were in water for no longer than 1 h).

In each case, thin median longitudinal (MLS) and appropriate cross sections (TS) were chosen under a

binocular microscope. Selected sections were suspended in a small drop of water on a plastic support and exposed to osmium tetroxide vapour (released from an open ampoule of 4% OsO_4 [JB EM Services, Montreal] in a sealed 100 ml jar) for 30 m. Sections were rinsed quickly, then soaked in distilled water for at least 1 h prior to lectin incubation. (If necessary, sections can be left soaking in water for longer periods, up to 24 h, without any apparent loss of reactivity to the lectin.)

Five to 10 osmicated hand sections were incubated in a 50 μl drop of lectin solution (12.5 mg fluorescein isothiocyanate [FITC]-Lotus purpureus lectin [Sigma] in 1 ml distilled water) for 30 m in a humid dark environment at room temperature. The Sigma lectin in 1 ml water gives a solution containing 1 mg lectin, 1.5 mg potassium phosphate, and 10 mg NaCl (ie. 1 mg lectin per ml phosphate buffered saline [PBS]). The following controls were used. 1) Non-osmicated sections with or without lectin labelling. 2) Osmicated sections incubated in phosphate buffered saline pH 7.2 (PBS). 3) Osmicated roots in lectin solution plus α -L-fucose (0.2 M, Sigma). 4) Osmicated roots in a mixture containing 50 μl of sonicated root cap mucilage (see General Materials and Methods, p. 11). 5) Osmicated root sections incubated in lectin solution plus p-nitrophenyl fucoside (0.2M, Sigma). 6) Osmicated

roots incubated in lectin solution plus α -D-fucose (0.2M, Sigma). 7) Osmicated root sections incubated in 50 μ m of FITC-labelled goat anti-rabbit γ -globulin (Antibodies Inc.). Following all incubations, sections were rinsed with PBS (twice in 10 m) to solubilize excess protein (lectin), then rinsed briefly in water to remove crystallized salts, and mounted in distilled water.

Sections were observed with a Zeiss epifluorescence microscope using an HBO-200 DC burner, BG 12 excitor filter, F1 500 beam splitter and barrier filter No. 50. Photomicrographs of sections showing either FITC-induced fluorescence or autofluorescence were recorded on Kodak-Plus X 35 mm film exposed for either 30 s. or 1 m. Photomicrographs of control sections were exposed for 3 m on the same film. All films were developed in full strength Microphen (Ilford) for 6 m at 20 C. The prints were made to show a contrast comparable to their respective negatives.

I.3 Results.

With the filter combination used in the present study, unreacted root tissues incubated in phosphate buffered saline show strong autofluorescence in the same colour band as fluorescein isothiocyanate. This autofluorescence is particularly intense on the outer surface of the epidermis (Fig. 1, see also Figs. 24 and 25, Clarke et al. 1979; and Fig. 2, Miki et al. 1980) and in cell walls of the root cap, epidermal, and cortical tissue (Fig. 1). Under these conditions it is

difficult, although it is just possible, to distinguish sites of lectin binding, particularly those associated with the epidermal mucilage (Fig. 2). Treatment of fresh sections with osmium tetroxide vapour effectively blocks autofluorescence in most areas of the root tip without apparent effect on subsequent binding of the lectin. Preosmicated tissue was used for all lectin experiments on fresh tissue.

In describing different portions of the epidermis of corn root tips, the designation (regions A, B, C, D) of Clarke et al. (1979, Fig. 1) is used. Briefly, region A cells include the irregularly polygonal protodermal cells over the tip cap junction; region B include those cells lying below the flanking root cap which are square to rectangular in face view but which are wider than they are long; region C cells include those cells which are elongated in the direction of the longitudinal axis of the root; region D cells include those cells which have elongated considerably (at least 8 times the length of cells in region C). Root hair primordia are evident as cell wall outgrowths from epidermal cells at the basipetal end of region D. These regions are illustrated in Diagram 1.

1.3.1 Lectin Labelling of Axenic Primary Roots.

Figures 3 to 10 show sections of axenic primary roots. Root cap mucilage is always strongly labelled by the lectin (Figs. 3, 4 and 6). Occasionally more

Diagram 1. Illustration of the relative positions of epidermal regions A, B, C, and D. The actual length of each region and the number of epidermal cells present in each region, vary considerably according to growth conditions. Region designations are determined by the shape of the epidermal cells, not by their distance from the tip-cap boundary. In the diagram the dark layer over the epidermis (Ep) is the epidermal mucilage. Tip-cap boundary (arrows), root cap (RC), sloughed root cap cells (SC), root hair primordia (RH). Individual cells and associated epidermal mucilage are illustrated for each region. After Clarke et al. 1979. Fig. 1.

intense threads in this mucilage are associated with sloughed root cap cells (inset Fig. 4). Walls of both attached and sloughed root cap cells are consistently strongly labelled (Figs. 3 and 4). This label is usually uniformly distributed but frequently there is asymmetric distribution with a concentration in the outer and tangential walls of the peripheral cells of the cap.

After inoculation with the lectin the epidermal surface shows a distinct, three-layered distribution of label which appears to correspond to the zonation of the extracellular material on the outer periclinal surfaces of columnar epidermal cells distinguished by other histochemical means (see Part II of thesis, p. 81; Miki et al. 1980). The outer periclinal wall of the epidermis and the outer portion of the epidermal mucilage are intensely fluorescent, the inner portion of the epidermal mucilage over the flanks of the root pole (regions A and B) is non-fluorescent (Figs. 3 and 6). The fluorescence of the outer layer (Fig. 3) can usually be followed down the flank of the root in the region where the cap abutts the epidermal mucilage (distal portion of region B) but occasionally this pattern is not observed (Fig. 4). The characteristic three-layered pattern is maintained over the columnar epidermal cells (region C, Fig. 7). However, the middle layer of the surface is always labelled (Fig. 8) in the

FIGURE LEGENDS:

Figures 1 - 26 show hand cut median longitudinal sections of fresh corn roots; Figs. 27 - 29 and 31 - 36 are transverse sections. Unless otherwise indicated, sections were exposed to osmium tetroxide vapour prior to incubation and (unless otherwise noted) were treated in FITC-lectin. In all figures showing longitudinal sections, the root tip is oriented towards the bottom. Exposure and printing times were standardized for comparative purposes.

Fig. 1. Axenic primary root. Note the intense autofluorescence of the tip-cap junction (small arrow), the epidermal mucilage (large arrow) and the peripheral root cap cells. Section was not treated with osmium tetroxide vapour. X 320.

Fig. 2. Axenic primary root incubated, without prior treatment, in FITC-lectin. Fluorescence is associated with both peripheral root cap cells (asterisk) and epidermal mucilage (arrow). This pattern is not clearly distinguished from root autofluorescence. X 140.

Fig. 3. Approximately 0.5mm proximal to the level of the tip-cap junction (region B). There is intense labelling of the cell walls (small arrow) and slime (asterisk) of the root cap. Label is also present in the outer periclinal walls of the epidermal cells (medium arrows) and in the outer layer of epidermal mucilage (large arrow). X 430.

Fig. 4. Approximately 0.5 mm proximal to the level of the tip-cap junction. In this root label associated with the epidermal mucilage cannot be traced acropetally around the flanks of the root proper (arrows) but the root cap cell walls and root cap mucilage are intensely labelled (cp. Fig. 3).

Inset: Sloughed cells at the edge of the root cap showing lectin-binding threads in the surrounding mucilage. Main Figure X 390.

Inset X 50.

Fig. 5. Section including region B incubated in lectin solution containing sonicated root cap mucilage. Both the root cap (asterisk) and epidermal mucilage (arrow) are relatively negative. Film was given 3 times the normal exposure.

Inset: Control section incubated in lectin solution containing 0.2 M α -L-fucose. The fluorescence seen is not as intense as in lectin treated sections and is associated with autofluorescence, which is not quenched with osmium tetroxide vapour, of very young cell walls. The tip-cap junction (arrows) stands out in this respect.

Main Figure X 170.

Inset X 195.

region where the epidermal cells begin to elongate (region C - D transition), except in grooves between tabular cells (Fig. 9) where a distinct three-layered label pattern may persist. In this region the epidermal mucilage in control sections is non-fluorescent (Fig. 10).

The fluorescence of the outer periclinal wall of the epidermal cells can be followed over the tip-cap junction as a very faint layer in lectin labelled tissues. However, the autofluorescence of this junction line is not completely eliminated by exposure to osmium tetroxide vapour. Thus it is not possible to determine if the lectin is binding to this region. Young cell walls of the root cap initials, quiescent centre, stelar initials and cortical cells are also more fluorescent in control tissue (inset Fig. 5) but autofluorescence of mature cell walls is blocked by osmium and the cells do not bind significant amounts of lectin.

Tissues incubated in lectin plus α -L-fucose, lectin plus p-nitrophenyl fucoside, or lectin plus sonicated root cap mucilage (Fig 5) do not fluoresce in regions other than the tip-cap junction and young cell walls. Tissues incubated in lectin plus α -D-fucose, when fluorescence is detected, have label in the epidermal mucilage and to variable extent in the root cap mucilage. Some sections showed no fluorescence.

Goat anti-rabbit FITC- γ -globulin was used as a

Table 1. Fluorescence of lectin treated and control incubations of preosmicated sections of axenic primary roots of Zea mays.

Incubation medium	Number of sections showing fluorescence ^a	Total number of sections observed
FITC-lectin (12.5 mg/ml)	91	91
PBS	1	60
FITC-lectin plus ^b α -L-fucose (0.2M)	18	73
FITC-lectin plus ^b root cap mucilage	3	23
FITC-lectin plus ^c α -D-fucose (0.2M)	6	14
FITC-lectin plus p-nitrophenyl fucoside (0.2M)	0	16
FITC-goat anti-rabbit γ -globulin	13	13

a - Sections showing fluorescence at any level above residual autofluorescence were scored to bring attention to some of the difficulties encountered in development of this technique.

b - Fluorescence in some of these cases can be attributed to problems of rinsing all of the unreacted lectin from the tissue particularly in areas where the solution may have dried to the glass slide beneath the section. Variability in section thickness may also be important.

c - The results of this control are somewhat baffling, although sample size is too small to allow for conclusions. The fact that α -D-fucose did not consistently inhibit the lectin binding is interpreted as showing that some other error had been introduced into the system in one of two experiments in which this control was used.

control to determine if there was non-specific binding of FITC labelled proteins to the root surface. Surprisingly, the sections showed strong overall fluorescence, particularly of the outer root cap mucilage, the outer regions of the epidermal mucilage, and the outer periclinal walls and cytoplasm of the epidermal cells.

These results are summarized in Table 1.

1.3.2 Lectin Labelling of Nodal Roots Below Soil Level.

The first nodal roots are developed from nodes situated below the soil surface. These roots, although broader, are anatomically identical to the axenically grown primary roots and differ very little from the latter with respect to label distribution (Figs. 11, 14). Exceptional are cells on the sides of the root cap, two or three cell layers below the surface, which invariably show an asymmetric distribution of fluorescence with the greatest intensity in the outer periclinal and tangential walls (inset Fig. 11). The walls of surface cells of these caps are uniformly labelled (Fig. 11).

The outer epidermal wall (of region B and C cells) is more intensely fluorescent than similar walls of axenic roots (cp. Figs 12 and 13 with Figs 7 and 8). Where these columnar epidermal cells are becoming tabular (region C - D transition) the three-layered

Fig. 6. Axenic primary root approximately 0.7 mm from the tip cap junction. Cell walls of sloughed peripheral cap cells (asterisk) and the outermost layer of epidermal mucilage (large arrows) are very strongly labelled. X 360.

Fig. 7. Axenic primary root approximately 1 mm proximal to the ~~level of~~ the tip-cap junction. The epidermal cell walls (small arrows) and outer layer of epidermal mucilage (large arrow) are intensely labelled. The inner layer of epidermal mucilage is not labelled and its apparent fluorescence is due to flare from the intensely labelled outer layer seen in this semi-grazing section.

X 530.

Fig. 8. Axenic primary root approximately 2 mm proximal to the level of the tip cap junction (region C). The epidermal surface is completely labelled. X 455.

Fig. 9. Axenic primary root approximately 4 mm proximal to the level of the tip-cap junction (region D). The entire epidermal surface is labelled except in grooves between epidermal cells where the three-layered pattern occasionally persists (arrows). X 390.

Fig. 10. Axenic primary root approximately 3 mm proximal to the level of the tip-cap junction, incubated in FITC-lectin plus α -L-fucose (0.2M). The epidermal mucilage (arrow) is not labelled. Film given 3 times the normal exposure. X 150.

Figs. 11 - 17 show longitudinal sections of young nodal roots initiated below soil level.

Fig. 11. Approximately 0.3 mm proximal to the level of the tip-cap junction. Lectin has bound to the walls (small arrows) and the mucilage of the root cap cells. The outer periclinal walls (large arrows) of the epidermal cells are also labelled.

Inset: Root cap cells which are distal to the tip-cap junction and close to the periphery of the root cap. Cells on the right hand side are 2 - 3 layers removed from the edge of the root cap. All cells show more label in their outer periclinal (right hand) and anticlinal walls than in their inner periclinal walls.

Main Figure X 290.

Inset X 125.

Fig. 12. The outer periclinal walls and the apical ends of anticlinal walls of columnar epidermal cells are strongly labelled as is the outer layer of the epidermal mucilage. Approximately 0.8 mm from the tip-cap junction (region B). X 360.

Fig. 13. Approximately 1 - 2 mm from the tip-cap junction (region C). The transitional epidermal cells and overlying mucilage show the same labelling pattern as the columnar epidermal cells (cp. Fig. 12). X 480.

Fig. 14. Approximately 4 mm proximal to the tip-cap junction. The tabular epidermal cells (region D) show uniformly intense label throughout their outer periclinal surface. X 340.

Figs.

15 - 18. Show increasingly older stages of root hair development. The initial bulge in the epidermal cell wall is strongly labelled (Fig. 15); the base of the young hairs is labelled similarly (Figs. 16 and 17). The walls of older hairs do not label (the fluorescent spot on the hair shown in Fig. 18 is debris). All Figures X 270.

Table 2. Neutral sugars present in root associated mucilages and surface structures of axenic primary roots of Zea mays. Data based on GLC analysis and given as % of total sugars. (Reid, personal communication.)

Neutral sugars	sample epidermal peels	root hairs	root cap
fucose	2	absent	18
arabinose	13	12	16
xylose	21	21	14
mannose	3	0.8	4
galactose	15	9	26
glucose	47	58	22

pattern persists (ie., the inner layer of mucilage is not fluorescent as it is in this region of axenic roots, cp. Fig. 13 with Fig. 8). The surface of labelled tabular cells is uniformly strongly fluorescent (Fig. 14).

Root hairs originate from fully elongated tabular cells approximately 1 cm from the apex. The youngest hairs, just visible as outgrowths of the epidermal wall are distinguished by a strongly lectin-binding surface (Fig. 15). As tip growth of the hair progresses, the lectin-binding material is restricted to the base of the hairs (Figs. 16, 17 and 18) and persists on the surface of all epidermal cells including those which gave rise to the hairs. The lack of fucose residues on root hair surfaces has been confirmed by GLC analysis of root hairs (Table 2). Roots fed with ^3H -fucose, however, have been shown to accumulate fucose residues in the apical tips of the developing root hairs (Forsyth 1982).

I.3.3 Lectin Labelling of Young Green Nodal Roots Which Have Not Penetrated the Soil Surface.

Roots initiated from the higher nodes of field corn are thick, with an extensive photosynthetic cortex. Exposed to air, these roots are characterized by a very dry, hard surface. The root tips are similar to those of nodal roots initiated below the soil except that the cap forms a persistent, intact mantle several

cell layers thick over the flanks of the root pole. The cap cells are covered by a thin, hard skin of material which swells extensively when hydrated and which strongly binds the FITC-lectin (Fig. 19). Label is concentrated in the outer periclinal and tangential walls of subsurface cells on the sides of the root cap (Fig. 20).

The epidermal surface material along the flanks of the apex corresponding to region B is not labelled by the lectin as it is in root tips which lie within the soil and in the tips of axenic primary roots (cp. Fig. 19 with Figs. 3, 4 and 11). However, higher up along the flanks (proximal region B) there is lectin binding in the epidermal wall and outer surface of the epidermal mucilage (Fig. 21). Over the columnar cells (region C) the inner layer of the epidermal mucilage is also labelled (Figs 22 and 23). As noted for other roots, this pattern persists (Fig. 24) over the tabular epidermal cells (region D). Frequently root cap mucilage is present over these cells (Fig. 25). All controls were negative (eg. α -L-fucose, Fig. 26).

I.3.4 Lectin Labelling of Older Green Nodal Roots Which Have Penetrated the Soil.

Like other roots of Zea these roots have persistent root hairs along their entire length within the soil (2 ft. or more). Below the soil surface there is no binding of lectin to these hairs and only very

Figs. 19 - 26 show median longitudinal sections of young nodal roots initiated above soil level. Figs. 27 - 29, 31 - 36 show transverse sections of mature nodal roots initiated above the soil surface. Fig. 30 is a median longitudinal section of such a root right at soil level.

Fig. 19. Approximately 1 mm proximal to the level of the tip-cap junction. In contrast to the case in primary roots, (Figs. 2, 3, and 4), the root cap here forms a persistent mantle over the columnar epidermal cells (E). Root cap cell walls and root cap mucilage are strongly labelled. The epidermal cell surface is not labelled in this case. X 660.

Fig. 20. Peripheral cells of the root cap closer to the tip of the cap in the same section showing strong labelling in the anticlinal walls and periclinal walls (arrows) closest to the root cap surface and in material presumed to lie between the wall and cell membrane. X 690.

Fig. 21. Approximately 2 mm proximal to the level of the tip-cap junction. The outer epidermal wall (small arrow) and outer layer of the surface mucilage (medium arrow) are labelled as are the outer periclinal walls of the abutting root cap cells (large arrows). X 515.

Fig. 22. Approximately 2.5 mm from the tip-cap junction. Labelling of the inner layer of the epidermal mucilage (asterisk) has almost eliminated the layered pattern of label of the immediately younger region (cp. Fig. 21). X 550.

Fig. 23. 3 to 3.5 mm from the tip-cap junction. The epidermal surface is completely labelled. X 500.

Fig. 24. Approximately 5 mm from the tip-cap junction. Here the epidermal surface, which is comparatively thin and irregular, is completely labelled. X 600.

Fig. 25. Region C - D transition of an above ground nodal root. Here the epidermal surface is covered by root cap mucilage (asterisk) and sloughed cells (arrows). In this case the three-layered labelling pattern of the epidermal surface persists (cp. Fig. 23).

X 185.

Fig. 26. A control section which has been incubated in lectin solution containing α -L-fucose. Film given 3 times the normal exposure.

X 200.

Fig. 27. Root hair-bearing region within the soil (these roots have root hairs along their entire length from the soil surface to within 1 cm of the tip) showing a small amount of lectin binding (arrows) to mucilage between the bases of the hairs. The lectin-positive object (asterisk) is probably of root cap origin (sloughed root cap cell).

X 270.

Fig. 28. Section similar to that shown in Fig. 27 except that incubation was in lectin solution containing α -L-fucose. In this region autofluorescence is not completely quenched by the osmium tetroxide vapour pretreatment.

X 290.

little binding to small clumps of material lying above the epidermis between the bases of the hairs (Fig. 27). The autofluorescence of all walls in this region is very strong and not easily quenched with osmium tetroxide pretreatment (Fig. 28). It is, however, possible to distinguish this autofluorescence from FITC fluorescence which is much brighter.

Lectin binding material is present between hairs of these roots right at soil level (Fig. 29). This material is frequently present as large, amorphous clumps on the surface in which sloughed cells, fungal hyphae, and particulate material are embedded (Fig. 30). Closer to the node from which the roots were initiated, surface-associated material is consistently present. This material is either amorphous, diffuse and only weakly fluorescent (Fig. 31), or condensed close to the root surface, strongly fluorescent and associated with sloughed cells (Figs. 32 and 36). Both types of material can be found in the same region of an individual root (Figs. 33 and 34). All controls were negative.

It is interesting to note that aerial portions of mature nodal roots are very slippery to the touch after a brief soaking in water. In some cases the slippery material is easily removed from the root as an intact "skin" which becomes intensely fluorescent when incubated with the FITC-lectin (Fig. 37); the

fluorescence is comparable to the fluorescence shown by the thick material present on the surface of sectioned root tissue (Fig. 36). Unstained mounts of the "skin" viewed with Nomarski or phase optics reveal numerous large bacterial colonies, fungal hyphae, sloughed cells and associated sand particles within an amorphous matrix (Figs. 38 and 39).

Lateral root primordia on the older nodal roots are comparable to the main root in their labelling pattern. Material present over the outer surface of the primordia binds FITC-lectin (Fig. 40). In primordia which have penetrated the parent cortex the epidermal surface is labelled in the characteristic three-layered pattern (Fig. 41). Mature regions of these lateral roots are labelled only in the grooves between epidermal cells (Fig. 42).

The results of lectin labelling experiments for all field grown roots are summarized in Table 3.

1.4 Discussion.

Autofluorescence is very strong in the cell walls and associated mucilages of the fresh root tips of corn (Clarke et al. 1979; Miki et al. 1980), wheat (Smith and O'Brien 1979), other grasses (Harris and Hartley 1976) and onion (Peterson et al. 1978). In wheat this autofluorescence has been attributed to the presence of ferulic and diferulic acid residues (Smith and O'Brien 1979). Elimination of autofluorescence from most sites

- Fig. 29. Section of a root similar to that shown in Figs. 27 and 28 but made at the level of the soil surface. Root hairs (arrows) are present and there is a large amount of lectin-binding mucilage adjacent to them. X 520.
- Fig. 30. Surface of a root (R) with lectin-positive material (asterisk) similar to that in Fig. 25. Sloughed cells (small arrow) resembling root cap cells, fungal hyphae (medium arrows) and indeterminate particles are present in the mucilage. X 240.
- Fig. 31. Large amounts of weakly lectin-positive, amorphous material on the surface of the above ground, green portion of a root. X 180.
- Fig. 32. Lectin-positive material (arrow) on the surface of a nodal root just below soil surface containing several sloughed cells (asterisks).
Inset: Lectin-binding material associated as a distinct, relatively thin surface layer on the above ground nodal root. One of the cells (arrow) is sloughed. Main Figure X 190.
Inset X 165.
- Fig. 33. Lectin-binding material (arrow) associated with the outer periclinal walls of the epidermal cells. X 325.
- Fig. 34. Surface immediately adjacent to that shown in Fig. 33. The inner regions of the mucilage associated with the outer periclinal walls of the epidermal cells and the outer regions of the mucilage are intensely labelled (arrows), the central region of the mucilage is only weakly lectin-positive. X 440.
- Fig. 35. Control section incubated in lectin solution containing α -L-fucose. Film given 3 times the normal exposure. X 130.
- Fig. 36. Lectin-positive, skin-like material on the surface of an above ground nodal root containing several apparently sloughed cells. X 310.

Figs. 37 - 39 show whole mounts of skin like material stripped from the surface of the above ground nodal roots following a brief soak in water.

Fig. 37. Skin material removed from the root surface prior to osmication and lectin incubation. The lectin is strongly bound by this material. X 90.

Fig. 38. Similar skin to that shown in Fig. 37 mounted in water and stained with aniline blue-phenol- acetic acid. Numerous sloughed cells (large arrows) and fungal hyphae (small arrows) are present. X 105.

Fig. 39. Unstained "skin" from an above ground nodal root viewed with Nomarski optics. Several sloughed cells (asterisks) and fungal hyphae are embedded in this material. X 240.

Fig. 40. Longitudinal section of a lateral root primordium (LR) which has grown approximately halfway through the parent cortex. There is lectin-labelled material (asterisk) associated with disintegrating cortical cells (C). Young root cap cells of the primordium show labelled cell walls (arrow).
Inset: Similar section of the same lateral root primordium incubated in lectin solution containing α -L-fucose. Main Figure X 150.
Inset X 50.

Fig. 41. LS of a just emerged young lateral root in the region of the parent cortex. The root cap mucilage is slightly labelled (asterisk) and the epidermal surface shows the characteristic three-layered pattern (arrows). X 160.

Fig. 42. Surface view of region D of an emerged lateral root. Grooves at the junctions of epidermal cells are labelled. X 430.

Table 3. Fluorescence of lectin treated and control incubations of preosmicated sections of field-grown roots of Zea mays.

location on root	incubation medium	sections showing fluorescence ^a		total number of sections
		MLS	TS	
root tips, below soil	FITC-lectin	15	20	35
	PBS	0	0	11
	FITC-lectin plus α -L-fucose (0.2M)	0	0	8
root segments all green, above soil	FITC-lectin	46	31	77
	PBS	0	0	9
	FITC-lectin plus α -L-fucose (0.2M)	0	0	27

a - Sections of mature root tissues in which autofluorescence was not quenched are not included in this data.

b - All MLS sections are of root tips which had not penetrated the soil surface.

c - All sections are of mature green regions of nodal roots formed from nodes of the stem situated above the soil surface. Only FITC associated fluorescence was scored as many sections showed residual autofluorescence following treatment with osmium tetroxide vapour.

in the corn root tissue by the osmium tetroxide vapour pretreatment may be due to the osmium binding to similar compounds since this fixative is known to react with dihydroxy phenols (Nielson and Griffith 1978). The osmium treatment effectively removes most autofluorescence except in the immediate region of the tip-cap junction where a low level of fluorescence is not quenched, and in mature, highly lignified regions. Autofluorescence and fluorescein isothiocyanate fluorescence can be distinguished in the latter areas, lectin binding in the tip-cap region where the two types of fluorescence are similar has not been considered. Osmication has no apparent effect on subsequent tissue reactivity towards the lectin.

The results reported here are restricted to the labelling pattern observed using fresh, osmicated material incubated in Tetragonolobus purpureus lectin. This lectin was chosen because it is available in purified form conjugated to the fluorescent tag fluorescein isothiocyanate. Previous work has shown that this lectin, as well as a lectin from Ulex europaeus which also binds terminal fucose, are suitable tools for the purpose of investigating the synthesis of root cap slime (Rougier et al. 1979). The results of the control incubations indicate that the fluorescence described in the present study is due only to binding of the labelled lectin to sites from which

it is restricted if it has been reacted previously with α -L-fucose. Thus the fluorescence appears to be linked only with sites containing α -L-fucose residues. Previous work (Allen et al. 1977) has indicated that the lectin used recognizes specifically α -L-fucose in terminal, non-reducing positions and does not recognize any monosaccharide internal to this position. Pereira et al. (1978) suggest that this specificity may involve short fucose terminal branches linked to galactose. It is assumed therefore that the fluorescence observed after FITC-lectin incubation of the corn root tissues indicates the location of polysaccharides bearing terminal fucose residues.

Biochemical analyses of corn root tips have indicated the presence of a high proportion of fucose residues which are assumed to be a constituent of the root cap mucilage polymers (eq. Harris and Northcote 1970; Wright and Northcote 1974; James and Jones 1979a). (Corn roots are apparently unique in this respect; Wright and Northcote [1974] found that neither wheat nor pea root tips contain significant amounts of fucose.) There is some discrepancy regarding the position of the fucose residues relative to the other monosaccharide units of the mucilage polymer. James and Jones (1979a) conclude that the fucose occupies terminal non-reducing ends of the branches of the acid fraction polymers. In contrast Wright et al. (1976)

conclude that the fucose is not necessarily terminal, since purified rat epididymal α -L-fucosidase preparation which was shown to cleave α -1 $\rightarrow$ 2 fucose linkage in fucoidin, did not release more than 17% of the radioactive fucose incorporated into the root cap mucilage. A recent report that an almond emulsion α -L-fucosidase does not act on fucose α -1 $\rightarrow$ 2 galactose linkage (Ogata-Arakawa et al. 1977) suggests that the results based on the treatment of root cap mucilage by this enzyme may be inconclusive. The acidic component of the root cap mucilage is thought to consist of a backbone of galacturonic acid and glucose to which the neutral sugar, fucose, is attached (Wright and Northcote 1974). The fucose-galacturonic acid linkage, like the fucose-galactose linkage may be protected against cleavage by this enzyme.

The lectin binding observed in the present study, while giving no clue to the presence of non-terminal fucose residues, does confirm the presence of a sizable number of terminal fucose residues in root cap polysaccharides. Controls in which lectin preincubated with sonicated root cap mucilage is unavailable to bind to tissue components provides additional confirmation for the presence of terminal fucose residues in the mucilage. It should be noted that while sonication treatment was utilized to reduce the viscosity of the mucilage, the treatment may have exposed internal

fucose residues (if present).

These results on sectioned materials extend the observations of Rougier et al. (1979) by showing that within the root cap the terminal fucose-containing polymers are not uniformly distributed, but predominate in the walls and surrounding mucilage of a few layers of peripheral cells on the flanks of the cap. The lectin-binding sites occur in a pattern similar to that observed for the incorporation of tritiated fucose (Paull and Jones 1975b; Rougier 1976), and to the locations of concentrations of polysaccharides with reactive 1,2 glycol groups (positive PAS reaction) and polyuronides (toluidine blue O-induced metachromasy) as indicated by histochemical means (Miki et al. 1980). However, these results are in contrast to those of Hinch and Clarke (1980) who did not detect a significant binding of the fucose-specific Ulex lectin to root cap mucilage in a different variety of corn. Only the elongation zone was found to bind this lectin strongly. Their findings have recently been confirmed (Guinel, unpublished) with the Ulex lectin. Both Seneca Chief and the variety originally used by Hinch and Clarke have been shown to be labelled by the Lotus lectin in the distinct pattern reported here, but not by the Ulex lectin. Although both lectins are thought to share a common specificity for terminal fucose residues (Allen et al. 1977); this discrepancy

indicates that the two lectins recognize fucose in different linkage positions (ie. subterminal vs. terminal, or short chain vs. long chain). It is interesting that the Ulex lectin does not label the root cap mucilage in either variety, particularly since fucose is present in relatively large amounts in this mucilage compared to the epidermal mucilage (GLC analysis; see also Harris and Northcote 1970).

Strong, distinctly-layered lectin-labelling of the outer periclinal walls and overlying mucilage of older columnar epidermal cells reflects the three-layered nature of this surface material as it is demonstrated by various staining methods and by polarizing and fluorescence microscopy (Clarke et al. 1979, Miki et al. 1980). However, the distribution of terminal fucose residues indicates that their appearance does not always parallel developmentally that of other properties of these layers. For example, in axenically grown roots, the three regions of the epidermal surface (ie. epidermal cell wall, inner and outer epidermal mucilage layers) are always clearly distinguished on the flanks of the meristem (region B) under the root cap (Clarke et al. 1979; Miki et al. 1980) by specific staining and optical microscopy methods. This layering is not always seen in comparable material labelled with the lectin (eg. cp. Fig. 4 with Fig. 22 but see also Fig. 3). Furthermore, the inner layer of the epidermal

mucilage of axenically grown roots which, even in the most distal portions of region B, is characterized by its strong birefringence and PAS-positivity, does not show the presence of terminal fucose residues until a position well back along the flanks of the meristem. Over a short distance basipetally along the root, the inner layer of the mucilage becomes labelled with the lectin (Figs. 8 and 9). Variability in plane of the hand sections may be involved but the relative frequency with which this characteristic was observed provides evidence that this change is real rather than artifactual. There is no comparable change over the same region in any of the observed staining or optical properties of the same area of mucilage (Clarke et al. 1979; Miki et al. 1980).

Although the late appearance of the lectin-binding could suggest that the terminal-fucose bearing polymer is secondarily secreted into the epidermal mucilage, the alternate explanation that fucose residues of this layer are progressively unblocked is equally valid. The presence of fucose residues in these areas has been verified by GLC analysis of epidermal strips (Table 2).

Earlier work (Rougier 1976) has shown that there is heavy incorporation of ^3H -fucose into epidermal cell cytoplasm, walls and overlying mucilage. However, since no autoradiographs of sufficiently low magnification were published, comparison of patterns with lectin

labelling sites identified in the present study is not possible.

Just recently, Forsyth (1982) has investigated the incorporation of L-fucose 6-³H at the optical level. Incorporation into the cytoplasm and outer periclinal cell walls of the epidermal cells and into the inner layer of epidermal mucilage adjacent to the cell walls was observed following a 60 m incubation. Epidermal mucilage over the flanks of the root pole (regions A and B) also appears to become heavily labelled (Figs. 45 and 49, Forsyth 1982). Although label eventually moves out into the rest of the epidermal mucilage, the outer layer of this mucilage is not noted to become heavily labelled even after a 90 m chase treatment (Figs. 117 - 119, Forsyth 1982). These sites of incorporation are not strictly comparable to those observed with the fucose specific lectin in the present study. Although label does appear throughout the epidermal mucilage over maturing epidermal cells (regions C and D), lectin-labelling sites are restricted in regions A and B to the outer periclinal epidermal cell wall and in region B to the outermost layer of the overlying epidermal mucilage. The difference in labelling patterns is likely a reflection of the restricted specificity of the lectin (which labels only terminal fucose residues) and to the limited chase period (90 m) used in the previous

study. Labelling patterns of the peripheral root cap cells with both the ^3H -fucose (90 m chase) (Forsyth 1982) and the fucose-specific lectin are comparable.

It has been well established that a fucose-containing polysaccharide associated with the root cap of corn is secreted by the cells of the outer regions of the cap (eg. Paull and Jones 1975b; Rougier 1976; Rougier et al. 1979). On the basis of lectin-labelling patterns it is apparent that the small amount of fucose extractable from segments of roots above the cap (Harris and Northcote 1970) is probably restricted in its distribution to the epidermal surface. There is, however, little hard evidence that the epidermal cells, like the peripheral cells of the root cap are involved in the synthesis and export of a specific fucose-containing polymer. On anatomical grounds it would appear likely that the components of at least the outer periclinal walls and the inner layer of mucilage must originate from epidermal cells and autoradiographs published by Dauwalder and Whaley (1974, Fig. 12 p. 25) and by Rougier (1976), as well as those obtained by Forsyth (1982), are consistent with this possibility. The absence of lectin-binding material from the walls of root hairs suggests that the older tabular epidermal cells may have lost the capacity to synthesize the fucose-containing polymer, or at least to fucosylate it. (Evidence of ^3H -fucose

uptake or incorporation by mature root hairs [Fig. 157b, Forsyth 1982] alternatively suggests that fucose is indeed present but not terminal.) The small amount of fucose-containing material that persists on the epidermal surface between the bases of the root hairs and in the grooves between epidermal cells of quite old roots could, therefore, be residue of either the polymer synthesized by columnar cells at the root tip or root cap mucilage.

It is difficult to explain the origin (and persistence) of the relatively large amounts of lectin-binding mucilage which accumulates around even very long nodal roots just at the point where they have entered the soil and on the aerial surface of these roots. While a microbial origin of this material has not been ruled out, the presence in it of cells strongly resembling sloughed root cap cells suggests that this mucilage may have been shed by the extensive root cap before it reached the soil and as it penetrated the soil surface. It is difficult to imagine, however, how this material persists in the field on basal regions of roots that have grown several feet into the soil.

The mucilage which accumulates on the nodal roots at and above the soil surface is probably the same as that analyzed by Floyd and Ohlrogge (1970). These authors describe the mucilage as "forming" on the root

surface when short, field grown nodal roots were soaked in distilled water. Analysis of this mucilage (Floyd and Ohlrogge 1970) indicated an approximate molecular ratio of 3:7:8:5:11 for uronic acids, galactose, arabinose, xylose and fucose respectively. This is at least qualitatively similar to results obtained for root cap mucilage (eg. Wright and Northcote 1974).

The concentration of fucose rich polysaccharides at specific locations on the surface of both axenic and field-grown corn roots suggests that this material may play an important role in recognition of these regions by associated microorganisms, both benevolent and pathogenic. In fine structure studies the presence of a relatively small characteristic microflora with soil grown roots has been observed (see Parts II, III and IV of thesis). These organisms are not randomly distributed but tend to aggregate around root cap mucilage and at the surface of the epidermis of mature roots between the root hairs. The fact that these organisms are almost inevitably absent from the surface of the epidermis distal to the youngest root hairs suggests that fucose residues may not be involved in their localization. Other factors, such as the strongly autofluorescent components present in this region of the root (Part II of thesis) may function to prevent colonization by all but a few resistant microbes.

Azospirillum sp. has been localized on the nodal

roots of corn in aggregations along the grooves between epidermal cells or as patches or streaks covering several cells. However, root hairs (which have been found to lack fucose residues (Table 2, Fig. 18) and apical regions of the root were bare (Patriquin and Dobereiner 1978). While the lack of mucigel, rather than the lack of fucose residues per se, could explain the absence of bacterial colonies on the root hairs, noncolonization of the apical epidermal surface suggests that fucose may not be involved in the recognition event. A recent study on rhizosphere microorganisms has shown a specific attraction of Azospirillum lipoferum to maize roots and it was suggested that the form of the carbohydrate substrate provided by the plant could play a role (Baldani and Dobereiner 1980). Matsumoto et al. (1979) have also shown that Azospirillum sp. is affected by maize root excretions. These authors identified stachyose as the main carbohydrate involved in the recognition, but have not excluded the possibility that other sugars are responsible.

Hinch and Clarke (1980) have demonstrated that Ulex lectin, which also binds terminal fucose residues, completely prevents adhesion of Phytophthora cinnamomi zoospores to the elongation zone of Zea mays roots. Further pursuit of similar lines of study are certainly required to elucidate these recognition phenomena.

Histochemical, histological and structural study of field-grown corn roots. Part II: Characteristics of the sheath free tips of young (to 20 cm long) roots of corn.

II.1 Introduction.

To this point the chemical composition of root cap mucilage of Zea mays has been extensively studied (see for example Wright and Northcote 1974, 1975 and 1976) and the exogenous factors controlling the amount of material produced by the primary root have been investigated (Morre et al. 1967, Jones and Morre 1973). As early as 1911, Price recognized a distinction between the root cap and root epidermis associated mucilages of desert grasses. Recently, ultrastructural and histochemical evidence has also indicated that the root cap mucilage differs in its staining reaction and ultrastructural appearance from materials present over the epidermal cells of the primary roots of corn (Clarke et al. 1979 and unpublished). Axenic primary roots of several other grasses, including barley and wheat, have also been shown to have a mucilagenous secretion over the epidermal cells which is distinct from the root cap mucilage (Miki 1977, Miki et al. 1980). The biochemistry of this epidermal surface layer has not yet been thoroughly considered, although in wheat roots phenolic acids are thought to be present as cross links between carbohydrate chains (Smith and

O'Brien 1979) in the epidermal mucilage.

Although the primary root lends itself well to experimentation, it is of little functional importance to the plant and is generally thought to be degraded (but see Kausch 1967) after the extensive adventitious root system has developed (Hayward 1967). In order to appreciate the role of various structural aspects of the corn root in its natural environment it becomes necessary to study the nodal roots (adventitious roots arising from the base of the intercalary meristem immediately above each node) of corn to a greater degree. Floyd and Ohlrogge (1970, 1971) have described the presence of a mucilaginous secretion over the surfaces of very young nodal roots in natural conditions. However, neither the origin of this material nor its anatomical and histochemical features were investigated by these authors. In the present report these characteristics are described for field grown, nodal roots of Zea mays var. Seneca Chief.

II.2 Materials and Methods.

For general techniques and fixation procedures, refer to General Materials and Methods p. 10 - 13 of thesis. A minimum of 8 roots were sampled at each area (root cap, apical mm, 1 cm, 2 cm and 3 or 4 cm from the root tip) of the root. Roots ranged in length from 2 to 20 cm.

II.3 Results.

In describing different regions of the corn root tips, the designation (A, B, C, D) of Clarke et al. will be used. These regions are illustrated in Diagram 1, Part I of thesis (p. 32).

The surface of even the youngest "in soil" nodal roots arising from the coleoptilar node and all subsequent nodes is clearly delimited into two zones (Fig. 1): a) An apical soil-free zone extending from just above the root cap to an average of 25 mm basipetally along the root pole and b) a basal zone in which the root is encrusted by a thick soil sheath. The clear zone generally increases in length with increase in root length (Fig. 2). The portion of any given root occupied by the sheath-free zone is highly variable throughout the growing season but does tend to decrease during this period (Fig. 2 - measured over a 3 w period). The following results deal only with the sheath free apical zone of young (up to 20 cm long) nodal roots.

II.3.1 Anatomy of the Sheathless Apical Zone of Field Grown Adventitious Roots.

The apical portion of the clear zone is identical in tissue arrangement to most grasses studied to date (Fig. 3). The protective root cap with its associated mucilage is clearly separated from the root proper at the tip-cap junction which becomes thickened over the

proximal region A epidermal cells, and epidermal mucilage is distinct over region B epidermal cells (Fig. 3).

The root cap cells of field-grown nodal roots show a progression of organelle and cell wall changes from the relatively undifferentiated initials (Figs. 3 and 4) through cells with large starch grains (Figs. 3 and 6) to peripheral cells lacking starch. Large amounts of secretory product accumulates between the walls and protoplast in these latter cells (Fig. 7). Ultimately the accumulated material is observed filling the void between the root cap and the soil which adheres to this mucilage (Fig. 4).

Microtubules are present throughout the life of each root cap cell. Initially they are involved in cell plate formation (Fig. 5) but subsequently appear lining the walls between the plasmodesmata (Fig. 6) at sites where the secretory product accumulates (Figs. 6 and 7). Rough endoplasmic reticulum (ER) is initially present as an extensive network of long strands lying parallel to the walls in columella cells which also possess large amyloplasts (Fig. 6). In these cells the ER is occasionally seen to connect to plasmodesmata (Fig. 6) and lies adjacent to the sites where small amounts of secretory product accumulates (Fig. 6). Endoplasmic reticulum is also present in all peripheral cells of the root cap but becomes somewhat less

FIGURE LEGENDS:

Fig. 1. Three week old corn plant. The primary root at the end of the mesocotyl (M) is no longer intact. Several mature seminal roots and coleoptilar nodal roots (arrows) are completely sheathed in soil; their tips have been severed. Two young nodal roots initiated at the first node of the stem show the clear demarcation between the upper sheathed zone (large arrow) and the soil-free apical zone of the root (brackets).

Fig. 2. (a) Length of the clear zone as a function of root length at three sample dates. The length of the apical clear zone generally increases with increased root length in any given sample group. For each sample date the average value of clear zone length given for roots <2.5, 2.5-5, 5-10 and 10+ cm long respectively.

(b) Graphic representation of the clear zone as a percentage of total root length at three collection dates. For each date the bars represent the average for roots <2.5, 2.5-5, 5-10 and 10+ cm long respectively. No clear pattern emerges.

Fig. 3. Median longitudinal section (MLS) of glycol methacrylate (GMA) embedded field grown root. Note the epidermal mucilage (large arrow) overlying the epidermis (Ep). The root cap (RC) is separated from the root proper by the thickened tip-cap junction (small arrows). The peripheral root cap cells secrete root cap mucilage (asterisk) into the surrounding soil (rinsed away). Numbers correspond to the figure in which the cells of the region indicated are shown. Stained by the periodic acid Schiff's reagent (PAS) followed by toluidine blue O (TBO). X 125.

Fig. 4. MLS of GMA embedded section demonstrating region A (A) and region B (B) epidermal cells, and epidermal mucilage (large arrows). Root cap mucilage (large asterisk) is secreted by peripheral root cap cells (small asterisk), filling the void between the root cap (RC) and soil (small arrows). Sand grains (S) are lost from the material during sectioning leaving holes. Note the periclinal and oblique cell divisions on the flank of the root cap (bracket) which will ultimately give rise to flanking peripheral root cap cells. PAS-TBO. X 265.

Figs. 5 - 21 are MLS of Zea roots prepared for electron microscopy, Figs. 22 and 23 are transverse sections.

Fig. 5. Root cap initial cell following cell plate formation. Note the short strands of rough endoplasmic reticulum (ER) (arrows), plasmodesmata (large arrow) and the numerous dictyosomes with small vesicles (D). X 5,880.

Fig. 6. Central root cap cell containing numerous amyloplasts (A), strands of ER (arrow) lining the regions next to the primary wall, dictyosomes (D) with small vesicles and the small amount of sequestered secretory product (SP) outside of the plasmamembrane which is lined by both ER and microtubules. Inset: Dictyosomes (D) and ER (arrows) close to the wall. Note the ER-plasmodesmatal continuity at the large arrow and the numerous microtubules lining the plasmalemma (bracket). Main Figure X 10,500. Inset X 10,000.

Fig. 7. Peripheral root cap cells. Strands of rough ER (arrows) and dictyosomes (D) with hypertrophied cisternae and numerous large vesicles (V) some of which lie close to the plasmalemma, are the most conspicuous organelles. Secretory product (SP) is present between the protoplast and the cell wall; its ultrastructural appearance is similar to that of the material contained in the vesicles. This cell is shown in lower magnification in Fig. 13. Main Figure X 13,000. Inset: Microtubules lining the plasmamembrane in the region shown by the box in the main figure. Inset X 29,250.

2

3

6

extensive as the cells are sloughed (cp. Fig. 8 and Fig. 6).

Although the dictyosomes are present in all cells of the root cap, they remain apparently "inactive" until the cell is displaced to the peripheral portions of the cap. Even in those columellar cells where some material is being secreted (Fig. 6) the dictyosomes are comparable to those present in the dividing cells (root cap initials); in both cases the vesicles associated with the dictyosome are very small (cp. Figs. 5 and 7). Peripheral cell dictyosomes become progressively more hypertrophied and in cells accumulating mucilage between the protoplast and the wall the entire Golgi apparatus consists of swollen cisternae, large associated vesicles and numerous free vesicles all containing fibrillar material comparable to the root cap mucilage (Fig. 7). Many similar vesicles are observed adjacent to the plasmalemma (Fig. 7) suggesting that fusion with the plasmamembrane precedes release of the dictyosome packaged material to the extracytoplasmic space. In sloughed root cap cells the dictyosomes revert to a morphological form comparable to that found in meristematic cells (cp. Figs. 8 and 5).

Accumulation of mucilage between the wall and plasmalemma prior to its release is usually markedly asymmetric being found against outer periclinal and

anticlinal walls of peripheral root cap cells. However, very active cells and peripheral root cap cells flanking the root pole accumulate secretory material along all the walls (ie. Figs 7 and 13) except at primary pit fields. Plasmodesmatal connections between root cap cells remain intact until the cells are sloughed (Fig. 9). These plasmodesmata may become occluded with electron dense material and are easily visible after the cells have sloughed (inset, Fig. 9).

The root cap initials and the distal layer of quiescent center cells are separated by a slightly thickened boundary, the tip-cap junction (Figs. 4 and 10). The protodermal cells of the flattened portion of the root pole are still connected to the root cap initials by plasmodesmata traversing thin wall areas in the tip-cap boundary (Fig. 11). As the protodermal cells become square in face view and the wall between the outer tier of the root pole and the root cap becomes markedly thickened (region A, Fig. 4) the frequency with which the plasmodesmata cross the boundary decreases. However, abruptly thinned regions in the boundary, traversed by plasmodesmatal channels, are still encountered (Fig. 12). Proximal region B cells and distal region C cells are no longer connected to the flanking root cap cells; both plasmodesmata and evidence of primary pit fields are lacking (Fig. 13). The walls of the epidermal cells and root cap cells are

at this point completely separated by a thick (4 - 5 μm) layer of epidermal mucilage (Fig. 13)

Like the root cap cells, epidermal cell maturation is marked by a series of distinct morphological changes. The most striking alteration is the gradual thickening of the tip-cap boundary over region A cells as the epidermal mucilage accumulates. Over region B cells the layer separating the root cap cell protoplast and epidermal cell protoplast measures 5 μm and consists of three more or less distinct layers: 1) the inner root cap cell wall, 2) the epidermal mucilage (4 - 4.5 μm thick) and 3) the outer periclinal epidermal cell wall (Fig. 13). Over region C cells where there are no flanking root cap cells, the epidermal surface still consists of three layers: 1) the outer periclinal wall of the epidermis, 2) the epidermal mucilage and 3) a thin, loose fibrillar material which is likely residual root cap mucilage (Fig. 14). The entire surface is an average of 6 μm thick (ranges from 2.49 to 9.8 μm were recorded) and is thickest over the distal C region cells. Frequently not all layers of the epidermal surface are easily distinguished even in median longitudinal section (for example the epidermal cell wall and epidermal mucilage in Fig. 15 are not clearly distinguishable although the residual root cap mucilage is easily identified).

The epidermal surface thins considerably

(2 - 2.5 μm thick) as the epidermal cells become tabular (region D) but the three layers persist, although not always easily distinguishable (Figs. 18 and 19). Epidermal mucilage remains distinct further from the tip (1 cm to the end of the sheath free portion of the root) but varies considerably in thickness and in reaction with the heavy metals used to stain thin sections (cp. Figs. 20 - 24). The epidermal mucilage is thickest in the groove between adjacent epidermal cells (Fig. 22) but even in these grooves is highly variable in its ultrastructural characteristics and often consists of three distinct regions including the epidermal cell walls, epidermal mucilage and an electron-lucent region in the base of the groove (which may be related to the plane of sectioning). Over the epidermal cells the mucilage is generally two-layered, lacking evidence of the residual root cap mucilage present over the surface of younger epidermal regions closer to the tip (cp. Figs. 20 and 22 with Fig. 19). Occasionally, however, large amounts of loosely-fibrillar material of undetermined origin is present above the epidermal mucilage (Figs. 23 and 24). This material is often associated with free living cells. Most of these cells were observed to possess electron dense structures within the walls presumed to be plasmodesmata (Fig. 23) and a full complement of organelles. Both bacteria and fungi are frequently

present in this layer (Fig. 24). Root hairs are also present but are not numerous and only occasionally encountered in the thin sectioned root tissue.

Early changes in the cells along the boundary between the root pole and cap are accompanied by changes in apparent number of organelles and structural changes in at least the dictyosomes and mitochondria, not unlike those observed in cell lineages in the root cap. Protodermal cells lying immediately above the root cap, initials, are densely cytoplasmic cells with numerous mitochondria (Fig. 11), several small vacuoles (Fig. 10), dictyosomes with small vesicles (Fig. 11) and short strands of rough endoplasmic reticulum. Microtubules are numerous and can be found along all walls including the tip-cap junction (Fig. 11).

As the protodermal cells become square in the tangential face view (distal region A) there is an abrupt increase in the number of dictyosomes and a gradual decrease in the number of plasmodesmata between the root cap and epidermal cells. Short strands of endoplasmic reticulum and proplastids also increase in number at this point. These organelles remain throughout the life of the epidermal cells (Figs. 14 - 19). Microtubules are frequently observed lining the outer plasmamembrane of epidermal cells (eg. Fig. 15) and are present even as high as 4 cm above the tip (Figs. 21 and 23).

Fig. 8. Sloughed peripheral root cap cell. The root cap mucilage has been extruded and is present as fibrillar material outside the wall (CW). The dictyosomes (D) are no longer hypertrophied and large vesicles are lacking. ER is present as small strands (arrow) in the peripheral cytoplasm.

X 22,350.

Inset: Dictyosomes (D) in a sloughed cell.

X 37,330.

Fig. 9. Peripheral root cap cells prior to being sloughed. The secretory product is present between the protoplast and cell wall except at primary pit fields (arrow). The plasmodesmatal connections between the left and right hand cell are intact (pd) although they are filled with material.

X 11,430.

Inset: Channels (arrow) in the wall of a sloughed root cap cell resembling plasmodesmatal sites.

X 12,000.

Fig. 10. MLS of the root quiescent center (QC), root cap initials (RC) and the flattened tier of cells (Ep) over the apical pole of the root. The root cap and root proper are separated by the slightly thickened tip-cap junction (arrows).

X 2,200.

Fig. 11. One of the flattened cells covering the root pole. Mitochondria (M), vacuoles (V) and short strands of ER (small arrows) are characteristically found in these cells. A plasmodesma connects this cell with the adjacent root cap initial (RC), numerous microtubules (medium arrows) line the plasmalemma and a coated vesicles (large arrow) is present.

X 26,000.

Inset: An example of the type of dictyosome present in the flattened tier of cells over the root pole.

X 14,000.

Fig. 12. Region A epidermal cell (Ep) and adjoining root cap cell separated by a progressively thickened tip-cap boundary (T/C) except at an abruptly thinned region traversed by numerous plasmodesmata (pd).

X 16,000.

Fig. 13. Region B epidermal cells (Ep) and flanking root cap cells (RC) (the lower root cap cell is shown in higher magnification in Fig. 7). The thickened tip-cap boundary is replaced by epidermal mucilage (EpMu). Root cap mucilage is secreted towards this boundary (arrow) as well as towards the outer periclinal walls of the root cap cells. X 2,000.
Inset: Epidermal cell dictyosome (D) with numerous large vesicles (V) associated with it. X 30,000.

Fig. 14. Region C epidermis. The epidermal surface is divided into three distinct regions: the outer periclinal cell wall (CW), the epidermal mucilage (Mu) and the residual root cap mucilage (arrow). Dictyosomes (D) with small vesicles are present in the cell. X 14,000.

Fig. 15. Region D epidermal cell (Ep). The three regions of the epidermal surface [cell wall (CW), epidermal mucilage (Mu) and residual root cap mucilage (arrow)] are not easily distinguished (cp. with Fig. 14). Note the large vesicles (small arrows) associated with the dictyosomes (D). X 8,620.
Inset: Microtubules (arrows) along the outer periclinal epidermal wall. X 32,000.

Figs. 16 & 17. Cytoplasm of region C epidermal cells. Long strands of rough ER (ER), numerous vacuoles (V), undifferentiated plastids (Pl) and dictyosomes (D) often with large vesicles (arrows) are characteristic. Nucleus (N).
Fig. 16 X 16,670.
Fig. 17 X 9,000.

There is some evidence that secretory activity (based on increase in size and nature of the contents of vesicles associated with the dictyosomes) in the epidermal cells is synchronous with accumulation of secretory product in peripheral root cap cells. Of 22 roots for which data was collected, 11 roots possessed dictyosomes with enlarged vesicles in both the epidermal and peripheral root cap cells (Fig. 7 and Fig. 13 show root cap and epidermal cells from the same root); 7 roots had dictyosomes with small vesicles at both sites. For the remaining roots, association could not be clearly determined. In addition to the synchrony in secretion between epidermal cells and root cap cells, there is indirect evidence of cyclic secretory activity of the epidermis. Enlarged vesicles with dark contents were observed simultaneously with microtubules lining only the outer periclinal plasmamembrane of epidermal cells. Of eighteen roots tallied, 11 roots lacked both active dictyosomes and microtubules while 9 roots lacked microtubules although dictyosomes were present with at least some larger vesicles. The association was not as lucid in more mature regions (2 - 4 cm from the tip) of the soil-free apical zone of the root.

II.3.2 Anomalies of Field Grown Roots.

Large groups of epidermal cells (all of regions B and C) of several of the field-grown roots sampled in

this study have extremely dense cytoplasm. Strands of dilated endoplasmic reticulum, a high density of free ribosomes and numerous vacuoles containing fibrillar material are characteristic of these cells. Large, globular, electron dense deposits line the plasmalemma and neither dictyosomes nor microtubules are apparent (Fig. 25). It is interesting, however, that even in these roots, the epidermal mucilage is always intact (Fig. 25), perhaps secreted by the epidermal cells before they died. Epidermal cells of region A are always intact (Fig. 26) and it is possible that much of the epidermal mucilage is produced by epidermal cells at this stage of maturation.

Some of the roots sampled (particularly early season primary and seminal roots but also a few late season nodal roots) are characterized by large wall-free areas of cytoplasm in the epidermal, cortical and stelar tissues of the root tip (Fig. 27) accompanied by some swelling of the root (Fig. 27). Most root cap cells are not affected. However, cells close to the tip-cap junction and root cap cells flanking the apical portion of the root pole may show evidence of disruption (Fig. 28). Cells in areas surrounding the wallless, cytoplasmic filled cavity are surrounded by a thin cell wall located within an extracytoplasmic space which is usually filled with loose, short, fibrillar material (Fig. 29). The

- Fig. 18. Region C - D transition epidermal cell. The outer periclinal cell wall (CW) and the epidermal mucilage (Mu) are not easily distinguishable but residual root cap mucilage is distinct (arrow). Note the numerous mitochondria (M) and dictyosomes (D) which have large associated vesicles. The dark band in the epidermal mucilage is a fold. X 14,400.
- Fig. 19. Region D epidermal cell. The numerous small vacuoles present in region B and C cells have coalesced into a single large central vacuole (V). There is also a clear distinction between the epidermal cell wall (CW) and epidermal mucilage (Mu). The amount of residual root cap mucilage (arrow) is markedly reduced (cp. with Fig. 18). X 6,670. Inset X 32,000.
- Fig. 20. Epidermal cell (Ep) 3 cm above the root tip. The outer periclinal cell wall (CW) and the epidermal mucilage are intact and of comparable thickness and there is a small amount of soil (S) present over the surface. A suberin lamellae has not yet formed in the wall between the epidermis and hypodermis (Hy). X 17,330.
- Fig. 21. Oblique section of an epidermal cell at 4 cm from the tip of the root. Numerous microtubules (arrows) are present near the cell wall. X 14,000.
- Fig. 22. Groove between two epidermal cells approximately 2 cm from the root tip. Epidermal mucilage (Mu) completely fills the groove between the cells and is therefore thickest at this point. X 6,750.
- Fig. 23. Epidermal cell approximately 4 cm above the tip with intact cytoplasm including a large central vacuole (V), mitochondria (M), plastids (Pl) and dictyosomes (D) with vesicles with dark contents. Microtubules are also present along the outer wall (but are too small to label at this magnification). The sloughed cell (SC) above the epidermis is embedded in a large amount of fibrillar material of unknown origin (asterisk). The cell has a large central vacuole (V) and mitochondrion (M) and scattered electron dense material (large arrows) in the wall which appears continuous with the fibrillar material. X 12,730.

Fig. 24. (a), (b) and (c) are all of median longitudinal sections of the epidermis.

(a) Approximately 1 cm from the root tip. The thin layer of epidermal mucilage is covered by a large amount of unidentified secretory material (asterisk) which is in turn bound to a large amount of soil (S) and in which a bacterial cell (arrow) is embedded.

X 8,570.

(b) Region D epidermal cell. The thick layer of epidermal mucilage (Mu) is covered by fibrillar material bound to the soil (S) and in which a bacterium (B) is embedded.

X 21,330.

(c) Approximately 2 cm from the tip-cap junction. The fibrillar material surrounds a fungal hypha in this case.

X 15,000.

Fig. 25. Region B epidermal cell showing an abnormally dense cytoplasm in which only small vacuoles (V) and apparently dilated ER are conspicuous. Electron dense material (arrows) lies between the plasmamembrane and the cell wall of this cell.

X 11,200.

Fig. 26. MLS of a GMA embedded root. The region B epidermal cells (Ep) are strongly autofluorescent due to the extremely dense cytoplasm. However, region A epidermal cells (bracket) are intact. Epidermal mucilage (arrow) is present over both regions. Note also the intense autofluorescence of accumulated root cap mucilage and walls of the peripheral root cap cells (asterisk). Autofluorescence is diminished further from the cap. The strong jade-green fluorescence of the epidermal mucilage is not captured on the black and white negative. Fluorescence optics.

X 230.

extracytoplasmic space occasionally appears to be connected to regions of dilated rough endoplasmic reticulum which, in turn, may appear to be continuous with the nuclear envelope. Neither the walls nor the fibrillar material are aniline blue positive (ie. they do not contain callose). In all affected areas, osmiophilic materials line the plasmamembrane and occlude the plasmodesmata (Figs. 29 and 30).

Nuclei are frequently observed in positions which suggest migration of the nuclei into a neighbouring cell through the primary pit field. This type of apparent migration of nuclei does occur across the tip-cap boundary (Fig. 28) but is more commonly restricted to large areas of the cortex close to regions where large cavities are found. Extreme nuclear abnormalities are present in some areas, particularly where a few files of cells parallel to the root axis are involved in cavity formation. In this case stretched and paracrystalline fragments appear coalesced into large masses of nuclear material in which both heterochromatin and nucleolar constituents are evident (Fig. 30).

II.3.3 Anatomy of the Apical Zone of Greenhouse-Grown Roots.

Although lacking a soil sheath, roots grown in the greenhouse are similar on an anatomical basis to field grown roots. The ultrastructure of the cells and

appearance of secretory activity is also similar (Fig. 31). (Exceptional are roots grown in pots in field soil. Although these roots show the typical cellular arrangement characteristic of corn roots, many of the cells of the root cap and quiescent center become compressed [likely due to the soil structure].) However, there is greater variation in the appearance of the epidermal mucilage which generally shows a much less dense arrangement of fibrils over the region B and C epidermal cells when grown in either vermiculite or Promix BX (Fig. 32). In regions further removed from the tip, epidermal mucilage is either absent (Fig. 36) or extremely expanded, occasionally with a boundary (Fig. 37). In addition, greenhouse-grown roots frequently have large bacterial colonies localized over grooves between cells (Fig. 34 and 37) but also over region B (Fig. 33) or C (Figs. 32 and 35) cells.

II.3.4 Histochemistry of the Sheathless Apical Zone.

The tip-cap junction of field-grown nodal roots is clearly distinguished (Table 1) from the walls of surrounding cells of both the root cap and quiescent center by its more intense autofluorescence and birefringence, and its greater Calcofluor positivity (Table 1), as well as by its stronger staining reactions with Nile blue (Fig. 39), Alcian blue (Fig. 40), PAS and toluidine blue O (Figs. 38).

Root cap mucilage is pink with PAS (Fig. 43),

Fig. 27. MLS of a root approximately 1 mm from the tip-cap junction. Numerous nuclei (small arrows) are scattered in a cytoplasm filled, wall-less cavity. The epidermal mucilage (Mu) is intact over the surface of the root which is only slightly swollen in the region of the cavity (large arrow). Toluidine blue O pH 11. X 330.

Fig. 28. Region A epidermal cell (Ep) and root cap cells (RC). A nucleus is migrating into the epidermal cell across a thin region (primary pit field) of the thickened tip-cap boundary (B). X 6,250.

Fig. 29. Intact nucleus (N) in a cell near a cytoplasmic cavity. The nuclear envelope is continuous with dilated ER (arrows) which is also continuous with the extracytoplasmic space. Residual plasmodesmatal connections across the thin cell wall are evident. X 9,000.

Fig. 30. Files of cells involved in cavity formation. Nuclear fragments (both nuclear and heterochromatin material) are coalesced into large disorganized masses (asterisk). X 2,670.

Figs. 31 - 37 show greenhouse-grown roots. Figs. 31 - 35 are median longitudinal sections, Figs. 36 and 37 are transverse sections.

Fig. 31. Region A epidermal cell of a root grown in vermiculite. Numerous microtubules (arrows), dictyosomes (D) with large vesicles (V) and rough endoplasmic reticulum (ER) are characteristic as was also observed for field-grown roots. X 41,250.

Fig. 32. Region C epidermal surface of a root grown in Promix BX. The epidermal mucilage is loosely fibrillar (cp. Fig. 14). Bacteria (B) are associated with this surface (shown in lower magnification in Fig. 35). X 35,000.

Fig. 33. Bacteria associated with the root surface over region B - C epidermal cell. A small amount of residual root cap mucilage (arrow) is present. Greenhouse-grown in field soil. X 21,710.

Fig. 34. Surface view of region D epidermal cells. A large bacterial colony (arrow) is present in the groove between epidermal cells (Ep). Phenol-aniline blue-acetic acid stain for microorganisms. Root grown in vermiculite. X 750.

Fig. 35. MLS of region C epidermal cells (Ep). Numerous bacteria (arrows) are associated with the surface. Toluidine blue O pH 11. Root grown in Promix BX. X 680.

Fig. 36. Epidermal cell at approximately 1 - 1.5 cm from the root tip of a root grown in vermiculite. Only the outer periclinal cell wall (CW) is intact over the epidermal cells. The cytoplasm of the cell is intact and contains both dictyosomes (D) and endoplasmic reticulum (ER). X 22,350.

Fig. 37. Similar region of a root grown in vermiculite as shown in Fig. 36. In this case the epidermal mucilage is present as a loosely-fibrillar material (Mu) in which bacteria (B) are embedded. X 12,000.

2

gives a metachromatic reaction with toluidine blue O at pH 4.4 (Fig. 45), is blue with alcian blue (Fig. 44) and light blue when stained with Nile blue. Tannic acid-uranyl acetate (for complex carbohydrates) stains the root cap mucilage light brown. Calcofluor-binding generates moderate fluorescence of the mucilage (Fig. 42) which is also strongly yellow-green autofluorescent (Fig. 26). In each case the peripheral root cap cells and accumulated root cap mucilage give the strongest reaction, the intensity of the staining reaction or fluorescence diminishes centrifugally. Only localized areas, corresponding to primary pit fields of the peripheral cells, are aniline blue positive (Fig. 41).

Epidermal mucilage over all regions (A, B, C, and D) is strongly PAS positive (Figs. 43 and 45), stained brown with tannic acid-uranyl acetate, intensely fluorescent with Calcofluor White M2R (Fig. 42) and birefringent. With both alcian blue (Fig. 44) and Nile blue epidermal mucilage is either faintly positive or unstained, although the outer periclinal wall of the epidermis and an outer layer of the epidermal mucilage (Fig. 44) stain blue. The metachromatic reaction of tissue treated with toluidine blue O is completely lacking with the epidermal mucilage which stains light blue-green, and is only vaguely apparent in younger epidermal cell walls. Generally the outer periclinal wall of the epidermis and an outer boundary layer of

Figs. 38 - 51 are median longitudinal sections of GMA-embedded field-grown nodal roots of corn. The inset, Fig. 47, is a longitudinal section of a field grown root embedded in Spurr's resin.

Fig. 38. The thickened tip-cap junction (arrows) separates the root pole from the root cap initials (RC). This boundary is strongly stained by the PAS-TBO procedure and stands out from the other walls in this respect as is demonstrated by the ease with which this layer is identified. The "V" shaped group of cells intruding into the root cap is an unusual configuration for the tier of cells covering the root pole. PAS-TBO. X 400.

Figs.
39 & 40. The tip-cap junction is stained blue with Nile blue (Fig. 39) and with alcian blue (Fig. 40) clearly distinguishing it from the walls of neighbouring cells. Fig. 39 X 312.
Fig. 40 X 220.

Figs.
41 - 43. Region A epidermis (Ep) and flanking root cap cells (RC).

Fig. 41. Regions in the peripheral root cap cell walls corresponding to primary pit fields. Aniline blue, fluorescence optics. X 390.

Fig. 42. Intensely fluorescent epidermal mucilage (large arrow). Root cap mucilage and the walls of the root cap cells are fluorescent but not as strongly. Calcofluor White M2R, fluorescence optics. X 260.

Fig. 43. Red epidermal mucilage (arrow) and lighter red to pink root cap mucilage (asterisk). Section stained by the PAS procedure. X 300.

Fig. 44. Region C epidermis stained with alcian blue. The outer periclinal epidermal cells wall (CW) is weakly stained and the epidermal mucilage (Mu) is unstained. A distinct boundary of the epidermal mucilage (large arrow) is strongly stained and is covered by root cap mucilage (stained blue, asterisk) and a small amount of soil (small arrows) which is also stained. X 480.

39

40

42

43

44

41

the epidermal mucilage are blue-green with this dye (Fig. 46 and 47). It is interesting that the outer boundary layer and epidermal cell walls in tissue embedded in Spurr's resin rather than glycol methacrylate are strongly stained (blue) with toluidine blue (pH 11) compared to the inner layer of epidermal mucilage (inset Fig. 47). The epidermal mucilage is strongly fluorescent (deep jade-green) and this fluorescence is distinct from the yellow-green fluorescence of the epidermal walls and root cap mucilage (Fig. 26).

These results are summarized in Table 1.

Basipetally along the surface of the root the staining reactions of the epidermal mucilage remain essentially comparable. In some cases (PAS - inset Fig. 45; Calcofluor - Fig. 51; birefringence - Fig. 49) the intensity of the reaction of the inner epidermal mucilage, but not of the outer periclinal walls of the epidermal cells and the outer "boundary" layer of the epidermal mucilage, is decreased. The reaction with toluidine blue (inset Fig. 46), Nile blue (Fig. 50) and alcian blue, and autofluorescence remain unchanged.

Any soil present is generally metachromatic with toluidine blue (Fig. 46), unstained but embedded in a faint pink matrix with PAS (Fig. 45), has strong affinity for Nile blue (Fig. 47) and is variably stained with alcian blue (Fig. 44). The soil is also

- Fig. 45. Proximal region B (B) and distal region C (C) epidermis stained with PAS. The epidermal mucilage is strongly stained, the root cap mucilage (asterisk) weakly stained and the soil (S) unstained. X 800.
Inset: Epidermis (Ep) in the center of the clear zone of the root. The intensity of the reaction of the epidermal mucilage is reduced although an outer boundary layer and the outer periclinal epidermal cell wall (arrows) are intensely stained. PAS. X 440.
- Fig. 46. Proximal region B (B) and distal region C (C) epidermis stained with TBO. The outer periclinal epidermal cell wall and outer epidermal mucilage layer are stained blue-green, the rest of the epidermal mucilage is stained pale blue-green. Root cap mucilage (asterisk) is stained mauve and the soil (S) is intensely metachromatic. X 870.
Inset: Epidermis in the centre of the clear zone, stained with TBO. X 730.
- Fig. 47. Region C epidermis. The epidermal cell wall and mucilage boundary layer (arrows) increase in the blue-green staining reaction to TBO (cp. Fig. 46). X 840.
Inset: The epidermal walls and outer mucilage boundary (arrows) are striking in this region C epidermis root tissue post-osmicated, embedded in Spurr's resin and stained with TBO pH 11. X 1,120.

Figs. 48 - 51 show field-grown root tissues sampled in the mid-clear zone portion of the root. All are MLS of GMA embedded tissues.

- Fig. 48. The entire epidermal mucilage layer (arrow) is phase light. Phase optics. X 760.
- Fig. 49. Only the inner region of the epidermal surface, corresponding to the cell wall is strongly birefringent (arrow) as are sand grains above the surface. Crossed polarizers. X 630.
- Fig. 50. With Nile blue, which does not stain the epidermal mucilage, the outer periclinal epidermal cell wall and the mucilage boundary layer (arrows) are strongly stained. X 575.

Fig. 51. The epidermal surface is completely Calcofluor positive at this level of the root but the intensity of the fluorescence is markedly reduced (cp. Fig. 42). Fluorescence optics. X 390.

strongly birefringent (Fig. 49) but only weakly autofluorescent (yellow brown) and only occasionally fluorescent when treated with Calcofluor White M2R.

These results are summarized in Table 2.

II.4 Discussion.

II.4.1 Dictyosomes and Secretory Activity.

The presence of a gradient of dictyosome "activity" from root cap initials in which small vesicles are associated with dictyosomes to the peripheral cells of the cap in which dictyosome activity culminates in the hypertrophy of dictyosome cisternae with formation of large kidney shaped vesicles has been documented in a series of publications (Whaley et al. 1959a, 1959b, 1960; Mollenhauer et al. 1961; Leech et al. 1963; Whaley et al. 1964; Clowes and Juniper 1964; Fineran 1973). The possibility of dictyosome involvement in polysaccharide mucilage secretion was further elucidated by histoautoradiographic techniques by which a transfer of radioactivity from labelled sugars to the root cap mucilage via the Golgi apparatus was demonstrated (Northcote and Pickett-Heaps 1966; Pickett-Heaps 1967, Dauwalder and Whaley 1974). Transfer of radioactive L-fucose 1-³H, which is not metabolized by root cap tissue, into root cap mucilage via dictyosome derived vesicles (Rougier 1975, 1976; Paull and Jones 1975) gave further evidence of dictyosome involvement in secretion

Table 2. Properties of the cell walls, epidermal mucilage and extraneous materials associated with the surface of field grown roots (at 1 cm and 2 cm from the tip).

portion ^a of root	property	cortex CM	hypodermis EpCM	epidermis EpCM	sloughed cells EpCM	sloughed cells EpCM	sloughed cells EpCM	sloughed cells EpCM	sloughed cells EpCM
1 cm	autofluorescence	++	++	+++	++	++	++	++	++
2 cm		++	++	+++	++	++	++	++	++
1 cm	birefringence	+++	+++	+++	+++	+++	+++	+++	+++
2 cm		+++	+++	+++	+++	+++	+++	+++	+++
1 cm	Calcofluor fluorescence	+++	+++	+++	+++	+++	+++	+++	+++
2 cm		+++	+++	+++	+++	+++	+++	+++	+++
1 cm	aniline blue fluorescence	pitfields	pitfields	pitfields	pitfields	pitfields	pitfields	pitfields	pitfields
2 cm		pitfields	pitfields	pitfields	pitfields	pitfields	pitfields	pitfields	pitfields
1 cm	periodic acid Schiff's reaction	+	+	+++	+++	+++	+++	+++	+++
2 cm		+	+	+++	+++	+++	+++	+++	+++
1 cm	toluidine blue staining	M to Bl	M to Bl	Bl	Bl	Bl	Bl	Bl	Bl
2 cm		M to Bl	M to Bl	Bl	Bl	Bl	Bl	Bl	Bl
1 cm	alcian blue staining	++	++	++	++	++	++	++	++
2 cm		++	++	++	++	++	++	++	++
1 cm	tannic acid uranyl acetate	++	++	++	++	++	++	++	++
2 cm		++	++	++	++	++	++	++	++
1 cm	mile blue staining	Bl	Bl	Bl	Bl	Bl	Bl	Bl	Bl
2 cm		Bl	Bl	Bl	Bl	Bl	Bl	Bl	Bl

Note: a- Sections of glycol methacrylate embedded material.

Key: +++ very strong, ++ strong, + average, + weak, - no reaction.

CM-cell wall, Ep-epidermis or epidermal, Mu-mucilage, RH-root hairs, a-sloughed, C-cell, SM-mucilage associated with the sloughed cells, O-outer, I-inner.

Bl-not present, Bl-blue, Gr-green, M-mauve, Y-yellow, ll-light, dk-dark.

of corn root cap mucilage to which the presence of fucose was thought to be unique (Wright and Northcote 1974). Subsequently the association of label with dictyosome membrane material was documented and transfer of radioactivity from dictyosome membranes to the plasmamembrane, a necessary event for extrusion of product compartmentalized in vesicles, has been demonstrated (Baydoun and Northcote 1980).

Examples of dictyosome hypertrophy are not restricted to the primary root; similar observations have been made for lateral root primordia (Karas and McCully 1973) and root primordia forming at the coleoptilar node (Martin and Harris 1976). Dictyosome hypertrophy is also one of the most striking changes of dividing quiescent center cells which form the new cap of a decapped root (Barlow and Sargent 1978). (It is interesting that in the latter case starch grain digestion was not observed concurrently with cisternal hypertrophy, therefore the swelling of cisternae does not necessarily reflect a sudden massive substrate availability from degraded amyloplasts as has been suggested [Juniper and Roberts 1964; Juniper and Pask 1973]). The observations on dictyosome activity in the root cap made in the present study are similar to observations made in previous studies and it is therefore likely that the dictyosomes in peripheral cells of the field grown nodal roots are active in the

synthesis or transfer of the root cap mucilage polysaccharide (but consider the excellent arguments against this conclusion presented by O'Brien [1972]).

In several of the field-grown roots studied, secretory product was evident near the cell walls prior to production of larger vesicles by the Golgi apparatus. While this observation does not prove that dictyosomes are not actively secreting at this stage, the close association of strands of endoplasmic reticulum with the secreted material suggests that the initial product is not transported to its extracytoplasmic site via dictyosome derived vesicles. Fucosyl transferases have been found associated not only with dictyosomes (Green and Northcote 1976) but also with ER membrane (James and Jones 1979b; Green and Northcote 1976) particularly in cold treated roots (James and Jones 1980), and polyprenol phosphate fucose or glucose derivatives, believed to be intermediates of slime synthesis, are located in ER (Green and Northcote 1979a). Although radioactivity has never been shown to be associated with ER in published histoautoradiographic studies (Paull and Jones 1975b), ER has been shown to export a polymer of low molecular weight (4,000) which does not contain fucose.

Endoplasmic reticulum and dictyosomes both appear to increase in number in actively synthesizing cells (ie. Fig. 7) and it is possible that both membrane

systems are involved in mucilage production by field-grown roots. Transferase activity (James and Jones 1979b; Green and Northcote 1979b) and slime intermediates (Green and Northcote 1978, 1979a, 1979b) are present in both membrane systems. Golgi-endoplasmic reticulum interconnections have been documented for corn roots (Mollenhauer and Morré 1976) particularly when roots are grown in the cold (Mollenhauer et al. 1975) implicating a transfer of slime intermediates from the ER to dictyosomes. Although a recent serial section study of Zea root cap dictyosomes has not been able to confirm the presence of dictyosome-ER continuities (Robinson 1980) and consequently questions the function of the entire endomembrane system in polysaccharide synthesis, the synthesis of some of the mucilage components by the ER, independent of the dictyosomes, is likely.

The close association of strands of ER and microtubules and of the plasmamembrane lining the extracytoplasmic space containing secretory product and microtubules is interesting and again may be interpreted as indicative of independent synthesis of mucilage material by the ER. However, the role of microtubules in the secretion of root cap mucilage is not clear. Numerous microtubules are present adjacent to most walls (Juniper et al. 1977) or at least adjacent to walls where mucilage is being accumulated

(Fig. 7) when secretion is occurring; however, disruption of microtubules with colchicine makes no difference to either the pattern of mucilage accumulation (Juniper et al. 1977) or to the accumulation and release of root cap mucilage for at least two cycles (Morre et al. 1967).

The change in dictyosome morphology apparent in proximal region A cells of the epidermis has also been observed in axenically grown roots by numerous authors (Mollenhauer et al. 1961; Leech et al. 1963; Clowes and Juniper 1964; Mollenhauer 1974; Mollenhauer and Mollénhauer 1978) and has been interpreted as indicative of secretory activity in the formation of the epidermal mucilage. Vesicles associated with these dictyosomes have been shown to incorporate label from galactose (Roberts and Butt 1969; Dauwalder and Whaley 1974) and from tritiated fucose (Kirby and Roberts 1971; Rougier 1975, 1976). While most of the label accumulates at the cell surface (Dauwalder and Whaley 1974) a small portion moves away into the associated mucilage. When epidermal cells are in division the outer layer is not labelled. (Dauwalder and Whaley 1974; Forsyth 1982) and the number of secretory vesicles associated with the dictyosomes is depressed during the cell cycle (Mollenhauer and Mollenhauer 1978). On the basis of these characteristics, it is conceivable that the epidermal cells are acting as

secretory glands as suggested long ago by Price (1911).

Diminished size of vesicles associated with the epidermal dictyosomes in field-grown roots (eg. cp. Figs. 14 and 15) may be associated with periods of cell division. During this period label from ^3H -fucose and other labelled sugars has been shown to become incorporated into the developing cell plate and wall rather than into the thickened outer periclinal wall (Dauwalder and Whaley 1974; Forsyth 1982). Cyclic secretory activity of both the root cap cells and the epidermal cells, which tends to be synchronous, may also be rationalized on the basis of informational continuity between cells. All necessary components are present - ER lines the walls of all cells and has frequently been observed connected to plasmodesmata which are present between all cells of the root cap until they are sloughed (Fig. 9). Plasmodesmata are also observed across the thickened tip-cap junction (Leech et al. 1963; Clowes and Juniper 1964; see also Fig. 12) such that the informational continuity required to maintain synchrony (Mollenhauer and Morré 1967) is possible between the epidermis and the root cap. However, root cap cyclic secretion is more likely reflective of the periodicity of cytokinesis of root cap initials (approximately 1 every 3 h [Clowes 1971; Paull and Jones, 1976]) and subsequent lateral displacement of these cells as a result of the activity

of the root cap initials (Paull and Jones 1976). Similar periodicity of epidermal cell cytokinesis (ie. 1 every 3 h, in phase with the root cap initials) would account for the synchrony of synthesis of the materials secreted to the outer peripheral walls of both cell types.

II.4.2 Epidermal Mucilage.

Incorporation into the thickened outer epidermal wall of labelled monosaccharides such as galactose (Roberts and Butt 1969), glucose and fucose (Forsyth 1982) suggests that the epidermal mucilage is composed of carbohydrates and for Zea roots, this has been confirmed by GLC analysis (see Part I of thesis). The intense PAS reaction (Table 1) demonstrates the presence of at least some carbohydrates possessing vicinal glycol groups (Pearse 1968; but see below) and Calcofluor positivity suggests a β -1,4-linkage (Hughes and McCully 1975); complex carbohydrates are also indicated by the positive tannic acid-metal salts reaction (Sannes et al. 1978). The negative reaction with alcian blue and the blue-green reaction with toluidine blue (Table 1) indicate that acidic, carboxylated sugars are either not present or are present in low concentrations. The capacity of pectin methylesterase to increase the metachromasy of this layer indicates that methylated pectins comprise a large portion of the epidermal mucilage (Miki et al.

1980). Chemical demethylation alone does not increase metachromasy of the epidermal mucilage, probably due to the extraction of uronic acids by this treatment (Miki et al. 1980). These results indicate that the acid radicals are present in the epidermal mucilage but that these sites are not available for reaction with either toluidine blue or alcian blue.

Diphenolic acid-mucopolysaccharide complexes generate a green colour reaction with toluidine blue as do phenols alone (Ramalingham and Ravindranath 1970) suggesting either that the metachromatic reaction is masked or that the carboxyl sites are not available and the metachromatic reaction never occurs. Some phenols also generate a positive PAS reaction (Geier 1980). It is therefore likely that the epidermal mucilage is a polysaccharide with cross-linked phenolic acids (Fry 1979). This conclusion is consistent with the autofluorescence of the epidermal surface layer (Table 1) and with the demonstration of phenols (and peroxidase enzymes) in the epidermal surface material of wheat (Smith and O'Brien 1979), onion (Peterson et al. 1978) and the roots of several other grasses (based on histochemical evidence [Miki et al. 1980]). (An excellent discussion concerning the presence of phenols in this layer is given in Miki et al. [1980]).

The relationship between mucigel, first described by Schwarz in 1883 (quoted in Russell 1977) and

subsequently frequently observed as a sheath surrounding the tips of young roots (Jenny and Grossenbacher 1963; Greaves and Darbyshire 1972; Old and Nicolson 1975; Guckert et al. 1975; Lopez-Fando et al. 1976; Bowen and Rovira 1976; Foster and Rovira 1976) is not clear. In several of the published micrographs (corn: Figs. 7 and 8, Lopez-Fando et al. 1976; barley: Fig. 1, Jenny and Grossenbacher 1963; Plates 1 and 5, Dayan et al. 1977) the positional relationship of the mucigel to the epidermal surface is identical to that of the epidermal mucilage over some of the older epidermal cells of field-grown roots of the present study. Except in the work of Jenny and Grossenbacher (1963), many of the published studies show either epidermal cells with peripheral cytoplasm (eg. Plate 1, Dayan et al. 1977) or completely degenerate epidermal cells devoid of cytoplasm (eg. Plate 3, 1 cm from the tip, Foster and Rovira 1976; Plate 1 Fig. 5, Old and Nicolson 1975; Fig. 1[c], Foster and Rovira 1973). In each case any fibrillar material on the epidermal surface is considered to be mucigel. Epidermal cells in the present study were invariably noted to possess distinct cytoplasm (but see anomalies below) even as high as 1 cm above the tip.

Microbes have been noted to be present in the mucigel layer of corn (Guckert et al. 1975) and barley (Dayan et al. 1977) and the mucigel present over the

tips of young roots has been found to increase in thickness when roots are grown in the presence of microbes (Greaves and Darbyshire 1972). In this respect, mucigel is not comparable to the epidermal mucilage of field-grown nodal roots of corn. Epidermal mucilage, for example, is actually somewhat thinner in field roots (an average of 6 μm compared to 10 μm thick for axenic primary roots [Clarke et al. 1977]). The more or less consistent thickness of epidermal mucilage on roots sampled at 2 week intervals during the summer reflects the stability of this material. In addition, apical regions of nodal roots are generally free of associated microorganisms although small, isolated colonies may be present in the root cap mucilage. Similar observations have been made for other roots (Marchant 1970; Rovira and Campbell 1975; Foster and Rovira 1976). Only corn roots grown in pots in the greenhouse show presence of bacteria associated with the epidermal surface within 1 cm of the tip (Fig. 35) as has previously been noted for corn roots (Guckert et al. 1975) under more restricted growing conditions.

Mucigel is often described as an uneven layer of granular and fibrillar material covering the root surface (Greaves and Darbyshire 1972; Dayan et al. 1977) or as a fibrillar structure containing granules of polysaccharide. The epidermal mucilage of field-grown corn roots lacks the loose, granular

material noted in other studies (eg. cp. Fig. 15 with Plate 1[b], Dayan et al. 1977). Under the fixation and staining procedures used in the present study, the epidermal mucilage of corn seems to consist of a compact layer of fibrils (eg. Fig. 14) [The presence of fibrils may account for the intense Calcofluor positivity of this region (Herth and Schnepf 1980).] overlain by looser fibrils which may not be of epidermal origin. The looser fibrils resemble the "Rhizoplane fibrils" extending from the walls of the cells of the root tips of axenic wheat roots (Leppard 1974; Leppard and Ramamoorthy 1975). [Micrographs in these papers show cells identified by the text as root surface cells; these cells have walls which are uniformly fibrillar and electron dense throughout and thus characteristic of root cap cell walls rather than epidermal cell walls which, together with the epidermal mucilage forms a distinctly layered surface in wheat roots (unpublished observations). The cells illustrated in Leppard (1974) and Leppard and Ramamoorthy (1975) may, therefore, be of root cap origin and the microfibrils splayed out from them, root cap mucilage.] It is thus strictly the positional relationship between mucigel and the epidermal cells that implies that mucigel is comparable to epidermal mucilage.

Recently Foster (1981) described, in some detail, the epidermal surface of wheat roots and interpreted

his micrographs and evidence previously published by Leppard (Leppard 1974; Leppard and Ramamoorthy 1975) to develop the hypothesis that much of the rhizosphere mucilage is derived from extracellular material on the soil-facing surface of epidermal cells. Foster (1981) describes this surface material as composed initially of two easily distinguished main layers, a largely fibrillar inner layer and a more amorphous outer layer overlain by a thin cuticle. These two layers clearly correspond respectively to the outer epidermal wall and epidermal mucilage observed in the present study (eg. cp. Fig. 2, Plate 1, Foster 1981 with Figs. 19, 20, 22, and 23). Foster refers to the outer layer as a primary wall and the inner underlying layer as a secondary wall, a designation which is unclear since both layers are present in very young, unexpanded root epidermal cells (eg. see Price 1911; Clarke et al. 1979; Smith and O'Brien 1979; Miki et al. 1980). Foster suggests that the outer layer becomes mechanically disrupted by the surrounding soil thereby becoming the loosely fibrillar material characteristic of the rhizosphere rather than the clearly defined layer characteristically found near the tip.

The presence of distinct layers in the epidermal surface has been previously described by Leech et al. (1963). Neither a distinct boundary (eg. see Dart and Mercer 1964) nor a cuticle (eg. see Scott 1969; Dayan

et al. 1977; Mollenhauer and Mollenhauer 1978; Foster 1981) is present in corn root tissue examined by transmission electron microscopy. However, a very distinct boundary (the outer layer of the epidermal mucilage, the outer part of the three-layered epidermal surface, eg. Clarke et al. 1979) is clear in the same material thick sectioned for light microscopy and stained with toluidine blue (pH 11) and in glycol methacrylate embedded tissue stained with toluidine blue (pH 4.4), Nile blue and alcian blue. These consistent staining reactions may reflect a chemical difference not demonstrable as a difference in electron density at the ultrastructural level. In wheat roots, a similar layer, called a pellicle, and the outer periclinal epidermal wall (which shares similar staining reactions with the outer mucilage layer in corn roots) are resistant to enzymes secreted by the wheat pathogen Gaeumannomyces which extracts the remainder of the epidermal mucilage (Smith and O'Brien 1979) perhaps reflecting a difference in "degree of phenolic substitution and/or in the type of linkage and phenolic entities between the inner and outer layers of the epidermal wall". (Smith and O'Brien 1979.)

II.4.3 Anomalies:

Dense cytoplasm of the epidermal cells (characterized by the presence of numerous free ribosomes, electron dense inclusions along the wall and

indistinct dictyosomes as observed in this study) have been reported for roots subjected to dehydration experiments (Ciamporova 1976, 1977; Nir et al. 1970a, 1970b). Roots sampled during the present study which show these symptoms were likely collected during a dryer period or were inadvertently dried after collection but prior to fixation.

Perhaps a more interesting anomaly is the presence of nuclear aberrations and apparent migration and the formation of a cytoplasmic soup in early season seminal and primary roots and in a few late season nodals. Lateral roots of corn (usually near most apical regions) have previously been found to flow together when transverse and some longitudinal walls breakdown and the cytoplasm becomes continuous (Leech et al. 1963). Histolytic polyploidy, accompanied by the formation of a large cytoplasmic cavity had previously been reported in some roots of Hopi Indian corn (Sass 1957).

It is not likely that the lytic cavity described in this report is formed in the manner suggested by Sass (1957) for Hopi Indian corn in which polyploid metaphase figures were observed in the enlarging cavity. The large nuclear configurations observed in the earlier study are likely comparable to the masses of coalesced nuclear fragments observed in the present study. The lysis of cell walls in the enlarging cavity

discussed by Sass (1957) was also observed here. The role of inhibited wall formation as an initial stage (Sass 1957) of multinucleate cavity formation, could not be ascertained in the ~~present~~ study.

The sequence of events giving rise to the large cytoplasm filled areas is not clear and it is not possible to determine whether aberrations in karyokinesis or cytokinesis or both, are involved. The presence of numerous spherical nuclei comparable to normal nuclei suggests that failure of cytokinesis following successive nuclear divisions, gives rise to the large cytoplasmic wall-free areas. Treatment with caffeine, which inhibits normal cell plate formation but not nuclear division is known to give rise to binucleate cells with incomplete wall fragments extending into the ~~cell~~ (Jones and Payne 1977; Hardham and McCully, 1982). Binucleate cells with wall fragments have also been reported to occur naturally in the epidermis of bulb scales of some onion varieties (Haissig and Stadelman 1969). These cells were thought to arise as a result of aberrant cell division. In abnormal corn roots the wall stubs are not strongly aniline blue positive (as is characteristic of developing cell plates) and the plasmalemma lines only the lengths of most wall stubs. Both characteristics indicate that wall formation is complete but that the cell walls break and the cytoplasm from the cells mixes

with that of the enlarging cavity.

It is interesting that regions adjacent to those areas where nuclear migration occurs, are characterized by broken wall fragments and pycnotic nuclei. These features suggest that the aberrations developed within intact cells following successful karyokinesis and cytokinesis and is perhaps indicative of programmed dissolution of a transient root system, replaced in mature plants by elaboration of the nodal roots. The possibility of a resorptive process in aging roots has been described for quiescent root meristems of Allium cepa (Bal and Payne 1972). In onion roots this process was characterized by the formation of masses of cytoplasm with free floating nuclei and proliferation of rough endoplasmic reticulum elements.

Histochemical, histological and structural study of field-grown corn roots. Part III: Characteristics of the sheathed zone and mature, sheath-free regions of field-grown nodal roots of corn.

III.1 Introduction.

Based on observations of the apical regions of young roots (less than 20 cm long) both of axenic grasses (Miki et al. 1980) and of field-grown corn (see Part II of thesis) it is clear that much of the fibrillar, mucilagenous material frequently described as sheathing the apical portions of roots (see for example Jenny and Grossenbacher 1963; Greaves and Darbyshire 1972) is actually epidermal mucilage and not of root cap origin. Histochemical reactions clearly indicate that the mucilage which is closely associated with the epidermal cells is distinct from root cap mucilage (Price 1911; Clarke et al. 1979; Miki et al. 1980) and ultrastructural evidence (Part II of thesis) in conjunction with histoautoradiography of the epidermal cells of Zea roots (eg. Kirby and Roberts 1971; Rougier 1975, 1976; Forsyth 1982) strongly implicate, but do not necessarily prove, the involvement of epidermal cells in secretion of the epidermal mucilage. The use of the term mucigel for the mucilages derived from the cells of the apical portions of the root under both axenic and natural conditions is misleading in that the different origins of the

mucilages are not taken into consideration.

The question then, is whether there is any additional mucilagenous material at least in part of root origin which could be called mucigel in the strictest sense (ie. associated with the root in nonsterile soil). Two observations suggest that such a mucilage is present, at least on field-grown corn roots. First the report by Floyd and Ohlrogge (1970, 1971) of material on young nodal root surfaces of field-grown corn which "formed" following soaking in distilled water and which was similar to, but not the same as, root cap mucilage of the same roots hints at the existence of a third mucilage (in addition to the root cap and epidermal mucilages). Second, the observation of a soil sheath surrounding basal portions of young nodal roots (thesis, Part II) indicates the presence of some type of adhesive material of undetermined nature, intimately associated with the root surface.

To attempt to answer the question of whether a third mucilage is formed along the root and to determine both the fate of epidermal mucilage and the mechanism of soil sheath formation, the histochemistry and histology of portions of both sheathed roots and mature roots (several feet long) lacking the soil sheath over their basal regions, were examined.

III.2 Materials and Methods.

Field grown roots less than 20 cm long, collected and prepared as described in General Materials and Methods (p. 10 - 12 of thesis) were sampled at the transition to soil sheath, mid-sheath and basal regions using a 1 mm long segment from each zone, quartered as necessary for broad roots. Most of these roots originated below the soil surface although a few roots which were initiated above the soil surface but which had subsequently grown into the soil were sampled. In addition, basal portions of old roots (at least 1 - 2 feet long) were also examined. These roots lacked a persistent soil sheath on at least their basal portions; the remaining apical portion of the root was severed during collection and the presence or absence of a soil sheath over that portion of the root was not determined. For each zone, at least 4 blocks were prepared for light microscopy and histochemistry and a minimum of 8 blocks were prepared for electron microscopy. The exception is the mature, sheath-free zone for which only 2 blocks for histochemistry and 2 blocks for electron microscopy were sectioned.

The section shown in Fig. 38 was prepared as described in Appendix B (p. 190).

III.3 Results.

In describing the regions sampled, the soil sheath will be referred to as the sheath and the transition

region from a sheath-free to a sheathed surface will be referred to as transition or transition to sheath. All histochemical results are restricted to field grown roots.

III.3.1 Macro Features of the Sheath.

The surface of the basal portions of all young nodal roots (less than 20 cm long and lacking emerged lateral roots) sampled is covered by a totally encrusted soil sheath (Fig. 1) continuous to the base of the root. In early season roots the transition to the soil sheath is abrupt (Fig. 1); late season roots have a more diffuse, elongated transition zone. Portions of mature roots (possessing emerged lateral roots) may actually lack a persistent sheath. In transverse section the sheath appears to effectively double the root diameter (Fig. 2) and following repeated washings (as would be encountered during fixation procedures) the sheath persists (Fig. 3) as a thinner band through which the many root hairs present on this portion of the root, extend (Figs. 3 and 4). Much of the soil sheath is poorly infiltrated with the embedding medium used (both GMA and Spurr's resin) and sections of prepared tissues are frequently damaged and unstable (eg. Fig. 4) even when cut on a diamond knife. Due to these difficulties many of the micrographs were made of areas where the soil sheath was either restricted in formation or where the soil

sheath had been removed by continued washing during fixation. The general relationships between the soil sheath and the epidermis can still be determined from this material and results are comparable to those obtained when heavily encrusted samples were considered.

III.3.2 Light Microscopy and Histochemistry of Sheath Regions of Nodal Roots.

The soil sheath is composed of a combination of sand grains, clays and silts and possibly organic matter (Fig. 4) held together by a matrix material which stains light pink with the periodic acid Schiff's reaction for polysaccharides (Fig. 5). Root hairs extend into the sheath forming a framework for the soil particles (Fig. 5). On casual observation, it appears that the root hairs give rise to the matrix material. The epidermal cell wall is overlain by a very thin layer which is strongly PAS positive and clearly distinct from the sheath matrix. The number of microbes present (Fig. 5) are also likely too low to generate such large amounts of mucilage.

Viewed with phase optics the sheath material is not distinguishable except in areas where it is displaced from soil particles and the epidermal mucilage, where its outline is barely discernable (Figs. 6 and 8). Sloughed cells within the soil sheath are distinguishable from root hairs by their different

phase characteristics (Figs. 6 and 9). The walls of these cells are only weakly birefringent compared to the walls of the root hairs and the soil (particularly sand) particles of the sheath (Fig. 7). The epidermal surface material, which is strongly phase light (seen as a white layer), is either not birefringent or only weakly so; all the epidermal walls, including those of the root hairs, are strongly birefringent (Fig. 7). The sheath matrix material is not birefringent. In areas where the soil sheath is somewhat restricted in extent (ie. not completely developed or lost during sample preparation) the matrix material in which the soil would be embedded exhibits stronger phase characteristics (Figs. 8 and 9) being particularly phase light and clumped where no soil is present but where sloughed cells occur above the intact epidermis (Fig. 9).

The walls of the sloughed cells are yellow-green autofluorescent although not as strongly as the walls of the epidermal cells. Neither the epidermal mucilage nor the sheath matrix material are autofluorescent (Fig. 10). Sloughed cells frequently possess small areas in the walls with a strong affinity for the fluorescent component of aniline blue (Fig. 11) a characteristic not associated with walls of either the epidermis or most of the root hair (except the tips) unless severely damaged. (Damaged tissues possess walls

FIGURE LEGENDS:

- Fig. 1. Apical portion of a young (lacking emerged lateral roots) nodal root. The apical clear zone (C) and sheath zone (S) are clearly separated at an abrupt transition region (arrows).
- Fig. 2. Cross section of a nodal root (R) through the soil sheath (S). The sheath (between arrows) approximately doubles the root diameter. This hand section was mounted in immersion oil and photographed through a 63 mm Luminar lens and the micrograph printed from a colour transparency. Photomicrograph by Dr. McCully 1981.
- Fig. 3. Section similar to that shown in Fig. 2 but following extensive washing. Much of the soil sheath (S) has been removed but a sizeable amount still adheres to the surface and approximates the situation following fixation, dehydration and embedment. The lateral root (L) lacks a sheath. Photograph prepared as described for Fig. 2 by Dr. McCully 1981.
- Fig. 4. Cross section of a glutaraldehyde fixed, Spurr's resin embedded root in the region of the soil sheath (S). Numerous root hairs (H) extend out beyond the sheath as in Fig. 3. Sand grains are poorly embedded and are lost from the section leaving holes (Sa). X 160.

Figs. 5 - 9, 12 - 19 are cross sections, Figs. 10 and 11 are median longitudinal sections of glycol methacrylate embedded, field-grown corn roots fixed in glutaraldehyde. Distances from the root tip at which the sections were made varies between 2.5 and 5 cm, Figs. 18 and 19 show sections cut at least 50 cm from the root tip. The epidermis (Ep) is intact, bears living root hairs (H) and is covered with a thin layer of epidermal mucilage (arrow). Living sloughed cells (asterisk) frequently lie in the rhizosphere. Soil (S) has often remained in situ during processing although sand grains (Sa) are usually lost (leaving holes). Bright field microscopy unless otherwise noted.

Fig. 5. Stained by the periodic acid Schiff's (PAS) reaction the epidermal mucilage is dark red and the mucilage surrounding the sloughed cells, root hairs and soil is light pink. The soil is essentially unstained. Some of the stained bodies (arrows) in the soil sheath are likely microorganisms but there are no distinct colonies present. X 460.

Fig. 6. Unstained section viewed with phase contrast optics. Epidermal mucilage is present as a phase light layer which does not extend beyond the base of the root hairs (arrow on left hand root hair). The wall of the single detached cell shows phase characteristics different from those of the epidermal cell walls. The mucilage in which the soil is embedded is phase light (large arrow). Phase light areas (circled) in the anticlinal walls of the hypodermal cells show the location of the Casparian strip. X 450.

- Fig. 7. Same section as that shown in Fig. 6 viewed through crossed polarizers. The soil sheath (S) contains strongly birefringent particles (quartz). Epidermal cell walls and root hairs are strongly birefringent, epidermal mucilage is very weakly birefringent (compare position of arrows on left hand root hair with those on the same hair in Fig. 6). The wall of the detached cell and the mucilage of the sheath are not birefringent. Note the areas in the hypodermal cell walls (circled) which show the location of the Casparian strip. X 450.

which are almost entirely aniline blue positive.) Walls of the sloughed cells are Calcofluor positive; the associated matrix is weakly positive (Fig. 12) to negative. Both epidermal and root hair cell walls bind some Calcofluor but the reaction is not marked. Epidermal mucilage is generally Calcofluor positive (Fig. 13). Sloughed cell walls are stained blue with alcian blue as is the associated material, contrasting strongly with epidermal walls and root hairs which are weakly stained, and the epidermal mucilage which is usually unstained (Fig. 14). The distinction between epidermal cells and sloughed cells is not clear with Nile blue which stains walls of both cell types equally. The matrix material associated with the sloughed cells is, however, stained a darker blue than the epidermal mucilage with the same stain (Fig. 15). The periodic acid Schiff's reaction likewise does not distinguish between root hairs and sloughed cells (Fig. 16). However, the matrix material associated with the sloughed cells (Fig. 16), like the sheath matrix (Fig. 5), is stained faint pink and the epidermal mucilage dark pink-red (Fig. 16). The different cell types and mucilages are clearly evident in cross sections stained with toluidine blue O. The walls of epidermal cells and root hairs are blue to blue-green, walls of the sloughed cells are mauve to mauve-blue, and the epidermal mucilage is light blue-green while

- Fig. 8. Region of a field-grown root from which most of the sheath (S) has been lost during preparation for microscopy. The sheath mucilage (large arrows) is visible with phase contrast optics but is not as phase light as the epidermal mucilage (small arrow). Phase contrast optics. X 600.
- Fig. 9. Section showing minimal sheath development. In this case the mucilage associated with the sloughed cell is phase light and clumpy (arrows). Some of the soil of the sheath may have been lost during fixation and embedment for microscopy. X 600.
- Fig. 10. Epidermal cell walls (small arrow) and the wall of the sloughed cell are yellow-green autofluorescent. Epidermal and sheath mucilages are negative. Fluorescence optics. X 430.
- Fig. 11. Section identical to that shown in Fig. 10. Spots (arrows) in the wall of the sloughed cell are intensely fluorescent when stained with aniline blue. Fluorescence optics. X 430.
- Fig. 12. Section showing a large amount of Calcofluor binding mucilage (Mu) over the root (R) but no sheath. Walls of detached cells also weakly bind Calcofluor. Fluorescence optics. X 520.
- Fig. 13. Root hairs and detached cells share a similar moderate affinity for Calcofluor. Epidermal mucilage (arrow) has a strong affinity for this fluorochrome. Fluorescence optics. X 175.
- Fig. 14. Section stained with alcian blue. Walls of sloughed cells and the surrounding mucilage are stained blue. Epidermal cell walls are weakly stained and the epidermal mucilage is unstained. X 710.
- Fig. 15. Section stained with Nile blue. All cell walls (both of the sloughed cells and the epidermal cells) are stained but the mucilage (Mu) associated with the soil sheath (darkly stained, S) is stained more intensely than the epidermal mucilage (arrow). X 455.

the sheath matrix is metachromatic pink (Fig. 17).

Roots lacking a sheath also lack the matrix material associated with the root surface where a sheath persists. Although root hairs are always present on the sheath free mature roots (Figs. 18 and 19) sloughed cells are only occasionally observed on these sheath free portions of mature roots. The characteristic staining reactions of the epidermal walls and root hairs - blue-green with toluidine blue O and pink with the periodic acid Schiff's reaction also persist. The extent of the epidermal mucilage is extremely variable from essentially lacking to thickened and with a boundary layer along the same area of the root (Fig. 18). Staining reactions of this layer are also variable. Stained with toluidine blue O the epidermal mucilage present over the epidermis of sheath free, mature portions of roots is stained pink (metachromatic) to light blue-green with a blue-green to pink boundary (Table 1), and treated by the periodic acid Schiff's reaction, the epidermal mucilage is stained red (Fig. 19) to light red-pink with a red boundary.

The presence of an "outer boundary" on the epidermal mucilage is a highly variable characteristic in the sheath region and is not consistently observed as it is in the apical portion of the root even with toluidine blue staining.

Table 1. Histochemical reactions of the transition to sheath, mid sheath and mature regions of *Zea mays*.

pos of root	proximity	cuticle	hypodermis	epidermis	SCW	DEPM	DEPM	DEPM	sloughed cells	SCW	cell/matrix
transition	autofluorescence	++	++	++	++	++	++	++	++	++	++
mid-sheath	++	++	++	++	++	++	++	++	++	++	++
sheath free	++	++	++	++	++	++	++	++	++	++	++
transition	birefringence	++	++	++	++	++	++	++	++	++	++
mid-sheath	++	++	++	++	++	++	++	++	++	++	++
sheath free	++	++	++	++	++	++	++	++	++	++	++
transition	Calcofluor fluorescence	++	++	++	++	++	++	++	++	++	++
mid-sheath	++	++	++	++	++	++	++	++	++	++	++
transition	aniline blue fluorescence	++	++	++	++	++	++	++	++	++	++
mid-sheath	++	++	++	++	++	++	++	++	++	++	++
transition	periodic acid	++	++	++	++	++	++	++	++	++	++
mid-sheath	++	++	++	++	++	++	++	++	++	++	++
sheath free	++	++	++	++	++	++	++	++	++	++	++
transition	toluidine blue staining	++	++	++	++	++	++	++	++	++	++
mid-sheath	++	++	++	++	++	++	++	++	++	++	++
sheath free	++	++	++	++	++	++	++	++	++	++	++
transition	alcian blue staining	++	++	++	++	++	++	++	++	++	++
mid-sheath	++	++	++	++	++	++	++	++	++	++	++
sheath free	++	++	++	++	++	++	++	++	++	++	++
transition	nil blue staining	++	++	++	++	++	++	++	++	++	++

Note: a-Mature root lacked a complete, persistent sheath over at least the basal portions of their length in the soil.

b-Section of glycol methacrylate embedded field grown roots.

c-On mature roots lacking a persistent soil sheath small amounts of soil and matrix material were occasionally present but were not included in the result for this Table.

Key: CW-cell wall, O-outer, I-inner, Ep-epidermis, Mu-mucilage, RH-root hairs, SC-sloughed cell, Gr-green, Y-yellow, R-red, Br-brown, Bl-blue, M-mauve, P-pink, Pu-purple, OH-dark, LI-light, ++ strong, + average, - weak, - no reaction, NP-not present, NA-not applicable, NS-not seen.

The above results are summarized in Table 1.

III.3.3 Ultrastructure of the Sheath Region.

The living epidermal cells at the transition to sheath region characteristically have a peripheral cytoplasm surrounding a large central vacuole. Undifferentiated plastids, mitochondria, short strands of rough ER and dictyosomes with vesicles with dark contents are present in these cells (Fig. 20). Microtubules lie perpendicular to the outer periclinal walls of many but not all epidermal cells. The outer periclinal wall of the epidermis (Fig. 20) is sometimes overlain by a fibrillar layer (epidermal mucilage, Fig. 20) which apparently expands and sticks to the soil of the sheath (cp. Figs. 20 and 21). The expanded fibrillar epidermal mucilage frequently possesses a dark outer boundary (Fig. 22) to which the soil binds. The epidermal mucilage is not always fibrillar or extended into the soil sheath of the transition region (Figs. 24 and 25). Frequently, epidermal mucilage is distinct as a fibrillar material (Fig. 23) which may or may not radiate out and follow the contours of the epidermal cells (Fig. 26). The epidermis persists throughout the life of the root although many of the epidermal cells eventually lose their cytoplasm (Fig. 26). The hypodermis, its cell walls distinguished by the presence of a suberin lamella, is present throughout the sheath zone (Figs. 6, 7, 19 and 26) and

Fig. 16. Section treated by the PAS reaction. The rhizosphere mucilage (Mu) and walls of the sloughed cells are moderately positive compared to the darkly stained epidermal mucilage. The PAS negative soil (S) contains traces of stained mucilage. X 380.

Fig. 17. Near adjacent section to that shown in Fig. 8, stained with toluidine blue O (TBO). The epidermal mucilage is faint green in sharp contrast to the walls of the sloughed cells and the associated rhizosphere or sheath mucilage which are bluish-mauve to dark pink. The soil (S) bound to the rhizosphere mucilage is intensely metachromatic. X 670.

Fig. 18. Cross section of a mature root (with emerged lateral roots). The basal portion of the root from which this section was cut lacked a persistent soil sheath. Root hairs are numerous in these regions (one root hair shown) but neither sloughed cells nor rhizosphere mucilage are present. The epidermal mucilage is variable, ranging from present as a thick blue-green (large arrows) or blue-mauve layer, to completely lacking (small arrows). Stained with TBO pH 4.4. X 840.

Fig. 19. Similar section to that shown in Fig. 18 but stained by the PAS procedure. Only root hairs and the epidermal mucilage which is still darkly stained (cp. with Fig. 16) are present at the root surface. Note the low level of staining of the anticlinal walls of the hypodermis (Hy) indicating the presence of a Casparian strip. X 430.

Figs. 20 - 50 are sections of glutaraldehyde fixed, post-osmicated root tissue embedded in Spurr's resin and prepared for electron microscopy. Unless otherwise stated, the micrographs show cross sections. All sections were cut with a diamond knife but chatter and holes could not be avoided in poorly infiltrated sheath regions.

- Fig. 20. Median longitudinal section at the transition to sheath region. The cell wall (CW) and mucilage (EpMu) of the intact epidermal cell are clearly evident. Cellular organelles include mitochondria (M), plastids (Pl), vacuoles (V) and endoplasmic reticulum.

X 22,105.

Inset: Many of the epidermal cells of the transition to sheath region possess large dictyosomes (D) with associated vesicles with dark contents (arrows).

X 13,000.

- Fig. 21. LS from a region basipetal to that shown in Fig. 20. The epidermal mucilage (EpMu) has apparently expanded and bound to the soil (S) (most of which was poorly infiltrated and lost from the section). The root hair (H) is not associated with mucilage.

X 5,500.

- Fig. 22. Section through the soil sheath just above the transition to soil sheath region. The epidermis (Ep) has a distinct outer wall (W) and epidermal mucilage (EpMu) which has become loosely fibrillar and expanded and possesses a dark outer boundary (arrow).

X 6,000.

- Fig. 23. Similar section to that shown in Fig. 22. In this case the distinct epidermal cell wall (W) is covered by an electron lucent layer corresponding to epidermal mucilage (arrow) and a thick layer of loosely fibrillar material (asterisk) which does not appear to be of epidermal origin.

X 12,000.

only infrequently becomes the outermost cell layer near the root base where epidermal cells are completely degraded (Fig. 27) in old roots.

The fibrillar material surrounds the soil particles, which are remarkably stable through the processing for microscopy when completely embedded in this matrix (cp. Fig. 28 with Fig. 21), and in regions where large amounts of fibrillar matrix material are observed, living sloughed cells are also frequently found embedded in it (Fig. 29 and 30). These cells have intact nuclei as well as plastids, mitochondria (Fig. 29), and small dictyosomes in the peripheral cytoplasm. The walls of these sloughed cells are "wavy" and appear to be intimately associated with the fibrillar material (Figs. 29 and 30). This is especially evident in Fig. 31 (this is actually a section of a soil clump associated with the epidermis of the sheath free apical portion of a root) where the fibrillar material lies above the clay particles pushed against the epidermal mucilage and which is therefore not likely of epidermal origin. In addition to the presence of their distinctive wall, the sloughed cells are also distinguished from epidermal cells by the frequently observed presence of dark channels in the walls (eg. Fig. 32). These channels resemble plasmodesmatal channels which have become plugged with electron dense material. In some sections profiles of the sloughed

- Fig. 24. LS at transition to sheath showing an epidermal cell (Ep) with a distinct outer wall (W) and electron dense epidermal mucilage (EpMu) which is only weakly fibrillar (cp. Fig. 20). The vacuole (V) of the epidermal cell is filled with electron dense material. X 14,950.
- Fig. 25. Transition to sheath region. The epidermal wall (W) and mucilage are not clearly distinguishable except in the groove. The epidermal mucilage lacks the loosely fibrillar nature observed in other samples (cp. Fig. 20). Note the oriented clay particles (arrows). X 24,440.
- Fig. 26. Section of a root with a soil sheath. The highly contorted epidermal cells (Ep) which may or may not be living (cp. Ep1 and Ep2) possess both an outer wall (W) and epidermal mucilage (large arrows). Rhizosphere mucilage (Mu) is present as fibrillar material which occasionally radiates out from the epidermal mucilage. The suberin lamella (small arrow) of the hypodermis is present in this preparation. X 13,410.
- Fig. 27. Basal region of a root showing the unusual case of a hypodermal cell (Hy) with an intact suberin lamella (arrows) forming the outer cell layer of the root proper. The old epidermal cell wall (W) forms a mucilage-like layer over the hypodermal cell. X 13,000.
- Fig. 28. Section through the epidermis (Ep) and sheath (S) of a root. The sheath soil is associated with fibrillar rhizosphere mucilage (Mu) which is distinct from the epidermal mucilage (arrows) and the outer periclinal epidermal cell wall (W). X 5,000.
- Fig. 29. Section of a soil sheath in which a detached cell (asterisk) is present above the epidermis (Ep). The thick cell wall (W) of the sloughed cell appears to be continuous with the rhizosphere mucilage (Mu) which binds the soil (S) of the sheath. Mitochondria (M) are present in both the detached and epidermal cells. X 13,640.

Fig. 30. Similar section to that shown in Fig. 29 demonstrating only the detached cell (asterisk) and the epidermis (Ep). The boundary of the epidermal mucilage (arrows) is not easily distinguished from the overlying fibrillar material (Mu) associated with the sloughed cell. X 12,630.

Fig. 31. Similar section to that shown in Figs. 29 and 30. A very thin band of oriented clay particles (small arrows) is positioned between the epidermal mucilage (large arrow) and the rhizosphere mucilage (Mu) associated with the detached cell (asterisk). The fibrillar mucilage could not have originated from the epidermis in this example. Note the plastids (Pl) in the intact epidermal cell (Ep). Microtubules lining the plasmamembrane adjacent to the outer periclinal cell wall are poorly visible at this magnification. X 6,430.

Fig. 32. Section through the outer sheath region of an older root (20 cm long). The cytoplasm of the detached cell (asterisk) is degenerate with only the remains of the nucleus (N) distinguishable. The wall of the cell is associated with only a small amount of fibrillar material (Mu, lower right) and has numerous electron dense channels running through it (arrows). X 5,000. Inset: Higher magnification of a portion of the wall of the same cell demonstrating the dark channels (arrows) and the association with fibrillar mucilage (Mu). X 15,000.

cells outnumber those of root hairs (Fig. 33 and 34) and this is particularly true at the base of young nodal roots (Fig. 33). The majority of sloughed cells in such a case are living (Fig. 34) although most epidermal cells lose their cytoplasm in this region of the root.

Root hairs with intact cytoplasm are also observed. Some root hairs may be associated with fibrillar material (Fig. 35). The latter association is not characteristic and root hairs are usually observed near the fibrillar matrix but not actually associated with it (Fig. 36). Likewise, soil may or may not be present adjacent to a root hair. Root hairs are most easily distinguished by their apparently two-layered wall in which the inner layer is not as electron dense as the outer layer (Figs. 35 and 36). This two-layered characteristic was not clearly evident in glycol methacrylate embedded tissues (eg. Fig. 18) of these below soil roots.

The metachromasy with toluidine blue O, alcian blue positivity, and the positive periodic acid Schiff's reaction of the sheath matrix material associated with the free-living sloughed cells suggests a similarity to root cap mucilage. This similarity is further confirmed by the positive labelling using FITC-lectin specific for fucose (Fig. 38) of the material surrounding the numerous free living sloughed

cells (Fig. 38) a labelling also characteristic of root cap mucilage (Vermeer and McCully 1981). This sheath matrix material is fibrillar and the sloughed cells within it are living (Fig. 39).

[Figures 37 - 39 are of adjacent sections from the same root approximately 4 cm from the root tip in a region where there was a soil clump present on the root but not in the soil sheath.]

The surface of mature roots (lacking a persistent sheath over their basal surfaces) also lacks the large amount of fibrillar material and the sloughed cells which would be present in the sheath region. Epidermal mucilage is intact and occasionally excessively thickened over the epidermal cells (Fig. 40) which are frequently devoid of cytoplasm (Figs. 40 and 41). The epidermal mucilage varies in ultrastructural appearance being either a dense layer not easily distinguished from the cell wall, or a loosely fibrillar region with embedded microorganisms (Figs. 41 and 42).

III.3.4 Greenhouse Grown Roots.

Like the sheath-free (mature) field-grown roots, which either develop only a weak soil sheath or which lack a persistent soil sheath, pot-grown roots have only a surface coating of epidermal mucilage which is usually fibrillar (Fig. 43) and restricted to within a micron of the epidermal surface except in grooves between epidermal cells where the mucilage layer is

- Fig. 33. Optical monitor section of the section shown in Fig. 34. A large number of detached cells (asterisks) are present in the rhizosphere of this mature portion of a root (as indicated by the secondarily thickened walls of the outer cortex (C)). X 275.
- Fig. 34. Portion of the rhizosphere of the root shown in Fig. 33. The sloughed cell (asterisk) possesses an intact peripheral cytoplasm and a thick undulating cell wall (CW) associated with the massive amount of fibrillar rhizosphere mucilage (Mu). X 9,000.
- Fig. 35. Oblique section through part of a living root hair (H) showing the characteristic two layered cell wall (outlined area is enlarged in inset). Fibrillar mucilage (Mu) lies between the hair and the epidermis (Ep) but does not surround the hair. Section shows a portion of the midsheath region of a root.
Main Figure X 6,550.
Inset X 11,700.
- Fig. 36. Portion of a degenerate root hair from the transition to sheath region of a root, with a characteristically two-layered cell wall. Part of the soil sheath (S) is closely pressed against the root hair. The soil particles were part of a large soil sheath intermeshed with fibrillar material, which largely disappeared due to instability under the electron beam. X 10,950.
- Fig. 37. Portion of the apical clear zone of a young root which was partially covered by a soil clump (S). Numerous sloughed cells (asterisks) and rhizosphere mucilage (Mu) were present above the epidermis (Ep) and between the root hairs (H). Outlined area shown in Fig. 38. X 425.

Fig. 38. Portion of the section shown outlined in Fig. 37 (following sodium methoxide treatment to remove Spurr's resin and incubation in FITC-lectin). Walls of the detached cells (arrows) and the associated mucilage (asterisk) were labelled by the lectin. Fluorescence was very weak because the section was both thin ($0.5\ \mu\text{m}$) and damaged by the sodium methoxide treatment. However, control sections incubated in lectin plus fucose showed no fluorescence and therefore the reaction is thought to be indicative of the presence of fucose residues. Fluorescence optics. Contrast copy (photograph of original print made on high contrast copy film).

X 2,040.

Fig. 39. Adjacent section to that shown in Figs. 37 and 38. The living detached cells (asterisks) and associated fibrillar material correspond to the lectin positive material shown in Fig. 38.

X 2,000.

somewhat thicker (Fig. 44). In some cases this epidermal mucilage may be granular (Fig. 45) and expanded (Fig. 46) and may possess a distinct outer boundary (Fig. 47). In only one case a distinct epidermal origin for the fibrillar material was not clear but the loosely fibrillar arrangement resembled that of epidermal mucilage in other preparations (cp. Fig. 48 with Fig. 43). Bacteria were frequently present within or near this material (Figs. 43, 44, 45, 46 and 47). There was no consistent difference between roots grown in vermiculite, Promix BX or field soil although all have surface features distinct from those of field-grown roots. In particular, greenhouse-grown roots develop only a weak soil sheath or no soil sheath although sloughed cells are present adjacent to the root surface of above soil nodal roots which have subsequently grown into the pot, just below soil level (Vermeer and McCully, 1982).

III.3.5 Microorganisms.

Figures 49 and 50 illustrate the range of microorganisms associated with the roots of field and greenhouse plants respectively. In both cases rod and cocci forms which tend to form colonies predominate. Field roots support a greater number of divergent forms (ie. different types of bacteria and more fungi and actinomycetes) than do greenhouse-grown roots. Actinomycetes (Fig. 49[h]) and fungi (Fig. 49[d]) were

- Fig. 40. Section of a mature (with emerged lateral roots) root lacking a persistent sheath. The epidermal mucilage is intact (arrows) over the entire epidermis (Ep) but is highly variable in thickness. X 2,890.
Inset: Thickened region of epidermal mucilage (EpMu) distinct from the outer periclinal wall (W) of the epidermis, with associated fungus (F - cross section) and bacterium or actinomycete. X 31,500.
- Fig. 41. Degenerate epidermal cell (Ep) of a mature sheath-free root, with an intact wall (W) and fibrillar epidermal mucilage (EpMu) in which bacteria have become embedded. The structure inside the epidermal cell is also a microorganism (M). X 31,110.
- Fig. 42. Intact, apparently living epidermal cell (Ep) of a sheath free, basal portion of a mature root. A bacterial cell is embedded in the loosely fibrillar epidermal mucilage (EpMu). X 9,480.

Figs. 43 - 48 and Fig. 50 are sections of greenhouse-grown roots. The micrographs represent mature regions of roots which, although lacking a persistent sheath, would likely have formed a sheath in these regions if grown in the field.

- Fig. 43. (a) Degenerate epidermis of a root grown in vermiculite. The intact cell wall (CW) is covered by fibrillar mucilage material of variable thickness (asterisk). X 11,330.
(b) Thick region of fibrillar material mucilage with embedded bacterium. X 7,000.
- Fig. 44. Intact epidermis (Ep) and a transverse section of a root hair (H) with only a single wall layer. The epidermal mucilage (arrows) is especially thickened in the grooves between epidermal cells. A small bacterial colony (B) is associated with the mucilage. Root grown in Promix BX. X 3,500.

- Fig. 45. Granular epidermal mucilage (Mu) with embedded bacterium (B) over a thick outer wall (W) of a degenerate epidermal cell (Ep). Root grown in Promix BX. X 26,580.
- Fig. 46. Expanded epidermal mucilage (Mu) with embedded bacterial cell (B). Note the suberin lamella (arrows) of the hypodermis (Hy). Root grown in garden plot soil. X 24,750.
- Fig. 47. Fibrillar epidermal mucilage (Mu) with a dark outer boundary (arrows). Root grown in Promix BX. X 17,750.
- Fig. 48. Granular epidermal mucilage (Mu) with associated bacteria (B) of a root grown in vermiculite. Note the suberin lamella (arrows) of the hypodermis. X 8,000.

Fig. 49. a - h. Examples of rhizosphere microorganisms associated with field grown roots.

- (a) Embedded in fibrillar rhizosphere mucilage.
- (b) With pili.
- (c) With fimbriae.
- (d) Fungi (F) and bacteria (B) associated with rhizosphere mucilage (Mu).
- (e) Colonial rod forms present within fibrillar mucilage of the root soil sheath away from the rhizoplane.
- (f) Colony of rod or coccoid bacteria embedded in the soil sheath without fibrillar mucilage.
- (g) Rhizosphere bacteria with fat bodies.
- (h) Actinomycetes (dark forms) and bacteria embedded in rhizosphere mucilage close to a sloughed cell (not shown).

Marker bar in each micrograph represents 0.5 μ m.

Fig. 50. a - i. Examples of microbial forms encountered in the rhizoplane and rhizosphere of greenhouse grown roots.

- (a) Bacterium showing marked polar attachment to a root hair (H). The small amount of fibrillar material (arrow) is probably of bacterial origin (vermiculite).
- (b) Polar attachment of a bacterium to epidermal mucilage (arrow) (garden plot soil).
- (c) Pair of bacteria in a naked groove (garden plot soil).
- (d) Single bacterium attached to the epidermal cell wall (W) by means of a peg (arrow) (garden plot soil).
- (e) Invasive fungus (F) penetrating the hypodermal wall of the host. The thickened wall has been formed by the host cell.
- (f) Embedded rod (R) and surface associated bacterium (arrow) (garden plot soil).
- (g) Similar rod forms embedded in the remnants of epidermal mucilage (garden plot soil).
- (h) Large colony of rod shaped bacteria invading the remnants of a lysed cell (arrow).
- (i) Two small bacterial colonies associated with a small pocket of rhizosphere soil (garden plot soil).

Marker bar on each micrograph equals 0.5 μ m.

encountered more frequently in field samples but the latter were also observed in greenhouse roots grown in field soil. Microorganisms are associated with epidermal mucilage (Fig. 49[a] - [d] and Fig. 50[b], [c], [d], [e], [f], [g]) or with what appears to be the fibrillar sheath matrix (Fig. 49[a] - [h]) especially in field-grown roots. This association of microorganisms with fibrillar material other than the epidermal mucilage was observed once in greenhouse roots grown in field soil (Fig. 50[i]). A few bacteria with pili (Fig. 49[b]), fimbriae (Fig. 49[c]) and amoeboid outer surfaces (Fig. 50[c]) were present. In greenhouse material only, bacteria were frequently observed to be adhered "tail first" to the surface of root hairs (Fig. 50[a]) or epidermal mucilage (Fig. 50[b]). In several samples of greenhouse-grown roots, microorganisms were also found associated with the outer periclinal wall of the epidermis on a somewhat extended peg which seems to originate from the host (Fig. 50[d]). None of these rhizosphere microorganisms were identified.

III.4 Discussion.

III.4.1 Epidermal Mucilage and Mucigel.

The epidermal surface associated mucilage is intact throughout the entire sheath region of field-grown roots and on the sheath free portion of roots which possessed emerged lateral roots. Although

many of the epidermal cells have dictyosomes with darker vesicles similar to those found in region B and C epidermal cells (cp. inset, Fig. 20 with Fig. 18 Part II of thesis) it is likely that this material (ie. the epidermal mucilage over the epidermis of roots possessing a soil sheath or over the epidermal cells of mature roots lacking a soil sheath) is residual epidermal mucilage from the apical portion of the root which has thinned considerably during cell elongation and is no longer secreted by the older epidermal cells. Additional histoautoradiographic studies and measurements of the volume of epidermal mucilage associated with the epidermis at any given stage would be required to determine the extent of continued epidermal activity in the secretion of epidermal mucilage.

The variability in the staining characteristics with toluidine blue O and the periodic acid Schiff's reaction (staining patterns with these procedures are consistent throughout the clear apical portion of the root) implies that some alterations in the properties of the epidermal surface has taken place. The occasional appearance of metachromasy in the epidermal mucilage (Table 1) and the somewhat lighter red stain of the same layer with the periodic acid Schiff's reaction suggests a gradual unmasking or removal of certain components. Gradual loss of phenolics would

account not only for the disappearance of the blue-green reaction with toluidine blue O in this layer (O'Brien and McCully 1981) but also in a decrease in the intensity of the periodic acid Schiff's reaction which may react with some phenols (Geier 1980) and in the autofluorescence of this layer. Phenolic feruloyl side groups "endow certain soluble polysaccharides with the property of forming gels" when oxidized (Fry 1979) and loss of phenols would account for loosening of the microfibrils of the epidermal mucilage as is ultrastructurally observed. Loss of the highly oriented arrangement of microfibrils would result in a loss of birefringence and perhaps in the decreased binding of the optical brightener Calcofluor White M2R New which binds oriented cellulose and other microfibrils (Herth and Schnepf 1980). [Calcofluor binding is not restricted to fibrillar polymers and will bind to pectins and callose as well (Hughes and McCully 1975).] Both characteristics were observed. It is interesting that the histochemistry of the outer periclinal epidermal walls remains essentially stable along the entire length of the root (Table 1).

Based on the striking differences in the histochemical reactions of the fibrillar epidermal mucilage and the fibrillar-matrix material of the soil sheath, it is clear that the epidermal mucilage alone does not account for all the fibrillar material found

within the rhizosphere although ultrastructural evidence is suggestive of the latter, particularly at the transition to sheath (Figs. 20, 21 and 23). Foster (1981) has postulated that the rupture of the cuticle (identified in the present study as the outer epidermal mucilage layer or the boundary layer) releases the primary wall (epidermal mucilage) and that it is this material and not a "specifically secreted mucilage" which fills the space between the epidermal cell and the soil particles much as was observed in some roots in the present study at an ultrastructural level (eg. Figs. 20 and 23).

It is also not likely that the root hairs, which are far more numerous in the soil sheath region than in the apical clear region of corn roots, are involved in mucilage secretion as root hairs are not consistently associated with fibrillar matrix material and root hairs are often observed in sheath free regions of roots with emerged laterals. Root hairs of Sorghum have been implicated in the formation of secretion of some type of mucilage (Werker and Kislev 1978) but in this same study root hairs of Zea were found to not have such a material. A mucigel has been described, however, as covering the outer surface and most root hairs of 16 agricultural species including corn (Greaves and Darbyshire 1972), and Head (1964) described the appearance of "droplets" on root hairs of young apple

roots (36 hours old) but in neither case was the nature of this material determined nor its site of synthesis investigated.

The marked similarity between histochemical reactions and FITC-lectin binding properties of the root cap mucilage and the mucilagenous-matrix material implies that the sheath matrix is actually residual root cap mucilage. However, it is difficult to understand how residual root cap mucilage becomes intimately associated with the root axis an average of 25 mm behind the tip rather than becoming bound to the surface immediately as root cap cells are sloughed. Since the average length of the clear zone decreases later in the growing season (casual observation, measurements not made) when the rate of root growth would also be reduced and maturation completed closer to the tip, the reassociation of fibrillar components with the root surface may reflect changes in the epidermal surface, coincident with a certain stage of differentiation of cells along this cross sectional plane of the root, which facilitates binding to the root surface. This surface change may be paralleled by the appearance of a suberin lamella in the walls of hypodermal cells of the sheathed portion of the root (eg. Figs. 26, 47 and 48); hypodermal cell walls within the clear zone always lacked the suberin lamella. It is not clear whether the tendency towards a loosening of

the fibrils in the epidermal mucilage, first seen in the transition to sheath region (eg. Figs. 20 and 21) is a necessary prerequisite for re-establishment of an intimate association between the root surface and the rhizosphere fibrillar material. Perhaps simple diffusible exudates, which account for 60 - 70% of all materials released from this portion of the root (Rovira 1969b) are involved in establishing binding.

The presence of sheath matrix material on axenic nodal roots remains to be investigated but Floyd and Ohlrogge (1970) have demonstrated the formation of a mucilagenous material over the surface of field grown roots soaked in water under laboratory conditions. The chemical similarity of this material to root cap mucilage (see Table 1, Barlow 1975) strongly suggests that it was of root origin, particularly since a high proportion of fucose was present. It is likely that it is this material which forms the sheath matrix of field grown roots. The present study also provides evidence for the root origin of this material since it is often present where microorganisms are lacking (but bacterial origin cannot be excluded).

Many of the rhizosphere organisms observed in the present study possessed some sheath or capsule material, but this was often visible only as a clear zone around the bacteria. Indeed, microbial exopolysaccharides are notable for their low inherent

electron density and low affinity for the heavy metals used for the contrast enhancement in electron microscopy (Costerton 1979). The clear zones observed around some of the rhizosphere microorganisms associated with the corn roots seem to correspond to the lytic zones for bacteria embedded in mucigel as demonstrated by Guckert et al. (1975) and Dayan et al. (1977). Foster and Rovira (1976) attributed the presence of mucilage materials in the sheath surrounding wheat nodal roots to the greater number of bacteria associated with the root and their activity in production of mucigels, rather than to the root cells which were noted to be collapsed and empty in this region of the root. Subsequently, however, Foster (1981) has theorized that while bacteria and fungi derived polysaccharides are present in the rhizosphere, much of the "so called 'mucigel' is morphologically derived from the primary wall [epidermal mucilage] of the epidermal cells", rather than from the sheaths and exopolysaccharides of the microbial population.

Many of the high resolution studies of the structure of the rhizosphere, dating from the pioneering work of Jenny and Grossenbacher (1963) with barley, and including a number of studies on other grasses, particularly wheat (see review by Oades 1978 and references therein; Foster and Rovira 1973; Old and Nicholson 1975; Leppard and Ramamoorthy 1975, Foster

1981) have shown fibrillar, apparently mucilagenous material at the root-soil interface and in the rhizosphere. Unfortunately, it is difficult to relate these studies to the present findings. There are several reasons for this. First, none of the foregoing studies included photomicrographs (indeed optical microscopy has not been routinely used) so that the identification of the region of the root from which sections were made is not possible. Secondly, most authors have not identified either the root regions or the type of root sampled. Frequently roots fixed in situ were sampled randomly and the alterations of surface features corresponding to root development and maturation were consequently not considered (eg. Foster 1981). Samples of axenically grown root systems of plants at the same age as sampled field grown plants have not been studied. In most cases an accurate determination of the origin of the fibrillar material or "mucigel" has thus not been possible. Finally, the presence of distinct root populations (ie. sheathed and unsheathed roots) has not been clearly noted in many earlier structural studies. Foster and Rovira (1976) did note the presence of a persistent sheath on nodal, but not seminal, roots of wheat but did not investigate its formation.

Regardless of the origin of the sheath matrix material or sheath mucilage, it is this material which

best fits the description of mucigel set by Rovira and co-workers (1979) following the original use of the word by Jenny and Grossenbacher (1963). Mucigel is defined (Rovira et al. 1979) as the "gelatinous material at the surface of roots grown in normal, nonsterile soils including plant mucilages, bacterial cells and their metabolic products such as capsules, glycocalyx materials and slimes and colloidal mineral and organic matter from the soil...mucigel is a product of the entire root-soil-microbial complex with its own distinctive morphological and biochemical properties." The present study shows that both the root cap mucilage and epidermal mucilage are present on field and axenically grown roots and neither the root cap mucilage nor the epidermal mucilage are active in establishing a root-soil-microbe complex in the immature, apical zone of field-grown roots. Therefore the indiscriminate use of the term mucigel in the literature for these readily distinguishable materials (eg. Leppard and Ramamoorthy 1975; Dayan et al. 1977) is misleading.

III.4.2 Sheath Formation.

Soil and sand sheaths have been observed frequently since Price (1911) first described them (see Troughton 1957 for a list of early reports; also Raju et al. 1972; Sprent 1975; Nambiar 1976a, 1976b; Fig. 4, Foster and Rovira 1976; Werker and Kislev 1978) and

rhizosheaths of certain xeric grasses have recently been investigated (Wullstein and Pratt 1979). The characteristic has been noted frequently for xeric grasses, implying that sheath formation is a morphological response to arid conditions (eg. see Troughton 1957). While xeric conditions are certainly not a factor in sheath formation on nodal roots of wheat (Foster and Rovira 1976) and of corn in the present study, imposed conditions of desiccation have been shown to increase the tenacity with which soil clings to the root or even to promote the development of a soil sheath on roots that would otherwise lack it. Sprent (1975) has noted that more sand clings to the tip and root hair zone of soybean roots grown under moisture stress regimes. Sand grains, while held against the root by root hairs, were thought to be bound by mucilage rather than by root hairs. Likewise, Nambiar (1976a) demonstrated the formation of a soil sheath encrusting that portion of the root lying within a dry layer of soil (portions of the same roots lying in moist soil were sheath free [ie. Fig. 1, Nambiar 1976a]). This sheath development was attributed to localized mucilage exudation which was particularly pronounced in the grasses studied (wheat and oats). The source of exudate was not determined although it was thought to originate from the root. Passioura (1980) has recently found that secondary roots of wheat formed

during imposed soil desiccation conditions differ markedly from the parent axis in having many adherent soil particles even after extensive washing.

It is interesting that wheat and barley were found to show an increase in the amount of "exuded" material (which included water soluble substances, nonsoluble mucilages, and sloughed cells) in response to drier conditions (Barber and Martin 1976); similar observations have been made for roots of lodgepole pine (Reid and Mexal 1977). Increase in the number of specific physiological groups of bacteria as rhizoplane and rhizosphere moisture decreased (Peterson et al. 1965) also reflects an increase in the exudations of nutrients (ie. carbohydrates, amino acids etc.) from the root. A tendency towards increase in fucose rich mucilage secretions has also been demonstrated for the algae Fucus grown under drought conditions (Percival 1967) suggesting perhaps, that this is a ubiquitous response.

Whether exuded mucilaginous materials themselves are sufficient for sheath formation is ~~not~~ clear. Frequently a case has been made for the involvement of root hairs in the enmeshing of sand grains leading to rhizosheath formation (eg. Wullstein and Pratt 1981) and Price (1911) mentioned this phenomenon for some grasses growing under xeric conditions (eg. Lygeum spartum, Schismus calycinus, Hordeum murinum). In these

cases the sand grains were thought to be entangled by the long root hairs and cementing mucilage was not present. It is interesting that Wullstein and Pratt (1981) while presenting striking pictorial evidence for the involvement of root hairs, also noted the presence of a coating of flaky material on sand grains of the sheath (Figs. 10 and 11, Wullstein and Pratt 1981) which was thought to resemble mucigel and suggested that this material could act as a biological bonding agent. Sources of this bonding agent were speculated, but now shown conclusively, to include "secretions, mucilages and exudates released from root hairs, root surfaces and root cap tissue" as well as from lysing cortical tissue and bacteria such as Bacillus polymyxa types (Wullstein and Pratt 1981). Micrographs of Paspalum notatum cv. batatais roots also show an extensive matrix material surrounding and embedding soil particles; this material was noted to be metachromatic with the toluidine blue O stain and was lacking from the surface of pensacola roots which also lack a sheath (Döbereiner et al. 1972). Sources of this material were not considered. Raju and co-workers (1972) observed that some roots of wild rye, wheat and triticale were coated with a mass of colloidal soil material that seemed attached both by root hairs and by material that may be bacterial slime. The actual source of this material was not determined.

From the foregoing it is evident that some type of mucilaginous material is involved in sheath bonding in most instances and in those cases where this material is lacking, sheaths do not become cemented into a cohesive structure (eg. Price 1911; Döbereiner et al. 1972) even in the presence of root hairs (Price 1911; Sprent 1975; Passioura 1980). Based on the present work it is obvious that root hairs, which are present throughout the sheath zone, must provide a framework for and strengthen the sheath but are not primarily responsible for sheath adhesion. This is demonstrated best by the lack of a soil sheath in the apical clear zone even though root hairs are found in this region (See Figs. 15 - 17, Part I of thesis). Soil residues in this area of the root, if present, are associated with small deposits of sloughed root cap material. [Affinity of soil for root cap mucilage is further demonstrated by the persistence of a thin layer of soil particles on the root cap surface throughout fixation procedures (eg. Fig. 3).] The observation that older roots with emerged lateral roots were often lacking a soil sheath, although numerous very long root hairs are present in these regions, provides further proof that root hairs are not responsible for adhesion. It is interesting that Wullstein and Pratt (1981) while attributing a major role in sheath formation to root hairs noted a lack of cohesiveness of the sheath on late season roots

even though root hairs were present. These authors suggested that this was a reflection of a decrease in availability of cementing substance.

III.4.3 Rhizosheath Functions.

The function of the soil sheath and fibrillar mucigel is not clear. Price (1911) has suggested that this system must both promote water absorption and protect the root and root surface against desiccation. Jenny and Grossenbacher (1963) suggested that the mucigel functions as a cation transport mechanism. Leppard and Ramamoorthy (1975) likewise postulate a role in the accumulation of cations. However, the suggestion of the latter authors is suspect because it is based on an ultrastructural study of a zone of cells which look like peripheral root cap cells rather than epidermal cells as implied by their paper; of all possible uptake sites, root cap cells are least likely to be functional in (root) nutrient uptake. Even under extremely xeric conditions, roots of a variety of plants which form a soil sheath reach 40% of the Zn uptake which would occur under optimal moisture regimes (Nambiar 1976b) indicating that when mucilage is present water and mineral uptake can continue even under very dry conditions.

Considering the remarkable stability under the beam of an electron microscope of sheath regions where small soil aggregates are completely embedded in

mucigel (large clumps of soil not impregnated are extremely unstable under the same conditions) there is little doubt that the fibrillar mucilage stabilizes the soil sheath. In so doing it may also contribute directly to enhancement of aggregate formation in soils. Although a rotation of corn has often been demonstrated to destroy soil structure (Rennie et al. 1954) a seasonal variation was noted in the percent of water stable aggregates found. This was highest in the spring and summer (corresponding to the season of active root growth) and the results were interpreted as demonstrating the influence of organic matter, lime, nutrient levels and climate, in addition to soil bacteria in the development of stable soil aggregates (Rennie et al. 1954). The general consensus among soil polysaccharides experts is that polysaccharides produced by bacteria and not plants is of prime importance (Acton et al. 1963; Lee and Loutit 1977) because complex materials with molecular weights of 4,000 or more are thought to be of microbial origin (Billès 1978); roots are believed to exude only simple sugars (Lee and Loutit 1977).

The difficulty with corn may be in rooting density rather than in the ability of mucilage to stabilize aggregates per se. If root derived mucilages are associated with very fine roots of other grasses (many of which possess a clear zone - sheath organization

similar to that of corn roots, unpublished observations) then plant derived mucilages could become important. In this respect it is interesting that soil structure is improved by following a corn crop with oats (Rennie et al. 1954) which have a much finer and more extensive root system. Involvement of plant derived mucilages in formation of stable aggregates warrants further investigation.

III.4.4 Microbial Adhesion and Surface Association.

The majority of microorganisms become embedded in the mucigel which probably acts as a substrate. However, the mucigel is not completely degraded even where bacteria are relatively numerous and it is possible that the mucigel complex provides a protective niche rather than substrate per se.

The polar attachment of certain cells to the epidermal and root hair surfaces of greenhouse-grown roots (Fig. 50) is interesting. Umali-Garcia et al. (quoted in Schmidt 1979) have studied the association of the nitrogen-fixing bacterium Azospirillum brasilense with roots of pearl millet and guinea grass and have shown that some of the bacteria were also oriented on the surface in a polar fashion. Polarity was suppressed when nitrogen ions were added to the medium.

Histochemical, histological and structural study of field-grown corn roots. Part IV: Characteristics of above ground portions of nodal roots (prop roots) of corn.

IV.1 Introduction.

The majority of nodal roots of corn are initiated from nodes situated below soil level. Following elongation of internodes during flowering however, some roots are formed above soil level. In order to generate a comprehensive overview of the characteristics of the nodal root system of corn, these roots were also investigated. Only characteristics of above ground portions of these roots are described herein.

IV.2 Materials and Methods.

Roots initiated from aerial nodes were collected and prepared for optical and electron microscopy as indicated in the General Materials and Methods (see p. 10 - 12). Tips of emerged nodal roots which had not penetrated the soil and 2 mm long segments taken just at soil level and at intervals between soil level and the root base of roots which had penetrated the soil, were used. None of the roots sampled possessed emerged lateral roots. For each region a minimum of 2 blocks were used for histochemistry and 2 blocks were prepared for electron microscopy.

For histochemical and microscopic procedures used

refer to General Materials and Methods (see p. 16 - 22).

IV.3 Results.

Nodal roots initiated from higher nodes of corn following stem elongation (in corn, stem elongation occurs after the floral apices have been initiated in the latter part of the growing season), are broad organs which are exposed above soil during their initial phase of growth (Fig. 1). Some of the roots grow actively and consequently penetrate the soil but roots initiated 5 cm or more above the soil level during the end of the growing season (ie. after the fruit matures) rarely complete their development (Fig. 1). Any of these roots which do penetrate the soil are similar in morphological and histochemical properties to those initiated below the soil. All above ground portions of these prop roots are green and have a hard, dry surface.

IV.3.1 Histochemistry of the Above Ground Portions of Prop Roots.

Unlike the root cap of roots initiated below the soil level, the root cap of the above ground prop roots forms an extensive mantle over the root pole and sloughed cells remain intimate with the epidermal surface well beyond 1 mm from the tip-cap junction (Figs. 3 - 6). These cells are embedded in a matrix material which is pink with the periodic acid Schiff's

reaction (Fig. 2), metachromatic with toluidine blue O pH 4.4 (Fig. 6), weakly fluorescent with Calcofluor (Fig. 3), weakly birefringent (Fig. 4) and strongly stained with alcian blue (Fig. 5). The striated, clumpy, accumulated mucilage and the sloughed flanking root cap cells overlies a distinct epidermal mucilage which is stained red with the periodic acid Schiff's reaction (Fig. 2), strongly fluorescent with Calcofluor (Fig. 3), strongly birefringent (Fig. 4), but unstained with alcian blue (Fig. 5) and stained pale blue-green with toluidine blue O pH 4.4 (Fig. 6).

Until they approach the soil these roots do not have root hairs. Roots which penetrate the soil form root hairs close to soil level (but above). Roots which have grown down into the soil for at least several centimeters retain on their above ground portions, sloughed cells which are embedded in the outer mucilage (which is of root cap origin). For the first few mm above soil level root hairs are also embedded in this mucilage. Several cm above soil level the root cap mucilage decreases in volume and although sloughed cells are present root hairs do not form in this region. The sloughed cells are usually devoid of cytoplasm on mature regions of these above ground roots.

Just above soil level, the outer mucilage layer is weakly fluorescent with Calcofluor (Fig. 7) weakly

Fig. 1. Nodal roots emerging from the nodes of elongated stem (S) above the soil. Several of the roots have subsequently penetrated the soil (arrows) but many of the other roots remain above the soil on this 2 month old plant.

Figs. 2 - 13 are glycol methacrylate embedded sections of fixed material. Figs. 2 - 4 and 6 are median longitudinal sections of region C epidermal cells (Ep) and flanking sloughed root cap cells (asterisks). Figure 5 is a median longitudinal section of proximal region B epidermal cells. Figs. 7 - 12 are cross sections just above soil level of roots which had penetrated the soil. Bright field optics unless otherwise noted.

Fig. 2. Section stained with the periodic acid Schiff's reaction followed by toluidine blue O pH 4.4. Sloughed root cap cells still flank the root pole. The detached cells are embedded in a very thick band of mucilage (RCMu) which is not as darkly stained as the epidermal mucilage (arrows). X 680.

Fig. 3. Comparable section to that shown in Fig. 2 stained with Calcofluor. The epidermal mucilage (arrows) demonstrates a much stronger affinity for this fluorochrome compared to the walls of the detached cells and their associated mucilage (RCMu). X 160.

Fig. 4. Epidermal mucilage (arrows) is more strongly birefringent than the overlying root cap mucilage. Walls of sloughed cells are weakly birefringent. Section viewed with crossed polarizers. X 240.

Fig. 5. Section stained with alcian blue. The root cap mucilage (RCMu) and root cap cell walls are strongly stained; epidermal mucilage is unstained (arrow). The area circled shows a thin region of epidermal mucilage across which an epidermal cell and flanking sloughed root cap cell are still joined. X 135.

Fig. 6. Section stained with toluidine blue O pH 4.4. The mucilage (Mu) covering the root pole is strongly metachromatic as are the walls of the sloughed cells. The epidermal mucilage (arrows) is stained pale blue-green. X 370.

yellow-green autofluorescent (Fig. 8), not birefringent (Fig. 9), stained dark pink by the period acid Schiff's reaction (Fig. 10) and is metachromatic with toluidine blue O (Fig. 12). Where it is abundant the outer mucilage is often clumpy (Figs. 10 and 12). In contrast, the epidermal mucilage is Calcofluor positive (Fig. 7), jade-green autofluorescent (Fig. 8), strongly birefringent (Fig. 9), red with the periodic acid Schiff's reaction (Fig. 10) and blue-green with toluidine blue O (Fig. 12).

On older regions of the above ground portions of prop roots where the mucilage of root cap origin has thinned considerably, numerous bacteria, actinomycetes and fungi are apparent in its place (Figs. 13 and 14) presumably having utilized the mucilage as substrate. Bacteria are initially observed as discrete colonies in the thick mucilagenous coat covering young regions of the above ground nodal roots (Figs. 10, 11 and 12).

Differences in histochemical reactions between the root hairs and sloughed cells observed below soil level still exist, but because there are so many contorted root hairs and because there is so much residual mucilage retained, it is often difficult to discriminate between sloughed cells and root hairs which become embedded in the outermost layer of mucilagenous matrix. Root hairs are strongly birefringent (Fig. 9) compared to the sloughed cells

Fig. 7. The strong affinity of epidermal mucilage (arrows) for the fluorochrome Calcofluor is somewhat reduced in this region of the root (cp. Fig. 3). The sloughed cells, root hairs and outermost mucilage surrounding them are at best only weakly Calcofluor positive. Fluorescence optics. X 300.

Fig. 8. All cell walls are autofluorescent (yellow-green). The jade green autofluorescence of the epidermal mucilage is not evident in this photomicrograph and the weak yellow-green fluorescence of the outer mucilage (Mu) is poorly evident. Fluorescence optics. X 330.

Fig. 9. Viewed with crossed polarizers, walls of the root hairs (H) are strongly birefringent, epidermal walls (arrows) are weakly birefringent. Epidermal mucilage, accumulated outer mucilage (Mu) and walls of the sloughed cells are not strongly birefringent. X 200.

Fig. 10. Section stained by the periodic acid Schiff's procedure. Walls of the root hairs (H) and sloughed cells (asterisks) are not easily distinguished with this stain. The accumulated mucilage surrounding the root hairs and sloughed cells is stained dark pink and the epidermal mucilage (arrows) dark red. X 400.

Fig. 11. The "clumpy" nature of the outer accumulated mucilage is clearly evident. Most of the cells embedded in it are root hairs (H) but two bacterial colonies are also present (arrows). Phase contrast optics. X 200.

Fig. 12. Section similar to that shown in Fig. 10 but stained with toluidine blue O. The blue-green stain of the epidermal mucilage is still distinct and the epidermal cells are still alive. The clumpy outer mucilage is metachromatic. Root hairs (H) and sloughed cells show slightly different staining reactions with the root hairs stained blue green and the walls of the sloughed cells walls blue. Root hairs can also be distinguished by their two layered wall (region outlined enlarged in the inset). Note the single large bacterial colony (B) and the numerous smaller colonies scattered in the outer mucilage (small arrows).

Main Figure X 1,010.

Inset X 2,185.

Figs. 13 - 23 are micrographs of fixed roots embedded in Spurr's resin.

Fig. 13. Cross section of a mature region of a root (just above soil level) as indicated by the secondarily thickened walls of the cortex (C). The epidermis (Ep) is intact and covered by a thin layer of epidermal mucilage (arrow). Numerous microorganisms (small arrows) occur in the rhizosphere (R) as do several sloughed cells (asterisk) and root hairs. Mature epidermal cells are devoid of contents and invasive microbes (eg. fungi - large arrow) frequently are found in these cells. Stained with toluidine blue O pH 11.
X 1,000.

Fig. 14. Cross section of an above ground nodal root which has subsequently penetrated the soil, taken just at soil level. The epidermis (Ep) is intact, although apparently devoid of contents, with an extremely thin covering of epidermal mucilage. A portion of the soil sheath (S) is evident between the root hairs occupying the site where accumulated mucilage was present above the soil. Large microbial colonies (likely actinomycete - A are also encountered. Toluidine blue O pH 11. X 425.

and show evidence of a two-layered construction (inset, Fig. 12). Just above soil level of roots which had penetrated several feet into the soil, root hairs and sloughed cells are contorted and collapsed and no longer distinguishable. Epidermal cells are also collapsed at this point.

Histochemical results are summarized in Table 1.

IV.3.2 Ultrastructure of the Above Ground Portions of Prop Roots.

The ultrastructural features of the root tip of roots initiated above the soil are identical to those observed for roots which have been initiated in the soil (see Part II of thesis) except that the fibrillar root cap mucilage persists as a thick layer even over region C epidermal cells (Fig. 15) and that the plasmodesmatal connections persist across the epidermal mucilage boundary even between region B epidermal cells and flanking root cap cells (Figs. 5 and 16). These channels are often apparently occluded and they occur within a thinner, almost pit like region of the epidermal mucilage (Fig. 16).

Like root cap cells below the soil level, cap cells of roots above soil level have numerous hypertrophied dictyosomes (Fig. 17) which revert to an apparently less active state (ie. they are smaller, Fig. 18) once the cells are sloughed. These root cap cells have a wavy outer wall continuous with the

Table 1. Properties of the cell walls and mucilages of nodal (prop) roots of corn which had been initiated above soil level.

portion ^a of root	proximity	cortex CW	hypodermis CW	epidermis CW	lenticels LEPa	oily OEpMa	RH	sloughed cells ^b CM	MyC
tip	auto fluorescence	+++	+++ YGr	+++	jade Gr	NS	NP	++	++ +
M-at soil		+++	+++ YGr	+++	++	NS	+++	++	+
M-serial		+++	+++ YGr	++	+	NS	++	RD	ND
tip	birefringence	++++	++ +	+++	++ +	NS	NP	++	++ +
M-at soil		++	++	++	+++ ^a	NS	+++	+	+
M-serial		++	+	++	7	7	RD	+	+
tip	Calcofluor fluorescence	+++	+++	++	+++	NS	NP	+++	++ +
M-at soil		+++	+	+	+++	NS	+	++	+
M-serial		+++ +	+	+	++	NS	NP +	NP +	+
tip	aniline blue fluorescence	-	-	-	-	-	tips	spots	-
M-at soil		-	-	-	-	-	tips	spots	-
tip	periodic acid Schiff's reaction	++	++	+++	+++	NS	NP	++	+++ +
M-at soil		++	-	+++	+++	NS	+++	few +	++++ clumps
M-serial		- +	-	+	+++	NS	NP	++ ^d	++ +
tip	toluidine blue staining	M	M	BlGr	BlGr	- to dkBlGr	NP	M to Bl	lim to M
M-at soil		Bl	BlGr	BlGr	- to lBlGr	- to BlGr	BlGr	few Bl	var M
M-serial		Bl to BlGr	BlGr	Bl	Bl to M	NS	NP	BlGr	var M
tip	alcian blue staining	++	++	+++	-	+++	NP	+++	+++ +
M-at soil		++	-	- +	++	++ +	-	++	++ , striated
M-serial		++ +	-	+	- +	NS	NP	+	- +

Note: a-Sections of glycol methacrylate embedded roots. Only the tip regions of prop roots which had not penetrated the soil and serial portions of mature roots which had penetrated the soil are included. The serial surfaces of mature roots were sampled just above soil level (M-at soil) and midway between the soil and root base (M-serial).

b-For the purposes of this Table, sloughed cells of the tip region refer to the free and attached root cap cells on the flanks of the root caps.

c-Where the reaction varies from marked to weak, a strong reaction was noted for mucilage not released from peripheral root cap cells and associated close to the root cap. This reaction becomes weaker centripetally away from the root cap.

d-Identification of these cells was extremely difficult because they were collapsed.

Key: +++ very strong, ++ strong, + average, + weak, - no reaction, 7 present but property could not be accurately determined. l-inner, o-outer, CW-cell wall, Ep-epidermis, M-mucilage, RH-root hair, NS-not seen, NP-not present, ND-no data, M-mature, Bl-blue, Gr-green, Y-yellow, li-light, dk-dark, var-variable.

fibrillar root cap mucilage (Figs. 17 and 18) like sloughed cells of below ground roots (Fig. 18). Sloughed cells with similar walls are associated with the root surface of the nodal prop roots along most of their length above the soil. Just above soil level sloughed cells present on the aerial surfaces of the root are outnumbered by the thick walled root hairs. The walls of these hairs have a dark outer layer (Fig. 22).

Throughout the above ground regions of roots which have subsequently elongated into the soil, the outer epidermal wall and epidermal mucilage are intact although the latter is often reduced to a very thin layer (Figs. 19, 20, 21, and 22). The outer mucilage layer (ie. above the epidermal mucilage) is fibrillar with granular material embedded in it in some areas (Fig. 19). It is this material which binds the soil particles (Fig. 20) and in which the bacteria and fungi become embedded (Figs. 19, 20, 21, 22 and 23). However, at least some of the fine, fibrillar material surrounding bacterial colonies (Figs. 21 and 23) may originate from the bacteria themselves; this possibility was not further pursued. The variability in the ultrastructural nature of mucilagenous material of root cap origin on above ground nodal roots is far greater than that encountered in similar material formed on roots below soil level.

Fig. 15. Median longitudinal section of distal region C epidermis (Ep) of an aerial root tip. Epidermal cells are identical to those of roots growing in the soil and dictyosomes with large associated vesicles with dark contents (arrows) are a consistent feature of these cells. The outer surface consists of three layers: the outer periclinal cell wall (CW), the epidermal mucilage (EpMu) and the fibrillar root cap mucilage (RCMu). X 5,410.

Fig. 16. Median longitudinal section of region B epidermal cell (Ep) and flanking root cap (RC) cell. Occluded plasmodesmata (pd) are present in a thin region of epidermal mucilage (EpMu) between the two cells. Note the dictyosomes (D) and associated vesicles with fibrillar contents (arrows) and the peripheral endoplasmic reticulum network (ER) of the root cap cell. X 13,530.

Fig. 17. Peripheral root cap cell demonstrating the hypertrophied nature of the dictyosomes (D) and the association of the cell wall (CW) with the fibrillar root cap mucilage (asterisk). X 14,400.

Fig. 18. Two living sloughed root cap cells. The peripheral cytoplasm, includes a nucleus (N) and dictyosomes (D) with flattened cisternae and small vesicles. The cell wall (W) is undulating, of variable thickness and apparently associated with the fibrillar root cap mucilage (asterisk). X 5,560.

Fig. 19. Cross section of a mature region (midway between soil level and the base of the root) of an above ground nodal root showing a dead epidermal cell (Ep), epidermal cell wall (W) and epidermal mucilage (arrow). The epidermal mucilage is covered by granular fibrillar mucilagenous material (asterisk) corresponding to the material which is metachromatic when stained with toluidine blue O (arrows in upper inset). Variable forms of microorganisms (lower inset) are embedded in this material.

Main Figure X 20,250.

Upper Inset X 200.

Lower Inset X 15,000.

Fig. 20. Portion of a dead epidermal cell (Ep) with intact wall (W) and epidermal mucilage (arrow). Bacteria are embedded in fibrillar mucilage (asterisk) lying above the epidermal mucilage. Soil (S) is also associated with this material. Section of a root initiated above soil level which had penetrated the soil, taken immediately below soil level.

X 8,570.

Fig. 21. Bacteria present in granular fibrillar mucilage at a region of the root comparable to that shown in Fig. 19.

X 8,000.

Fig. 22. Bacteria (B) and fungi (F) associated with an above ground root. A fungal hyphae (F) is present in one of the epidermal cells. The large cell at the upper right edge of the photomicrograph is a root hair (H) with a two layered wall. There is a suberin lamella present in the hypodermal cell wall (Hy) but it is not clearly seen at this magnification.

X 4,000.

Fig. 23. Examples of bacteria (B) and a multicellular organism (asterisk) present in the outermost granular fibrillar mucilage on the root surface.

X 12,140.

IV.4 Discussion.

Strong histochemical evidence of similarity between the outer mucilagenous material lying over the epidermal mucilage on mature regions of above ground roots and accumulated root cap mucilage encasing the apical pole of the same roots above ground, suggests that the two materials are at least chemically similar. The presence in these materials of sloughed cells bearing strong resemblance to flanking, peripheral root cap cells provides additional evidence that this material is in fact residual root cap mucilage. While chemical analysis would be necessary to bear this hypothesis out, it is interesting to note that Floyd and Ohlrogge (1970) who examined nodal roots associated mucilage which "formed" when roots were placed in water, found that the gelatinous coat was qualitatively, but not quantitatively, similar to root cap mucilage as analyzed by others (eg. see Table IV, p. 44, Barlow 1975). The material associated with above ground nodal roots in the present study was observed to assume a gelatinous nature after it had been soaked in water or an aqueous solution. This is likely a reflection of the ability of this material to hydrate to a high degree, a characteristic shared with root cap mucilage (Miki et al. 1980). Hydration of the mucilage upon soaking in water would account for its apparent formation as observed by Floyd and Ohlrogge (1970).

The mucilage overlying the epidermal mucilage provides a favourable niche for microorganisms which are relatively abundant in this material. In addition, the outer mucilage strongly binds soil particles at and slightly above soil level. These features are similar to those noted for the mucigel, or sheath matrix materials, of roots below soil level.

Root caps of epiphytic plants (such as Cattleya) are characterized by secretory activity similar to that described for Zea and on these roots the peripheral root cap cells along with layers of secreted mucilage form a protective mantle over the root tip (Mollenhauer 1967). These roots are characterized by a tough outer surface which, as is the case for above ground nodal roots in this study, swells into a mucilage ball when placed in water. The presence of mucilage on the aerial roots suggests a role of water balance for the dictyosome derived materials (Mollenhauer 1967). Under dry conditions the mucilage becomes hard and dry, perhaps preventing moisture loss from the root; under moist conditions the mucilage takes up large amounts of water.

The function of these aerial roots in corn is not clear. They may function as prop roots in anchoring the large plant late in the growing season. Alternately, they may represent nothing more than the development of the nodal root system which does not cease until the

plants are destroyed by frost at the end of the growing season.

GENERAL CONCLUSIONS

What has emerged from this study is not just a description of cellular structure along the length of a root but rather a recognition and some clarification of the variety of microenvironments making up the rhizosphere and rhizoplane along the length of roots of field-grown corn plants. Although observations have been restricted to young nodal roots (less than 20 cm long and lacking emerged lateral roots) much of what has been described can be utilized as a basis for describing structures present in the rhizosphere of mature roots following a season of growth and maturation.

The apical 1 mm of field-grown roots is sheathed by two distinct mucilages corresponding to the root cap mucilage and epidermal mucilage described for axenic seedling roots (Clarke et al. 1979; Miki et al. 1980) and to the mucigel sheathing the apical tip of crop plants in culture (Jenny and Grossenbacher 1963). Beyond the apical 1 mm the root cap mucilage thins considerably and by 2 - 3 mm is only occasionally encountered on the root surface. The epidermal mucilage also thins considerably (Clarke et al. 1979) being most distinct within the first 1 cm of the root as it also is in legumes (Dart and Mercer 1964) but persisting over the root surface in both the apical clear zone and over the soil sheath region.

Both mucilages are present on corn roots regardless of conditions of growth and the histochemistry of the two distinct mucilages is always comparable (regardless of conditions of growth). However, the ultrastructural characteristics of the epidermal mucilage varies considerably and shows some dependence on the growth media used (see also Samtsevitch and Romano 1975). Thus greenhouse-grown roots were found to have a loosely fibrillar epidermal mucilage compared to field-grown roots and this was particularly evident with vermiculite and Promix BX grown roots. While roots of corn plants grown in pots of field soil most closely resembled field-grown roots collected during the summer, characteristic association of microorganisms with the epidermal mucilage within a few cm of the tip and the marked compression of the root cap cells and quiescent centre cells were not observed in field-grown roots. In addition the establishment of a persistent soil sheath was not encountered in greenhouse-grown roots (although some soil did cling to the roots). Foster and Rovira (1976) have noted that there is no difference in rhizosphere structure between field and pot grown roots of wheat. However, in an earlier review Rovira (1975) acknowledged that considerable differences exist between the natural environment and model systems and that caution is required in extrapolating functions of

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OF / DE

the materials present on axenic roots to field conditions. Evidence of doubled exudation rates for roots grown in solid compared to liquid medium (Bowen and Rovira 1976) suggests that the variations seen in the present study are real.

The presence of a "boundary" layer in the epidermal mucilage of field and pot-grown roots is highly variable. Outer boundary layers have been observed, in material fixed for electron microscopy, associated with legumes (Dart and Mercer 1964), barley (Dayan et al. 1977), wheat (Foster 1981) and in some cases with the greenhouse-grown roots (eg. Fig. 47, Part III of thesis) and field-grown roots (eg. Fig. 22, Part III of thesis) in the present study. The boundary layer has been identified as a cuticle (Scott 1963, 1965; Mollenhauer 1974; Shen-Miller et al. 1978; Foster 1981).

In the present study, large portions of the root were observed to lack any increase in the electron density of the outer layer of epidermal mucilage suggesting that the boundary layer, when present, is formed by an interaction between the epidermal mucilage and the environment. On the other hand, the presence of a boundary layer detectable on the outermost region of the epidermal mucilage using histochemical means is consistent. This distinctly stained layer is particularly evident in the apical clear zone of corn

roots regardless of the conditions of growth or the fixation or embedding procedures used. A difference in the chemistry of the outer epidermal mucilage layer is suggested and this is borne out by the resistance of this layer (in wheat roots) to attack by Gaeumannomyces enzymes (Smith and O'Brien 1979) and by its distinct labelling with FITC-lectin (see Figs. 3 and 7, Part I of thesis); the inner epidermal mucilage is attacked by this pathogen's enzymes (Smith and O'Brien 1979) and is not labelled by the lectin. Reaction with toluidine blue O and alcian blue hints at the presence of acid groups and the sensitivity of this layer in barley to pectinases (Dayan et al. 1977) indicates that galacturonic acids of pectin are involved in the staining reactions observed. While the presence of carboxyl groups may be functional in establishing a cation exchange capacity for the roots (which is increased under compaction when root exudation rates also increase [Kulkarni and Savant 1970]) there is evidence that cation contents of plants do not show significant correlation to root cation exchange capacity (Mane et al. 1970). The function of the outer epidermal mucilage boundary layer is thus not clear.

In addition to the root cap and epidermal mucilages, the roots of field grown corn plants also have a third carbohydrate matrix associated with the surface. This material has not been observed associated

with the primary axenic roots of maize (Clarke et al. 1979) or other grasses (Miki et al. 1980). Based on the intimate association of this third matrix with both the rhizosphere soil and microorganisms it can be termed mucigel (Rovira et al. 1979). The mucigel was evident in the soil sheath region of roots growing in the soil and over most of the aerial surface of above ground nodal (prop) roots.

The presence of numerous free, living sloughed cells in the mucigel of the soil sheath has not previously been described. The literature does contain numerous references to the presence of dead root surface cells (Greaves and Darbyshire 1972), sloughed cell material (Hale et al. 1971), cortical sheets, tissue fragments and individual sloughed cells (Griffin et al. 1976), sloughed epidermal cells (Umali-Garcia et al. 1978) and organic debris consisting of loose epidermal and cortical cells (Gadgil 1965). However, these cells have generally been considered as nothing more than organic matter contributed by the root to the rhizosphere. Rogers and co-workers (1940 quoted in Rovira 1965) demonstrated intense phosphatase activity associated with a gelatinous material surrounding maize roots. Although this material contained nuclei and empty cell walls of sloughed root cap cells, the phosphatases and the gel were thought to originate directly from the root (Rogers et al. 1940, quoted in

Rovira 1965). It is interesting that for field-grown roots of corn the mucigel was generally most abundant where these sloughed cells were present and living, as indicated by an intact peripheral cytoplasm. This association suggests an active and direct involvement in establishment of the rhizosphere.

The source of detached cells in corn rhizospheres is somewhat of a mystery. They are somewhat elongated cells in longitudinal section and range in size from 15 - 20 μm x 10 μm . The cell walls are undulating with true plasmodesmata and the peripheral cytoplasm is usually intact. Casual observations by Dr. McCully following completion of this research, have shown that many of these cells found at locations relatively distant from the root cap possess intact nuclei and streaming cytoplasm. The most likely source for the free-living cells is the root cap. Corn root cap cells are present in two fairly distinct populations. Tip cells are isodiametric with collapsed cytoplasm whereas peripheral cells flanking the root pole become elongated (10 - 40 μm x 10 μm), retain a peripheral cytoplasm (Batenchuk and McCully, unpublished) and possess an undulating cell wall with true plasmodesmata. It is interesting that these cells show β -glucosidase activity while apical cap cells do not (Figs. 17 and 18, Ashford and McCully 1970), weak inosine dephosphatase activity, which may demonstrate

UDP-type reactions concerned with polysaccharide synthesis (Dauwalder et al. 1969) and readily incorporate labelled sugars such as galactose (Fig. 12, Dauwalder and Whaley 1974) and fucose (Fig. 30, Kirby and Roberts 1971; Forsyth 1982). Tritiated thymidine (Fig. 7a, Bednara 1974) and ^3H -uridine (Barlow 1970, cap cell location not distinguished) have also been shown to be incorporated by these cells. In above ground roots of corn where root cap cells remain associated with the root surface in the absence of the sloughing action of the soil, these cells can be observed over older epidermal cells and here the relationship bears strong resemblance to that encountered with sloughed cells and the epidermis in the soil sheath.

The common assumption that sloughed root cap cells die goes back to the work of Jost (1907). The presence of β -glycerophosphate in root cap cells of pea is thought to be involved in cells autolysis (Sexton and Sutcliffe 1969), and the presence of lysosome-like organelles in corn root cap cells (Berjack 1968; Matile 1968; Matile and Moor 1968; Berjack and Villiers 1970) which contain a number of hydrolytic enzymes, has been described as functional in root cap cell senescence. There are a surprising number of hints, however, that root cap cells do not die either before or immediately after becoming separated from each other. Leiser (1968), for example, demonstrated the presence of

living detached cell caps 35 - 50 mm behind the cap of mist grown Rhododendron roots and this falls within the area (25 mm +) where detached cap cells are first seen associated with roots of corn. Samtsevich (1968) has described sloughed cells of corn which remain in living condition in the soil for 1 or 2 days (quoted in Balandreau and Knowles 1978). Even more phenomenal is the longevity of sloughed cells in culture noted by Knudson (1919). In his experiments sloughed root cap cells of corn were alive following 45 days in culture and cells sloughed from pea root caps were reversibly plasmolyzable following 71 days in liquid culture, despite contamination. These remarkable experiments have only recently been repeated and it has been found that peripheral root cap cells of corn can be maintained in tissue culture for at least one month (McCully, unpublished).

Association of root cap cells on the root surface of axenic clover roots has been described. These cells were found to occur at random along the root and their continuous walls were noted to possess true plasmodesmata (Mosse 1975). Furthermore, this author speculated on the likelihood of a similar situation in field-grown roots resulting in packets of cells becoming "attached to soil particles or the root surface providing small pockets of localized substrate". Sloughed epidermal cells of Panicum shown

by Umali-Garcia et al. (Fig. 3, 1978) look exactly like the detached cells of corn suggesting that association of sloughed root cap cells with mature portions of young roots may be a universal phenomenon. Homology of the detached cells and the peripheral root cap cells, in addition to morphological and anatomical similarities, is further indicated by the fact that detached cells and peripheral root cap cells (cp. Fig. 2 Part I and Fig. 38 Part II of thesis) are both strongly labelled by FITC-lectin specific for fucose. The presence of fucose in root associated mucilage is a characteristic considered peculiar to corn roots (Rougier et al. 1979).

Staining reactions of the sheath mucigel of field grown corn roots with toluidine blue, alcian blue and the periodic acid Schiff's reaction indicate chemical similarity between this material and root cap mucilage and a definite distinction between epidermal mucilage and the surface of root hairs. Root cap mucilage accumulated on aerial portions of roots (see Part IV of thesis) also shows identical histochemical and ultrastructural characteristics and this similarity between aerial surface mucilage and sheath mucilage further indicates root cap origin. Binding patterns of fucose-specific lectins (Part I) supports this conclusion. Finally, chemical analysis of sugar components in a gelatinous matrix which formed on Zea

nodal roots which were soaked in water demonstrated a qualitative similarity to root cap mucilage and demonstrated the characteristic presence of fucose (Floyd and Ohlrogge 1970, 1971). This material is presumably equivalent to the sheath mucilage found on roots growing within the soil.

Providing that the root cap mucilage is protected from degradation in the soil, much of the sheath mucilage may be residual root cap mucilage. Protection mechanisms do exist. For example, complexing of neutral and acidic polysaccharides with clays in soil at pH 5.8 (Parfitt and Greenland 1970), and formation of metal complexes or tanning by soil humic substances (Cheshire 1977) may be responsible for stability of the root cap mucilage. Phenolic glycosides may also provide some protection. The presence of phenolics linked to the epidermal mucilage of wheat has been discussed (Smith and O'Brien 1979). Complexing of the root cap mucilage and sloughed cell walls with phenols is indicated by the autofluorescence of these areas. Such complexes would surround the rhizosphere soil and subsequently be involved in sheath formation.

While formation of some type of mucilage complex would explain the inaccessibility of root cap mucilage to microbial degradation and therefore its resistance, a number of puzzling questions arise. How does the material, for example, become attached to the roots

remote from the root tip? Why is there an absence of a soil sheath on the white tips of the roots? At present the best explanation lies in the rapid elongation likely to be taking place in the clear apical zone. Sloughed cells and residual mucilage would not have the opportunity to bind to this region and would bind only to a region with a reduced growth (by elongation) rate. Binding may reflect an increase in root diameter (Price 1911), an alteration in epidermal mucilage, or a change in the type of exudate released from this differentiated region of the root where the sheath first binds. Any of these factors alone or in combination would facilitate binding between the root, residual root cap mucilage, detached cells and the soil of the rhizosphere.

The possibility that some of the rhizosphere mucilage is produced in part by the detached cells is intriguing particularly in light of the observation that these cells are living (Part III; McCully unpublished). Nutritionally there is probably no barrier to the survival of detached cells or to their ability to synthesize polysaccharides. All roots release simple sugars, amino acids, and organic acids and vitamins and hormones are either released from roots or are synthesized by microorganisms of the root environment (eg. see Hale and Moore 1979). Root hairs and sloughed cells may be sources of auxins (Scott

1972). Brown et al. (1950) have shown that exudates from both sorghum and corn roots contain a factor which at very low concentrations stimulates the growth of isolated root segments. There is no evidence to suggest that detached cells would not be able to utilize these substances for the maintenance of cellular integrity as well as in secretion. It is interesting that where numerous living sloughed cells are present (Figs. 33 and 34, Part III of thesis) there is also a large amount of fibrillar mucilage; where these cells are no longer living there is very little mucilage present regardless of the presence or absence of microorganisms. The presence of large amounts of mucigel on nonsterile roots may indicate that mucigel is produced continuously along the root (Brown 1975; Bowen and Rovira 1976). Sloughed cells could be responsible for this continued synthesis.

Although the microorganisms are not consistently associated with the sheath mucilage and sheath mucilage is frequently absent where large microbial colonies are present, the possibility of a microbial origin of at least some of the mucigel cannot be entirely excluded. Microbial polysaccharides contain many of the sugars present in root mucilages and could give rise to histochemical reactions similar to those of the sheath matrix. There is also strong consensus in literature that much of the soil polysaccharides are of microbial

rather than plant origin (eg. Parfitt and Greenland 1970; Tan and Loutit 1977). Raju and co-workers (1972) speculated that the mass of colloidal soil on roots of wild rye, wheat and triticale is held by root hairs and bacterial slime. Based on differences in content of free and bound sugars, amino acids, organic acids and uronic acids between exudates of sterile beans, beans grown in soil suspension and beans grown in media inoculated with Xanthomonas, the gel sheath of roots is at least in part of bacterial origin (Lasik and Kofláková 1971).

Circumstantial evidence of bacterial association with a mucigel layer of batatais roots, which stains metachromatically with toluidine blue, and the lack of both a mucigel layer and bacteria in pensacola roots (Döbereiner et al. 1972) further suggests at least some microbial synthesis. Umali-Garcia et al. (1980) have likewise noted that inoculated pearl-millet and guinea grass produced a more extensive mucilagenous sheath. It is interesting that in this case axenic roots were found to lack both sloughed cells and root cap mucilage indicating that while microorganisms may have stimulated sheath formation, they did not necessarily contribute directly to this material. Evidence of extremely low bacterial and fungal coverage (Rovira et al. 1974), particularly on young roots (Rovira and Campbell 1974) suggests that microbial association is

not significant enough to account for formation of the completely encasing soil sheath.

Regardless of the origin of mucigel its function in the soil remains obscure. The increased formation of mucigel under dry conditions (Nambiar 1967a) suggests a function in the maintenance of continuity between the solution in fine soil pores and the solution within the root. Such continuity is important both in water uptake and nutrient availability. On drying, soil and roots shrink away from each other forming gaps of 10 μm or greater, disrupting water uptake except in areas where mucigel helps retain root-soil contact (Tinker 1976). Measurements of the water uptake distribution patterns in roots show maximum uptake rates at 5 to 5.5 cm from the root tip (So et al. 1978). Soil sheaths on field-grown roots of corn are present at this distance from the root tip indicating that the sheathed roots are functional in water and nutrient uptake. Passioura (1980) has shown that only a portion of any root is active in continued water uptake. Microscopic examination of the root system revealed that 30% of any root system was contorted and encrusted by strongly adherent soil particles. The author explained that, "it is tempting to equate the contorted root length with the calculated active root length". (Passioura 1980). This contorted region of wheat roots may be equivalent to the sheathed corn roots observed in the present

study.

Sloughed root cap cells from roots of blue grama plants amount to about 0.07% of the root carbon during a 30 day growing period (Bokhari et al. 1979) and sloughed root cap cells from peanut plants account for 0.26 - 0.73 mg of carbon per plant per week (Griffin et al. 1976). This sloughed cell material, which is generally not measured in liquid exudate studies (Rovira 1969b), contributes a significant amount of organic materials to the soil. This has implications both in nutrient availability (Rovira 1969b; Newman 1978) and as a source of nutrients for microorganisms (Rovira 1969b). Although there is microscopic evidence that bacteria do not readily degrade this layer (Newman 1978) loss of organic carbon from roots represents a significant energy input into the soil ecosystem which is available for microorganisms.

Numerous publications concerning the association of microorganisms capable of acetylene reduction (and therefore nitrogen fixation) have appeared in recent years. Healthy maize plants in an otherwise chlorotic field were found to show acetylene reduction activities associated with the presence of Enterobacter in large amounts of its own slime on the root surface (Raju et al. 1972). Colonies of bacteria capable of N₂ fixation have been localized in the mucigel of batatais roots (Azotobacter, Döbereiner et al. 1972), in mucigel of

the root cap, granular material on the root hair and fibrillar material of the root axis of pearl millet roots (Umali-Garcia et al. 1980), in mucigel of various grasses as determined by antibody techniques (Azospirillum, Schank et al. 1979), in the mucous of certain wheat roots (Neal and Larsen 1976; Larsen and Neal 1978), in the rhizosheaths of xeric grasses (Bacillus polymyxa, Wullstein et al. 1979), and in the mucigel of apices of axenic roots of rice but not of field-grown roots (Diem et al. 1978). In other cases, activity was shown to be related to photosynthetic activity of plants (Döbereiner and Day 1974, Ahmad 1979) and to be stimulated by the addition of a carbohydrate source such as glucose (Ruschel et al. 1978; Wahab and Wareing 1980). Genetic manipulation of wheat has been shown to enhance the establishment of nitrogen-fixers in the rhizosphere (Neal et al. 1973; Neal and Larsen 1976; Larsen and Neal 1978) demonstrating control of the plant genome in this rhizosphere effect. High variability between and within lines for nitrogen fixation in the rhizosphere of corn roots has been observed (Von Bülow and Döbereiner 1975) indicating that the possibility exists for genetic manipulation of corn in regards to increasing efficiency of associative N_2 fixation.

There is some debate as to the agricultural significance of associative nitrogen fixation

(Mishustin 1970) particularly in temperate regions where soil temperature is the apparent limiting factor (Von Bülow and Döbereiner 1975; Döbereiner 1978). However, increases in the dry weight of Zea and Setaria plants inoculated with Azospirillum (Cohen et al. 1980) and significant uptake of nitrogen either completely or partially sufficient for the plants requirements have been noted (Döbereiner and Day 1974; Rusche et al. 1978; Ahmad 1979). Yield increases following inoculation has also been reported for Panicum and Digitaria during three consecutive years of field trials (Smith et al. 1978).

The form of the carbohydrate substance provided by the plant may play a role in association of specific microorganisms with the root surface (Baldani and Döbereiner 1980) and association of Azospirillum with pearl millet roots has been shown to be enhanced by root exudates (Umali-Garcia et al. 1980). This was considered to be compatible with the idea of lectin recognition, as has been postulated for Rhizobium-legume interactions (Stacey et al. 1980). The possibility that a similar interaction could exist between corn roots, which do have lectins (Gietl and Ziegler 1979) as well as a prominent mucigel layer, and associative nitrogen-fixing bacteria such as Azospirillum has important implications in the nitrogen nutrition of field-grown corn.

The capacity of mucigel to bind soil particles of the sheath suggests a function in soil aggregate formation. Generally, aggregate stabilization has been demonstrated to be enhanced by periodate sensitive polysaccharide materials (Greenland et al. 1962) particularly in cropped and young pasture soil. While this effect is ephemeral (Moavad et al. 1976), interaction with metals and organic matter (Moavad et al. 1976; Cheshire 1977) or montmorillonite (Martin 1971) would generate more stable aggregates. Polysaccharides of microbial origin (Tisdall et al. 1978; Ferham and Weaver 1978) and fungal hyphae and their amorphous materials (Sutton and Sheppard 1976; Tisdall and Oades 1979) have been considered to be most significant. However, plant materials (Gadgil 1965; Cheshire and Anderson 1975) and unique new heteropolysaccharides formed from both microbial and plant polymers (Cheshire 1977) are also involved. It is particularly interesting that associations between the fibrillar mucilage of Zea mays epidermal cells and montmorillonite have been observed (Guckert et al. 1975). In this case the polysaccharide was seen to be adsorbed to the clay surface and to penetrate between the layers of the clay (Guckert et al. 1975).

~~The work reported in this thesis only begins to~~
touch on the complexity of the rhizosphere of corn roots. The root boundary of mature axenic plants

remains to be considered and its features compared with the root surfaces of field grown plants to determine whether the rhizosphere mucilage is present only under natural conditions. Observation of greenhouse grown roots may help to elucidate environmental responses. The changes in the root boundary along the entire length of both young and mature roots of field grown plants must be mapped, to clearly elucidate surface alterations which facilitate binding of the sheath to the root surface, the locations of sloughed cells along the surface and changes in the association of microbes with the root surface as the roots mature. The microbes associated with the root surface must be identified (of particular interest is the possibility that associative nitrogen-fixing organisms may be present) and the continued secretion of the sheath mucilage by living sloughed cells should be explored, first by demonstrating that these cells are indeed living and metabolically active and subsequently by demonstrating the release of labelled carbon compounds by these free-living cells. Finally, the presence of the sloughed cell-rhizosphere mucilage association on sheathed roots of other plants must be determined.

APPENDIX A: Alternative Method for the Fixation of Corn Root Tissue.

Following initially satisfactory results (Clarke, unpublished) obtained by the use of Piperazine-N-N¹-bis (2-ethanol) sulfonic acid (PIPES) buffer as the vehicle for the fixative (Salemo and Brandao 1973) an experiment was initiated to assess the use of this technique for the fixation of field-grown roots of corn.

Appropriate tissue segments were fixed overnight on ice in each of three fixative solutions: 1) 5% (v/v) glutaraldehyde in PIPES buffer (0.08M), pH 8.0. 2) 3% (v/v) glutaraldehyde in PIPES buffer (0.03M), pH 8.0. 3) 3% (v/v) glutaraldehyde in phosphate buffer. The latter fixative solution was routinely used for root tissue fixation.

Tissues fixed in the PIPES-buffered glutaraldehyde solutions were rinsed with 0.2M PIPES buffer, pH 6.8 (4 times in 15m); postfixed with 2% osmium tetroxide in 0.2M PIPES buffer pH 6.8 for 1 h on ice, rinsed several times with the same buffer, dehydrated according to the standard procedure and embedded in Spurr's resin (Spurr 1969).

The PIPES buffers were prepared as stock solutions using the following formulations: 1) PIPES 0.2M, pH 8.0: 6 g PIPES (Sigma) plus 0.75N NaOH to give a volume of 45 ml, made up to 50 ml with distilled water.

following pH adjustment to 8.0 with 1N NaOH. 2) PIPES 0.4M, pH 6.8: 6 g PIPES (Sigma) plus 0.5N NaOH to give a volume of 20 ml, made up to 25 ml with distilled water following pH adjustment with 1N HCl or 1N NaOH.

Results and Discussion.

Materials fixed in PIPES-buffered glutaraldehyde are generally acceptable (eg. Fig. 2) but show no real advantage over the routine fixation techniques used throughout the rest of this work (eg. Fig. 4). Meristematic cells fixed in 5% glutaraldehyde in PIPES buffer frequently show cytoplasmic retraction from the walls (Figs. 1 and 3) except at the primary pit fields (Fig. 3). Membranes of the cellular organelles, including the sensitive tonoplast, are usually intact (Fig. 2).

Older portions of roots fixed in the same fixatives show even less distinction in maintenance of cellular structure between roots fixed with 3% glutaraldehyde in either phosphate or PIPES buffer; roots are not well fixed in 5% glutaraldehyde in the PIPES buffer. Extracytoplasmic spaces are occasionally present in phosphate-buffered material (Fig. 6) and are not observed in PIPES-buffered tissues fixed in 3% glutaraldehyde (Fig. 5).

As suggested (Salemo and Brandao 1973) 5% glutaraldehyde in PIPES buffer is a good fixative for meristematic cells while 3% glutaraldehyde in PIPES

buffer works well with highly vacuolated tissues. However, 3% glutaraldehyde in phosphate buffer provides adequate fixation of both cell types and appears to penetrate the tissue faster than does glutaraldehyde in solutions where PIPES is the vehicle for the fixative. Since much of the published work on roots to date has been carried out using phosphate buffer as the vehicle for glutaraldehyde fixation, and since PIPES buffer, which is cumbersome to prepare, provides no apparent advantages, it is suggested that the standard glutaraldehyde-phosphate buffer solutions are satisfactory for the fixation of field-grown root tissues.

FIGURE LEGENDS:

Figs. 1 - 6 are micrographs of root tissues fixed in either PIPES or phosphate buffered glutaraldehyde.

Fig. 1. Region B epidermis (Ep) and flanking root cap. The epidermal cell cytoplasm has retracted from the cell wall resulting in the formation of a large extracytoplasmic space (asterisk). Median longitudinal section (MLS). 3% glutaraldehyde in PIPES buffer.
X 5,140.

Fig. 2. Peripheral root cap cell with well preserved cytoplasm. Note particularly the dictyosomes (D) with large dark vesicles (arrows). MLS. 5% glutaraldehyde in PIPES buffer. X 7,600.

Fig. 3. Tip-cap junction (arrows). Cytoplasm has retracted from the walls except at the primary pit fields (large arrows). The nuclear envelope (small arrows) and the tonoplast (T) are at least partially intact. MLS. 5% glutaraldehyde in PIPES buffer.
X 12,380.

Fig. 4. Region C epidermis (Ep). Both the cytoplasm and epidermal mucilage (EpMu) are well preserved. MLS. 3% glutaraldehyde in phosphate buffer. X 9,710.

Fig. 5. Soil particles of the sheath (S) and bacteria in the rhizosphere of an intact epidermal cell (Ep). The epidermal cytoplasm, including the dictyosomes (D), is well preserved and the epidermal surface is intact. Transverse section. 3% glutaraldehyde in PIPES buffer.
X 15,000.

Fig. 6. Similar section to that shown in Fig. 5. The formation of an extracytoplasmic space (asterisk) in the epidermis is only occasionally observed using phosphate buffer. Transverse section. 3% glutaraldehyde in phosphate buffer. X 15,000.

APPENDIX B: Use of Lectins for Identification of Carbohydrates.

Theory.

Lectins are proteins (often, but not necessarily glycoproteins) which lack enzymatic activity but which have the capacity to recognize sugar residues. Frequently, specificity is reported as being restricted to one or two particular monosaccharides (eg. see review by Goldstein and Hayes 1978) but the critical component in the binding reaction appears to be the arrangement of the hydroxyl groups (Lis and Sharon 1973) and the C1 oxygen atom (Gold and Balding 1975) and consequently any one of a group of sugars with similar contact faces will potentially be bound.

The ability of lectins to bind with polysaccharides makes them rather unique reagents for the study of surface glycoproteins and glycolipids (Lis and Sharon 1973). Any of the marker molecules used in immunohistochemistry can also be used to tag lectins. Hence labels such as ferritin-antilectin antibodies (Franz et al. 1979), ferritin alone (Nicholson and Singer 1971), radiolabelled iodine (Warr et al. 1977) and colloidal gold (Roth and Wagner 1977) have been used. The majority of these labels are best suited for either scanning or transmission electron microscopy. For visualization at the level of the light microscope, lectins can be tagged with fluorescein dyes which emit

80 - 95% of absorbed light as green fluorescence (Goldman 1968).

Fluorescein isothiocyanate has a receptive group towards proteins which forms the basis for its stable binding to lectins. Epsilon amino groups of lysine and the terminal amino groups of protein chains are major sites for the reaction but accessible sulfhydryl groups may also be involved (Goldman 1968).

Fluorescein absorbs light in the 495 nm (blue) band and has a maximum fluorescence band at 517 nm (green). The tagged lectin upon incubation with the tissue recognizes and binds to its specific monosaccharide. Following rinsing away of unreacted lectin with phosphate buffered saline, the bonding sites are visualized by exciting the tissue with blue light. Green fluorescence identifies the binding site and, ideally, occurs only when the proper sugar residues are accessible (Fig. 1). Tissue autofluorescence should be checked as a control. To determine specificity of lectin binding, comparable tissue is incubated in a lectin solution containing an excess of the sugar for which it is specific. In this case, all lectin binding sites become occupied by the monosaccharide in solution and lectin sites are subsequently not available for binding to the tissue. Following a rinse with phosphate buffered saline to remove the unreacted lectins, the tissue is excited

with blue light. If lectin binding is specific, fluorescence would not be observed (Fig. 2).

Fresh tissues are suitable for lectin labelling but where greater resolution is required, labelling can be carried out following fixation and glycol methacrylate embedment (Kraenbuhl and Jamieson 1974; Craig *et al.* 1979; Tronchin *et al.* 1979).

Labelling of Fixed and Embedded Tissues.

1.) Glycol methacrylate. Axenic root tips of corn were fixed in 4% paraformaldehyde (10 ml - 10% paraformaldehyde [2.0 g paraformaldehyde heated to 60 - 65 C in 20 ml distilled water, 1N NaOH added dropwise till solution clear, cooled], 5 ml phosphate buffer [0.025 M], 10 ml distilled water) for 2 h on ice to reduce glutaraldehyde-induced autofluorescence. Tissues were dehydrated and embedded in glycol methacrylate following standard procedures (O'Brien and McCully 1981). Subsequent to sectioning (1 μ m - 2 μ m thick sections) sections were affixed to glass slides and incubated in 4% (v/v) aqueous osmium tetroxide to further reduce autofluorescence and rinsed thoroughly with distilled water. Sections were incubated in the appropriate lectin and control solutions (see Part I of thesis) overnight in a dark, humid environment. Following several rinses with phosphate buffered saline (to remove unreacted lectin) and a distilled water rinse (to remove excess salts), sections were mounted

in immersion oil and observed with epifluorescence optics.

Autofluorescence is reduced in all osmicated samples but is not completely eliminated. Nonetheless, fluorescein-fluorescence is distinguishable and present in all treated sections. Sections incubated in lectin alone show only marginally brighter fluorescence (Figs. 3, 4 and 5) than do sections incubated in lectin plus fucose (Figs. 6, 7 and 8) but this difference is probably insignificant. Although the root cap mucilage binds FITC-lectin the distinct labelling of the epidermal mucilage noted in fresh material (see Part I of thesis) is not pronounced in glycol methacrylate embedded material. A tenfold decrease in section thickness (from fresh to fixed tissue) may in part be responsible for some of the problems encountered, but does not account for lack of specificity. Nonspecific lectin binding was particularly pronounced in some experiments, in which the starch grains of the central root cap cells also were labelled by the lectin.

2). Spurr's Resin Embedded Tissue. Glutaraldehyde fixed tissue embedded in Spurr's resin has been successfully utilized for antigen localization with peroxidase following removal of the resin (Rodning et al. 1980). Based on this report, thick optical sections of Spurr's resin embedded corn root material was used in a lectin labelling experiment.

Sections showing blue to green interference colours (0.5 - 1 μ m thick), affixed to glass slides, were treated for 2 m with sodium methoxide to remove the resin matrix (Sutherland et al. 1976) rinsed thoroughly with running water and air dried. Sections were then incubated in a 1 ml drop of lectin solution in a dark, humid environment at room temperature for 2 h. Lectin plus α -L-fucose and phosphate buffered saline (PBS) were included as control incubations. Following incubation, sections were rinsed with PBS and distilled water, then air dried and mounted in immersion oil.

Initial results were encouraging. A pronounced specificity was obtained and control sections were completely negative. This technique is restricted by the minimal amount of lectin binding material available for binding in the thin (0.5 μ m thick) sections of Spurr's embedded tissue routinely used for light microscopy. For this reason, positive reactions are difficult to record and require long exposures, even with a 100 X objective. Fluorescein-fluorescence appears to extinguish under these conditions, further complicating the results. For the figure shown (Fig. 38, Part III of thesis) the contrast was enhanced by copying a print onto high contrast copy film and using the resultant negative in place of the original. Despite the weak reaction, the use of Spurr's embedded tissue for higher resolution of lectin labelling

patterns is promising.

Fig. 1. Diagrammatic representation of lectin binding to fresh tissue. The fluorescein (black dot) tagged lectin (L) is incubated with the tissue. The lectin recognizes and binds to its specific monosaccharide at the root surface. Following excitation with blue light (495 nm) the fluorescein emits light in the green (520 nm) band allowing visualization of the binding site. ^

Fig. 2. As a control for reaction specificity, the lectin is mixed with an excess of its specific monosaccharide resulting in the blockage of all available binding sites (right hand side of upper reaction). Subsequent incubation with the tissue does not allow for lectin binding and thus, excitation with blue light shows that no lectin has bound to the tissue.

Figs. 3 - 5 are median longitudinal sections of glycol methacrylate embedded root tips of corn incubated in FITC-lectin. Fluorescence optics.

Fig. 3. Walls of the sloughed root cap cells and root cap mucilage (asterisk) are labelled. The epidermal mucilage (arrows) over the region B epidermis (Ep) is weakly labelled but has a slightly brighter outer layer (large arrow).
X 360.

Fig. 4. Region C epidermis (Ep). The outer periclinal wall and the outer layer of epidermal mucilage are labelled (small arrows). The fluorescence of the epidermal mucilage (large arrow) is not as bright.
X 360.

Fig. 5. The tip-cap junction (arrows) and walls of the surrounding cells are labelled by FITC-lectin.
X 360.

Figs. 6 - 8 show sections comparable to those shown in Figs. 3 - 5 respectively but incubated in lectin plus α -L-fucose (0.2M) control solution. Fluorescence optics.

- Fig. 6. The walls of the sloughed root cap cells and the root cap mucilage (asterisk) are labelled although the label of the epidermal mucilage boundary (large arrow) is very weak compared to that of FITC-lectin incubated tissue (cp. Fig. 3). X 360.
- Fig. 7. Region C epidermis. Label pattern is similar to that shown in Fig. 4 but the overall fluorescence is somewhat reduced. X 360.
- Fig. 8. Fluorescence of the tip-cap junction (arrows) and surrounding cells is not reduced in this control (cp. Fig. 5). X 360.

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