1

National Library
of Canada

Acquisitions and
Bibliographic Services Branch

395 Wellington Street
Ottawa, Ontario
K1A 0N4

Bibliothèque nationale
du Canada

Direction des acquisitions et
des services bibliographiques

395, rue Wellington
Ottawa (Ontario)
K1A 0N4

Votre file - Votre référence

Our file - Notre référence

NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Reproduction in full or in part of this microform is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30, and subsequent amendments.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

La reproduction, même partielle, de cette microforme est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30, et ses amendements subséquents.

Canada

**THE WATER BALANCE OF BRANCH ROOTS OF CORN RELATED
TO THEIR STRUCTURE**

XUELIAN WANG, B.Sc., M. Sc.

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

Doctor of Philosophy

**Department of Biology
Carleton University**

January 1994

©copyright

1994, Xuelian Wang

National Library
of Canada

Bibliothèque nationale
du Canada

Acquisitions and
Bibliographic Services Branch

Direction des acquisitions et
des services bibliographiques

395 Wellington Street
Ottawa, Ontario
K1A 0N4

395, rue Wellington
Ottawa (Ontario)
K1A 0N4

Your file *Votre référence*

Our file *Notre référence*

The author has granted an irrevocable non-exclusive licence allowing the National Library of Canada to reproduce, loan, distribute or sell copies of his/her thesis by any means and in any form or format, making this thesis available to interested persons.

L'auteur a accordé une licence irrévocable et non exclusive permettant à la Bibliothèque nationale du Canada de reproduire, prêter, distribuer ou vendre des copies de sa thèse de quelque manière et sous quelque forme que ce soit pour mettre des exemplaires de cette thèse à la disposition des personnes intéressées.

The author retains ownership of the copyright in his/her thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without his/her permission.

L'auteur conserve la propriété du droit d'auteur qui protège sa thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

ISBN 0-315-89779-1

Canada

Name

Xue-lian WANG

Dissertation Abstracts International is arranged by broad, general subject categories. Please select the one subject which most nearly describes the content of your dissertation. Enter the corresponding four-digit code in the spaces provided.

Biology

SUBJECT TERM

0433

SUBJECT CODE

U·M·I

Subject Categories

THE HUMANITIES AND SOCIAL SCIENCES

COMMUNICATIONS AND THE ARTS

Architecture 0729
Art History 0377
Cinema 0900
Dance 0378
Fine Arts 0357
Information Science 0723
Journalism 0391
Library Science 0399
Mass Communications 0708
Music 0413
Speech Communication 0459
Theater 0465

EDUCATION

General 0515
Administration 0514
Adult and Continuing 0516
Agricultural 0517
Art 0273
Bilingual and Multicultural 0282
Business 0688
Community College 0275
Curriculum and Instruction 0727
Early Childhood 0518
Elementary 0524
Finance 0277
Guidance and Counseling 0519
Health 0680
Higher 0745
History of 0520
Home Economics 0278
Industrial 0521
Language and Literature 0279
Mathematics 0280
Music 0522
Philosophy of 0998
Physical 0523

Psychology 0525
Reading 0535
Religious 0527
Sciences 0714
Secondary 0533
Social Sciences 0534
Sociology of 0340
Special 0529
Teacher Training 0530
Technology 0710
Tests and Measurements 0288
Vocational 0747

LANGUAGE, LITERATURE AND LINGUISTICS

Language 0679
 General 0289
 Ancient 0290
 Linguistics 0291
 Modern
Literature 0401
 General 0294
 Classical 0295
 Comparative 0297
 Medieval 0298
 Modern 0316
 African 0591
 American 0305
 Asian 0352
 Canadian (English) 0355
 Canadian (French) 0593
 English 0311
 Germanic 0312
 Latin American 0315
 Middle Eastern 0313
 Romance 0314
 Slavic and East European

PHILOSOPHY, RELIGION AND

THEOLOGY

Philosophy 0422
Religion
 General 0318
 Biblical Studies 0321
 Clergy 0319
 History of 0320
 Philosophy of 0322
Theology 0469

SOCIAL SCIENCES

American Studies 0323
Anthropology
 Archaeology 0324
 Cultural 0326
 Physical 0327
Business Administration
 General 0310
 Accounting 0272
 Banking 0770
 Management 0454
 Marketing 0338
Canadian Studies 0385
Economics 0501
 General 0503
 Agricultural 0505
 Commerce Business 0508
 Finance 0509
 History 0510
 Labor 0511
 Theory 0358
Folklore 0366
Geography 0351
Gerontology
History 0578

Ancient 0579
Medieval 0581
Modern 0582
Black 0328
African 0331
Asia, Australia and Oceania 0332
Canadian 0334
European 0335
Latin American 0336
Middle Eastern 0333
United States 0337
History of Science 0585
Law 0398
Political Science
 General 0615
 International Law and Relations 0616
 Public Administration 0617
Recreation 0814
Social Work 0452
Sociology
 General 0626
 Criminology and Penology 0627
 Demography 0938
 Ethnic and Racial Studies 0631
 Individual and Family Studies 0628
 Industrial and Labor Relations 0629
 Public and Social Welfare 0630
 Social Structure and Development 0700
 Theory and Methods 0344
Transportation 0709
Urban and Regional Planning 0999
Women's Studies 0453

THE SCIENCES AND ENGINEERING

BIOLOGICAL SCIENCES

Agriculture 0473
 General 0285
 Agronomy
 Animal Culture and Nutrition 0475
 Animal Pathology 0476
 Food Science and Technology 0359
 Forestry and Wildlife 0478
 Plant Culture 0479
 Plant Pathology 0480
 Plant Physiology 0817
 Range Management 0777
 Wood Technology 0746
Biology 0306
 General 0287
 Anatomy 0308
 Biostatistics 0309
 Botany 0379
 Cell 0329
 Ecology 0353
 Entomology 0369
 Genetics 0793
 Limnology 0410
 Microbiology 0307
 Molecular 0317
 Neuroscience 0416
 Oceanography 0433
 Physiology 0821
 Radiation 0778
 Veterinary Science 0472
Zoology
 General 0786
 Medical 0760

EARTH SCIENCES

Biogeochemistry 0425
Geochemistry 0996

Geodesy 0370
Geology 0372
Geophysics 0373
Hydrology 0388
Mineralogy 0411
Paleobotany 0345
Paleoecology 0426
Paleontology 0418
Paleozoology 0985
Palynology 0427
Physical Geography 0368
Physical Oceanography 0415

HEALTH AND ENVIRONMENTAL SCIENCES

Environmental Sciences 0768
Health Sciences
 General 0566
 Audiology 0300
 Chemotherapy 0992
 Dentistry 0567
 Education 0350
 Hospital Management 0769
 Human Development 0758
 Immunology 0982
 Medicine and Surgery 0564
 Mental Health 0347
 Nursing 0569
 Nutrition 0570
 Obstetrics and Gynecology 0380
 Occupational Health and Therapy 0354
 Ophthalmology 0381
 Pathology 0571
 Pharmacology 0419
 Pharmacy 0572
 Physical Therapy 0382
 Public Health 0573
 Radiology 0574
 Recreation 0575

Speech Pathology 0460
Toxicology 0383
Home Economics 0386

PHYSICAL SCIENCES

Pure Sciences
Chemistry
 General 0485
 Agricultural 0749
 Analytical 0486
 Biochemistry 0487
 Inorganic 0488
 Nuclear 0738
 Organic 0490
 Pharmaceutical 0491
 Physical 0494
 Polymer 0495
 Radiation 0754
Mathematics 0405
Physics
 General 0605
 Acoustics 0986
 Astronomy and Astrophysics 0606
 Atmospheric Science 0608
 Atomic 0748
 Electronics and Electricity 0607
 Elementary Particles and High Energy 0798
 Fluid and Plasma 0759
 Molecular 0609
 Nuclear 0610
 Optics 0752
 Radiation 0756
 Solid State 0611
Statistics 0463

Applied Sciences

Applied Mechanics 0346
Computer Science 0984

Engineering
 General 0537
 Aerospace 0538
 Agricultural 0539
 Automotive 0540
 Biomedical 0541
 Chemical 0542
 Civil 0543
 Electronics and Electrical 0544
 Heat and Thermodynamics 0348
 Hydraulic 0545
 Industrial 0546
 Marine 0547
 Materials Science 0794
 Mechanical 0548
 Metallurgy 0743
 Mining 0551
 Nuclear 0552
 Packaging 0549
 Petroleum 0765
 Sanitary and Municipal 0554
 System Science 0790
Geotechnology 0428
Operations Research 0796
Plastics Technology 0795
Textile Technology 0994

PSYCHOLOGY

General 0621
Behavioral 0384
Clinical 0622
Developmental 0620
Experimental 0623
Industrial 0624
Personality 0625
Physiological 0989
Psychobiology 0349
Psychometrics 0632
Social 0451

The undersigned hereby recommend to
the Faculty of Graduate Studies and Research
acceptance of the thesis,

The Water Balance of Branch Roots of Corn Related to Their Structure

submitted by
XUELIAN WANG, B.Sc., M.Sc.

in partial fulfilment of the requirements
for the degree of Doctor of Philosophy

Chair, Department of Biology

Thesis Supervisor

External Examiner

Carleton University

Abstract

The recent discovery of the delayed maturation of the late metaxylem (LMX) in the main roots of corn has suggested that most of the water used by the plant must enter old, mature portions of the root via branch roots. This has recently been confirmed: the branch roots of corn collect over 80% of the water for the plant. My study focuses on whether these branch roots have any special anatomical features associated with their role in water uptake. My results show that branches are miniature versions of main roots in the maturation of LMX, and of the hypodermis and endodermis, but they have some unique features compared to the main roots. Measurements of relative water contents showed that branch roots are the most responsive part of the root system to drying by transpiration. The outer epidermal walls of branch roots in contact with the soil have a more loose, and fluffy texture than their counterparts in the main roots, or in any other epidermis in the literature. Such walls are likely more open for water and nutrient diffusion. Most branch roots retain a living epidermis throughout their life, in contrast to the main roots, in which the epidermis and root hairs die and are shed some 30 cm behind the tip. A living epidermis might be important for some root functions because it is not suberized like the hypodermis, and because the plasmodesmata joining it to the hypodermis would disappear following its death. The branch roots of corn growing in the field are surprisingly short (mode 6-10 mm). They lose their apical meristem at an early stage, and stop growing, and their tissues mature right to the dead end. This maturity includes the formation of Casparian strips and suberized lamellae in the hypodermis and endodermis, which form distal to the maturation of the LMX. The frequency of pits on the outer tangential wall of the hypodermis is 0.3 per 100 μm^2 , and about twice this on the corresponding wall of the endodermis. The number of pits through the two layers is the same. The xylem vessels become open (70% of the root length) for conducting close to the distal end, which is

closed either by living, immature xylem or by mucilaginous plugs. I found these plugs to come from the xylem parenchyma, instead of from decaying xylem walls, as assumed in the literature.

Acknowledgements

I would like to thank Dr. Margaret E. McCully and Dr. Martin J. Canny for their supervision and encouragement throughout the course of this study, and for their time and patience in correcting this thesis. Also, I want to express thanks to my advisors, Dr. J. Sinclair and Dr. C. Charest for their timely advice and suggestions; Hank Datema, Ed Bruggink and Art Goodenough for providing greenhouse services; Agriculture Canada for the use of the field plot at the Central Experimental Farm. I thank the Chinese Government for an Overseas Graduate Scholarship, and Carleton University for a Graduate Scholarship.

Working with my colleagues in the Laboratory gave me friendship and joy during this study at Carleton. I express my thanks to all of them, especially to Wayne England, Kathleen Ismond, and Zhongmin Dong for their help with the plates, and diagrams; Michelle Watt, and Cathy Bayliss for helping with the proof reading. Finally, I would like to acknowledge my wife, Meijun Wang, for her understanding and encouragement.

TABLE OF CONTENTS

	Pages
Acceptance Sheet	ii
Abstract	iii
Acknowledgements	v
Table of Contents	vi
List of Tables	x
List of Figures	xii
<u>Chapter 1:</u> General Introduction	1
References	7
<u>Chapter 2:</u> The water status of the roots of soil-grown maize in relation to the maturity of their xylem	15
Introduction	16
Materials and Methods	17
Results	20
Discussion	22
References	26
<u>Chapter 3:</u> The maturation and openness of xylem conduits in the first-order branches of field-grown roots	35
Introduction	36
Materials and Methods	37
Plant material	37
Anatomical study of LMX maturation	38

Determination of openness of LMX elements	39
1. From thin sections of embedded material	39
2. Particle diffusion	39
Progression of cell death at the tips of determinate branches	40
Microscopy and photomicrography	41
Results	41
Numbers and sizes of LMX conduits in branch roots	41
Structure of immature and mature LMX elements	43
Development of openness of LMX	44
Progression of cell death in determinate branches	44
Discussion	47
References	50
<u>Chapter 4:</u> Development of the epidermis, surface features and suberization	
of the hypodermis and endodermis in first-order branches	67
Introduction	68
Materials and Methods	73
Plant materials	73
Development of the epidermis	73
Preparation of epidermal strips	73
Embedded materials	74
Preparation of whole mounts	74
Autofluorescence of the epidermal cell walls	75
Anatomical study of the surface of the branches	75
The suberization of the hypodermis and endodermis	76
Relationship between suberization in the cortex and LMX	
maturation	77

Suberization of hypodermis and endodermis	77
The maturation of the LMX	77
Pit frequencies on the outer tangential cell walls of hypodermis and endodermis	77
Macerated materials	78
Embedded root tissues	79
Microscopy and photomicrography	79
Results	80
Branch roots retain a living epidermis	80
Development of the epidermis	81
Changes in the epidermal cell outer surface during root elongation	83
Epidermal cell outer surface in the mature region	84
Autofluorescence of the epidermal cell walls	85
Other surface features in branch roots	86
Soil sheaths	86
Detached root cap cells	86
Microorganisms on the root surface and suberization of epidermal cell walls	87
Suberization of the hypodermis and endodermis	88
LMX maturation in relation to suberization of the endodermis and hypodermis	91
Pits and plasmodesmata in the outer tangential walls of hypodermis and endodermis	92
Discussion	94
Branch roots retain a living epidermis	94

	Page
Branch roots have a loose, open outer surface	95
Other features of the branch epidermis	96
The frequency of pits in hypodermis and endodermis	97
Water flux through each pit	99
Comparison of suberin development between branch and main roots	100
Relationship between suberization and LMX maturation and water uptake	101
References	102
<u>Chapter 5:</u> General discussion and conclusions	129
Similarities of branches to main roots	130
1. Maturation of late metaxylem	130
2. Maturation of apoplastic barriers	131
3. Water flux	131
Differences of branches from main roots	132
1. Further stages of maturation	132
2. More intense response to transpiration	132
3. Retention of epidermis and root hairs	133
4. Open texture of outer epidermal cell walls	133
5. High proportion of high resistance roots	134
6. Consistent numbers of pits through apoplastic barriers	135
Conclusions	136
1. Significance of living epidermis	136
2. Diffusivity in epidermal cell walls	136
3. Structure and activity of the grooves	137
4. Role of class I roots	137

5. Plugged determinate roots	137	^
References	138	

Chapter 2:

Chapter 3:

Chapter 4:

Table 1. Percentage of branch roots with living epidermis	113
Table 2. Epidermal cell volume at different distance from the roots tips of branches	114

Table 3. Sizes of branch epidermal cells and the proportions of them bearing root hairs	115
Table 4. Frequency of pits in hypodermis and endodermis of corn roots	116
Table 5. Water flux through single pit	117

	Pages
<u>Chapter 2</u>	
Fig. 1. Relative water content in relation to water potential	34
<u>Chapter 3</u>	
Fig. 1. Anatomy of a class 3 branch root	60
Figs. 2-6. Structure of developing LMX elements	61
Figs. 7-10. Determination of openness of LMX by India ink diffusion	62
Figs. 11-14. Tip structure in determinate branch roots	63
Figs. 15-19. Cell death and decay in determinate branch roots	64
Figs. 20-25. Xylem plugging in determinate branch roots	65
Figs. 26-29. Xylem parenchyma involved with xylem plugging	66
<u>Chapter 4:</u>	
Diagram 1. Positions in different root types where developmental stages 1 to 3 of the hypodermis and endodermis begin	118
Diagram 2. Suberization of hypodermis and endodermis in relation to xylem maturation	119
Figs. 1-5. Contrasting epidermal features in branch and main roots	120
Figs. 6-10. Nuclear and wall fluorescence in epidermal cells of branch roots	121
Figs. 11-17. Epidermal surface development in branch roots	122
Figs. 18-19. Long and short cells in the epidermis of branch roots	123

Figs. 20-23. Detached root-cap cells, soil aggregates and microorganisms at the root surface	124
Figs. 24-29. Hypodermis and endodermis development	125
Figs. 30-32. Passage cells in hypodermis and endodermis	126
Figs. 33-36. Suberized lamellae in stage 3 hypodermis and endodermis	127
Figs. 37-42. Pits and plasmodesmata in hypodermis and endodermis	128

CHAPTER 1.
GENERAL INTRODUCTION

Almost all green plants are differentiated into two interdependent systems: the above ground shoot and underground root. Neither of these two systems can survive and function well without the proper functioning of the other. The leaves of the shoot system absorb energy from the sun and transform it into organic matter of the plant body, and support the root system with carbohydrates. In turn, the root system, distributed in the soil as a well-branched network, absorbs water and nutrient ions for the whole plant, and supplies anchorage for the shoot system.

Plant roots penetrate deeply downwards into the soil. Weaver (1919) has described, for example, the panic grass grown in loose, sandy soil, spreading only a little near the soil surface, but to a maximum depth of over 9 feet, many roots having a diameter of 3 or 4 mm. Because of the difficulty of recovering the root system from the soil, few data about root systems in their entirety are available. Weaver (1926) showed that a 5-week old corn plant, grown in rich loess soil with a nearly optimum water content, had 19 main roots with 1462 first-order, and 3221 second-order branch roots. The main roots contributed only 4 or 5% of the total length, the first-order branches 47-67%, the second-order branches 29-48%. By careful counts of extensive samplings, Dittmer (1937, 1938) found that a fully mature winter rye plant grown in a box of soil had approximately 13,800,000 roots with a total length of 380 miles and a surface area of about 2,550 square feet. When the length and surface area of the root hairs were added in, the totals for the entire system were nearly 7,000 miles and 7,000 square feet. Miller (1981) also showed that in an one-month-old corn plant, 99.3% of the roots have a diameter of less than 400 μm , and the most abundant size class is that with a diameter of 150 μm . Such an astonishing root system spreads through the soil in response to limited water in soil and the

extreme dilution and immobility of soil nutrients (Epstein, 1973). However, Brewster and Tinker (1972), and Gillespie (1989) have also shown that roots can access water and many nutrients up to 2 mm from the root surface. Therefore, fine roots would exploit a larger volume of soil relative to their mass than larger roots. From all these points of view, the finer roots would appear to be most important to the plant in water and nutrient uptake.

Early study (Miller, 1938) has shown that a single corn plant transpired over 200 liters of water during its life, or 100 times of its own fresh weight. It was further shown that about 225 kg of water was transpired by corn plants for each 1 kg of dry plant materials, including roots, stems, leaves, cobs and kernels, and some 600 kg of water was transpired to yield 1 kg of kernels (Hanks, 1983). Of all the water taken by plants, over 95% is lost by transpiration and only 5% or even less is used in plant metabolism and growth (Salisbury and Ross, 1985). All the water used by the plant is taken up through the root system.

McCully and Canny (1988) have shown that the apical regions of the nodal roots of corn grown in the field (and many other grasses as well) bear a thick rhizosheath for 20+ cm from their tips, except at the growing tip (called here sheathed roots). Back along the root in the mature regions, these sheaths are lost and roots become bare (called bare roots). It is now clear that the rhizosheath is a developmental phenomenon, and these two root types are different developmental stages. The sheathed roots have living, immature large late metaxylem (LMX) elements, and these living cells are accumulating nutrient ions taken up from the soil. When they finally mature to open vessels, the rhizosheaths are shed (St. Aubin, Canny and McCully, 1986). The hydraulic conductivity through these immature xylem elements is close to zero. Only a small volume of water can pass to the shoot through the narrow early metaxylem (EMX). McCully and Canny (1988) proposed that

most of the water used by the plants must enter the older, mature, proximal regions, via the branch roots. Earlier Maertens (1971) had reported that most of the water that entered a corn plant did so in the old, proximal regions of the roots. These studies have called my attention to the branch roots.

The earlier studies quoted above showed that most of a plant's roots are small roots. In a recent study of root length of field-grown corn, Pallant *et al.* (1993) found that more than 56% of the total root length occurred in roots with diameter smaller than 0.175 mm, and less than 24% of the total length were in roots larger than 0.275 mm. Previous studies have also revealed that the branch roots of corn are mostly short (mode 6-10 mm) (Varney *et al.*, 1991), and only elongate for an average 2.3 days (Cahn, Zobel and Bouldin, 1989). They soon lose their root tip and apical meristem and become determinate (called here determinate branches) (Fusseder, 1987; McCully, 1987; Varney and McCully, 1991), leaving most of their tissues mature right to their dead tips (Varney and McCully, 1991), except those on the apical regions of the main roots which retain a normal root tip (called here indeterminate branch roots). They are the driest portions of the corn root system (Wang, Canny and McCully, 1991 and Chapter 2 in this thesis). The first-order branch roots can be classified into 4 size classes based on their diameter, and the number and diameter of the LMX elements (Varney *et al.*, 1991). The relative water flux entering unit surface area is the same in main and branch roots (Varney and Canny, 1993), but the total length of the first-order branch roots in a mature corn plant is about 35 times that of the main roots (Varney *et al.*, 1991). These branch roots contribute over 80% of the total surface in the root system, and thus collect over 80% of the water for the plant, the axial roots the rest (Varney and Canny, 1993). My study of them concentrates on the question whether branch roots of corn are similar to main roots in structure and development, and whether these branch roots have any special anatomical features related to their role in water

uptake and transport.

Epidermal cells play a very important role in root-soil exchanges and have been studied extensively, especially in grasses (see review in Abeysekera and McCully, 1993, and Introduction in Chapter 4 in this thesis). However, all these studies have been done on the young primary roots or nodal roots, and nobody has attempted to study the development of the epidermis and surface features in the branches.

Most plant roots have an endodermis which is the innermost layer of the cortex and has long been considered a barrier between the cortex and the stele. The wall modifications constituting this barrier develop over some centimetres as the root matures (Esau, 1965; Fahn, 1982; Kroemer, 1903; Van Fleet, 1961). Kroemer distinguished three developmental stages (detailed descriptions of these stages will be given in the Introduction to Chapter 4). Another barrier is found in the hypodermis, the subepidermal cell layer of the root. Similar cell wall modifications to those of the endodermis have been reported in a wide range of plant species including corn (Clarkson *et al.*, 1978; Clarkson *et al.*, 1987; Enstone and Peterson, 1992; Esau, 1965; Fahn, 1982; Perumalla and Peterson, 1986; Perumalla, Peterson and Enstone, 1990; Peterson, 1987; Peterson, Emanuel and Wilson, 1982; Peterson and Lefcourt, 1990; Peterson, Murrmann and Steudle, 1993; Peterson and Perumalla, 1990; Peterson, Peterson and Robards, 1978; Wilson and Robards, 1978; Wilson and Peterson, 1983). These wall modifications include deposition of Casparian strips in the radial and transverse walls, suberin lamellae in all the cell walls, and additional cellulose layers (Esau, 1965; Fahn, 1982; and references listed therein). All these studies of hypodermis and endodermis have been with corn primary roots. There have been no

comparable studies of the branch roots.

Water (and nutrients) collected by the epidermis and root hairs moves through the cortex and scler cells in the symplast and finally gets into the xylem where upward water transport occurs as the transpiration stream. However, it won't happen unless the xylem there is mature and open and also is connected to open, conducting xylem in main roots. The differentiation and development of the xylem in main roots has been studied (St. Aubin *et al.*, 1986; Wenzel, McCully and Canny, 1989) as described earlier in this chapter. No such work has dealt with branch roots. Varney and McCully (1991) have also shown that most first-order branch roots lost their tip and meristem and have their tissues mature to the broken ends. But, they left unclear whether the xylem conduits are open to the end of the branches and, if so, how the roots stop air bubbles and other foreign materials (soil particles, microorganisms, etc.) from entering and plugging the xylem.

To date, almost all available information of plant root anatomy is, for herbaceous roots, and mainly for the primary roots of young, laboratory-grown seedlings. Even this work does not cover the regions beyond several millimetres to a few centimetres proximal to the growing root tip. Nor does it give any clue to the heterogeneity of the structure of the whole root system. Only field-grown roots can represent the root structure in nature, and the information from laboratory-grown plants may be misleading. In an early study of root systems, Pavlychenko (1937) showed that the average root length of Marquis wheat measured at emergence from the soil was 12.62 inches in a greenhouse and twice that length when grown in the field. The corresponding figures for Banner oats were 6.6 and 14 inches. Studies on field-grown maize roots also revealed many unique features which can not be seen in roots of laboratory-grown seedling roots. One such is the rhizosheath (McCully and Canny, 1988; Vermeer and McCully, 1982). This is only weakly developed

in greenhouse-grown plants (Vermeer-Macleod, 1982). Other features are, more nodal roots (Hoppe, McCully and Wenzel, 1986), and more (but mostly short) branch roots (Varney *et al.*, 1991, and Chapter 3 in this thesis). It is, therefore inadequate to apply the information from laboratory-grown seedling roots to field situations.

My study of the first-order branch roots in field-grown corn includes: (1), the relative water content of different regions of the main and branch roots in rapidly transpiring field-grown plants. This work is based on the hypothesis that the roots which are most influenced by transpiration would have lower and fluctuating relative water content, and vice versa; (2), the xylem development in mature branch roots, and especially to find to what extent their xylem vessels are open for conduction; (3), seeking any features of the root surface which might be related to the role of branches in water and nutrient uptake; and (4) the development of barriers in the apoplast of the root cortex (i.e., suberized lamellae and Casparian strips in the cell walls of the hypodermis and endodermis).

REFERENCES

- Abeysekera RM and McCully ME. 1993a.** The epidermal surface of the maize root tip I. Development in normal roots. *New Phytologist* **125**: 413-429.

- Brewster JL and Tinker PBH. 1972.** Nutrient flow rates into roots. *Soils and Fertilizers* **35**: 355-359.
- Cahn MD, Zobel RW and Bouldin DR. 1989.** Relationship between root elongation rate and diameter and duration of growth of lateral roots of maize. *Plant and Soil* **119**: 271-279.
- Clarkson DT, Robards AW, Sanderson J and Peterson CA. 1978.** Permeability studies on epidermal-hypodermal sleeves isolated from roots of *Allium* (onion). *Canadian Journal of Botany* **56**: 1526-1532.
- Clarkson DT, Robards AW, Stephens JE and Stark M. 1987.** Suberin lamellae in the hypodermis of maize (*Zea mays*) roots; development and factors affecting the permeability of hypodermal layers. *Plant, Cell and Environment* **10**: 83-93.
- Dittmer HJ. 1937.** A quantitative study of the roots and root hairs of a winter rye plant (*Secale cereale*). *American Journal of Botany* **25**: 654-657.
- Dittmer HJ. 1938.** A quantitative study of the subterranean members of three field grasses. *American Journal of Botany* **24**: 417-420.
- Enstone DE and Peterson CA. 1992.** The apoplastic permeability of root apices. *Canadian Journal of Botany* **70**: 1502-1512.

Epstein E. 1973. Roots. *Scientific American* **228**: 48-58.

Esau K. 1965. *Plant Anatomy*. Second ed. John Wiley and Sons, Inc., New York/London/Sydney.

Fahn A. 1982. *Plant Anatomy*. Third ed. Pergamon Press, Oxford, New York, Toronto, Sydney, Paris, Frankfurt.

Fusseder A. 1987. The longevity and activity of the primary root of maize. *Plant and Soil* **101**: 257-265.

Gillespie AR. 1989. Modelling nutrient flux and interspecies root competition in agroforestry interplantings. *Agroforestry Systems* **8**: 257-265.

Hanks PJ. 1983. Yield and water-use relationships: an overview. In *Limitations to Efficient Water Use in Crop Production*. ASA-CSSA-SSSA, 677 South Segoe Road, Madison, Wisconsin, USA.

Hoppe D, McCully ME, Wenzel CL. 1986. The nodal roots of *Zea*: their development in relation to structural features of the stem. *Canadian Journal of Botany* **64**: 2524-2537.

Kroemer K. 1903. Wurzelhaut, hypodermis and endodermis der Angiospermenwurzel.
Bibliotheca Botanica **12**: 1-159.

Maertens C. 1971. Étude expérimentale de l'alimentation minérale et hydrique du Maïs.
Capacité d'absorption des parties basales et apicales de racines de *Zea mais*.
Comptes Rendues del' Academie des Sciences (Paris) **273**: 730-732.

McCully ME. 1987. Selected aspects of the structure and development of field-grown roots with special reference to maize. In *Root Development and Function*, ed. Gregory PJ, Lake JV and Rose DA, pp.53-70. Cambridge University Press, Cambridge.

McCully ME and Canny MJ. 1988. Pathways and processes of water and nutrient movement in roots. *Plant and Soil* **111**: 159-170.

Miller DM. 1981. Studies of root function in *Zea mays*. II. Dimensions of the root system. *Canadian Journal of Botany* **59**: 811-818.

Miller EC. 1938. *Plant Physiology* Second Ed. McGraw-Hill, New York.

Pallant E, Holmgren RA, Schuler GE, McCracken KL and Drbal B. 1993.
Using a fine root extraction device to quantify small diameter corn roots (≥ 0.025 mm) in field soils. *Plant and Soil* **153**: 273-279.

Pavlychenko TK. 1937. Quantitative study of the entire root systems of weed and crop plants under field conditions. *Ecology* **18**: 62-79.

Perumalla CJ and Peterson CA. 1986. Deposition of Casparian bands and suberin lamellae in the exodermis and endodermis of young corn and onion roots. *Canadian Journal of Botany* **64**: 1873-1878.

Perumalla C, Peterson CA, and Enstone DE. 1990. A survey of angiosperm species to detect hypodermal Casparian bands. I. Roots with a uniseriate hypodermis and epidermis. *Botanical Journal of the Linnean Society* **103**: 93-112.

Peterson CA. 1987. The exodermal Casparian band of onion roots blocks the apoplastic movement of sulphate ions. *Journal of Experimental Botany* **38**: 2068-2081.

Peterson CA, Emanuel ME and Wilson C. 1982. Identification of a Casparian band in the hypodermis of onion and corn roots. *Canadian Journal of Botany* **60**: 1529-1535.

Peterson CA and Lefcourt BEM. 1990. Development of endodermal Casparian bands and xylem in lateral roots of broad bean. *Canadian Journal of Botany* **68**: 2729-2735.

- Peterson CA, Murrmann M and E Steudle. 1993.** Location of the major barriers to water and ion movement in young roots of *Zea mays* L. *Planta* **190**: 127-136.
- Peterson CA and Perumalla CJ. 1990.** A survey of angiosperm species to detect hypodermal Casparian bands. II. Roots with a multiseriate hypodermis or epidermis. *Botanical Journal of the Linnean Society* **103**: 113-125.
- Peterson CA, Peterson RL and Robards AW. 1978.** A correlated histochemical and ultrastructural study of the epidermis and hypodermis of onion roots. *Protoplasma* **96**: 1-21.
- Salisbury FB and Ross CW. 1985.** *Plant Physiology*. Third Ed. Wadsworth Publishing Company, Belmont, California.
- St. Aubin G, Canny MJ and McCully ME. 1986.** Living vessel elements in the late metaxylem of maize roots. *Annals of Botany* **58**: 577-586.
- Van Fleet DS. 1961.** Histochemistry and function of the endodermis. *The Botanical Review* **27**: 165-220.
- Varney GT and Canny MJ. 1993.** Rates of water uptake into the mature root system of maize plants. *New Phytologist* **123**: 775-786.

- Varney GT, Canny MJ, Wang XL and McCully ME. 1991.** The branch roots of maize. I. First-order branches, their number, sizes and divisions into classes. *Annals of Botany* **67**: 357-364.
- Varney GT and ME McCully. 1991.** The branch roots of *Zea*. II. Developmental loss of the apical meristem in field-grown roots. *New Phytologist* **118**: 535-546.
- Vermeer-Macleod J. 1982.** *Histochemical, histological and structural study of field-grown corn roots*. MSc. Thesis, Carleton University, Ottawa, Canada.
- Vermeer J and McCully ME. 1982.** The rhizosphere in *Zea*: new insight into its structure and development. *Planta* **156**: 45-61.
- Wang XL, Canny MJ and McCully ME. 1991.** The water status of the roots of soil-grown maize in relation to the maturity of their xylem. *Physiologia Plantarum* **82**: 157-162.
- Weaver JE. 1919.** *The Ecological Relations of Roots*. Carnegie Institution of Washington, Washington.
- Weaver JE. 1926.** *Root Development of Field Crops*. McGraw-Hill Book Company, Inc., New York.
- Wenzel CL, McCully ME and Canny MJ. 1989.** Development of water conducting capacity in the root systems of young plants of corn and some other C-4 grasses. *Plant Physiology* **89**: 1094-1101.

Wilson AJ and Robards AW. 1978. The ultrastructural development of mechanically impeded barley roots. Effects on the endodermis and pericycle. *Protoplasma* **95**: 225-265.

Wilson CA and Peterson CA. 1983. Chemical composition of the epidermal, hypodermal, endodermal and intervening cortical cell walls of various plant roots. *Annals of Botany* **51**: 759-769.

CHAPTER 2.

THE WATER STATUS OF THE ROOTS OF SOIL-GROWN MAIZE IN RELATION TO THE MATURITY OF THEIR XYLEM¹

1. This chapter is in large part the subject of a published paper (Wang, Canny and McCully, 1991)

INTRODUCTION

The two types of root region found on field-grown maize (and indeed on the grasses in general), sheathed roots with strong, persistent soil sheaths, and bare roots with no closely adhering soil, are distinguished by a constellation of properties, anatomical, physiological and microbiological (McCully and Canny, 1988). The fundamental difference from which most of the other differences arise is the closed immature late metaxylem (LMX) of the sheathed roots, and it is at the point in the development of the root at which the LMX opens to form vessels that the soil sheath is lost and the root becomes bare (St. Aubin, Canny and McCully, 1986). The sheathed zone of the roots is thus connected to the negative water potential of the leaves (ψ_{leaf}) only through the narrow early metaxylem (EMX). Then, with the opening of the LMX, the bare zone becomes connected to ψ_{leaf} by a low-resistance channel, the wide vessels of the LMX. The drying effect of this far-negative water potential produces the next most basic difference between the two zones which is consistently observed in transpiring plants in the field, that the sheathed zones are wet (turgid and easily snapped, juicy when squashed), and the bare zones are relatively dry, stringy and flexible. Other differences between them, like the presence of mucigel in the rhizosphere (Vermeer and McCully, 1982; Guinel and McCully, 1986) and the differences in the associated microflora (Gochnauer, McCully and Labbé, 1989), derive directly from the differences in water content. This chapter explores the hypothesis that bare roots should be more responsive to transpiration than sheathed roots. It reports the quantification of the differences in water status between sheathed and bare root zones in terms of Relative Water Content (RWC).

RWC, the proportion of water in a particular piece of tissue compared with the maximum it contains when equilibrated with water, depends on the balance of water fluxes before sampling. Thus, the RWC of a root sample is the integrated result of the water fluxes that prevailed in the period just prior to its collection, fluxes which were determined by all the gradients of ψ and the resistances across which these gradients acted. It combines the effects of the supply of water from the soil, the evaporative loss from the shoot, and the resistances to flow from the root surface to the leaves. It is the last of these factors which is different between sheathed and bare roots, and which might be expected to be manifested as a difference in RWC. Since the RWC of a sample changes with any loss or gain of water, precautions must be taken to minimize such exchanges between collecting a sample and measuring it. Further, since the comparison of RWC between different tissues may be complicated by inherent differences in wall imbibition and elasticity (see Kramer, 1983), it is important to establish that the bare and sheathed roots have the same RWC when they are held at the same water potential, if possible over a range of potentials. I have therefore measured the water potentials of tissues of different RWC for both sheathed and bare roots, and for the branches on bare roots.

MATERIALS AND METHODS

Plants of *Zea mays* L. cv. Seneca Chief were grown, either in pots in the Environmental Laboratories, Carleton University with regular water and fertilizing and with supplemental illumination ($200-400 \mu\text{mol m}^{-2} \text{s}^{-1}$) from Optimarc High Intensity Discharge lights (Duro-Test Corp., 2321 Kennedy Blvd., North Bergen, NJ 07047), or in field plots at the Experimental Farm of Agriculture Canada, Ottawa, in sandy loam soil with no added fertilizer, and natural rainfall. As described by Hoppe, McCully and Wenzel (1986), the roots are produced successively from stem nodes up to node 7. Roots were harvested from

plants aged 50 to 78 days. Roots from the early nodes (1-4) were by then bare, while those from later nodes (5-7) were still sheathed for most of their length. It is difficult to recover from the ground complete single roots whose distal portion is immature and sheathed and whose proximal portion is mature and bare, so in the absence of such desirable samples, comparisons were made between sheathed and bare regions of roots from the different nodes of the same plant and between plants. The numbers of plants from which the root samples were taken are listed in the tables and ranged from 4 to 17 per sample.

The RWC measured was

$$\text{RWC} = \frac{\text{fresh weight} - \text{dry weight}}{\text{turgid weight} - \text{dry weight}} \times 100 \%$$

This was determined as follows: plants were detopped to stop transpiration. Root pieces about 10 to 15 cm long were quickly cut from freshly excavated axile roots, and each was placed immediately in a tared small vial and sealed. The sheathed root pieces had much soil adhering to them, the bare root pieces only a few grains. Branch roots were collected as follows: as soon as it was cut off, each piece of axile bare root was covered with the surrounding soil to prevent water loss and divided into 3 segments. Each segment in turn was uncovered, and all its first-order branches were cut off quickly and placed in a tared vial. Soil samples were collected in other vials to determine the relation between fresh weight and dry weight of the soil. The vials were weighed upon their return to the laboratory (1/2 h), giving values for each root piece of (fresh weight + soil) = A. The vials with the root pieces were filled with water and equilibrated for 3/4 h. Measurements of weight change after different periods of equilibration showed that the uptake of water had

slowed to a very low rate after this time. If left soaking for longer times, the pieces slowly increased in weight due to the infiltration of air spaces by water. Root pieces were brushed clean of sheaths and soil while still in the vials and water, removed to clean, tared vials and re-weighed to give the turgid weight (B) of the root. These vials and samples were placed unsealed in an oven with circulating air at 70°C and dried for at least 8-12 h. Drying curves showed that no further weight loss occurred after 8 h. After drying, the vials were cooled in a desiccator and re-weighed to determine the dry weight (C). Meanwhile, the soil fresh weight (D) was determined. The vials containing the water and soil were drained by gently sucking the water away from the soil particles with a fine hypodermic needle. These vials were then dried in the same oven as the roots, and the dry weight of soil associated with each root sample was determined. This was converted to a soil fresh weight from a calibration curve of soil fresh weight vs. soil dry weight, prepared from the collected soil samples. RWC was calculated for each sample by:

$$\begin{aligned} \text{RWC} &= \frac{\text{FW}(\text{root}+\text{soil}) - \text{FW}(\text{soil}) - \text{DW}(\text{root})}{\text{turgid weight}(\text{root}) - \text{DW}(\text{root})} \times 100 \quad \% \\ &= (A - D - C) / (B - C) \times 100 \quad \% \end{aligned}$$

The relation between RWC and Ψ was explored for the 3 root types by psychrometric measurements. Roots were collected from 27 pot-grown, 3-month-old plants. The large number was necessary because few sheathed roots were found. Six sets of samples were measured for each root type. Each set consisted of 8 samples, and each sample consisted of 15 to 20 cm of root cut into 1- to 3-cm pieces. All root pieces were initially equilibrated in distilled water for 3/4 h (the same period as the RWC samples) to reach saturation. Progress curves (data not shown) confirmed that Ψ had reached a stable value at this time, and did not become more positive even after 3 h soaking. A set of 8 samples was set to dry in the laboratory, and the samples were collected successively

during a period of up to 1 h, to provide a series of decreasing RWC. After its drying period, each sample was sealed in a tared Eppendorf test tube and weighed. Its water potential was then determined psychrometrically (Decagon SC-10A and NT-3 nanovoltmeter, Decagon Devices, Inc., P.O. Box 835, Pullman, WA 99163; KCl solution calibrations). After this measurement, RWC of each sample was determined as above.

RESULTS

Comparisons of water status between different tissues using RWC need to be justified by showing that the tissues are not inherently different in their capacity to hold water and respond to changes in water potential. As a test of this, RWCs of bare and sheathed root regions were compared under conditions of no transpiration and plentiful water supply. Four separate experiments were carried out with pot-grown large plants. To eliminate transpiration their shoots were cut off late in the day, and the pots liberally watered. At 0900 next morning the root systems were sampled and the RWC of bare and sheathed roots determined (Table 1). Both kinds of root attained RWCs of 80 to 90%, and there was a small but consistent difference in RWC between the 2 kinds of root. Bare roots had always 3 to 5% lower values. An inherent difference between the 2 types of root under these conditions was therefore detectable, but small.

This relationship was further tested over the range of RWCs by measurements of Ψ vs. RWC for samples of sheathed and bare roots and of the branches of the bare roots. The results are shown in Fig. 1. Analysis of covariance shows that all sets of root samples measured were indistinguishable: there was no evidence that the regression lines were not members of the same population ($F = 2.6$ and $F_{0.05, 2, \infty} = 3$). The regression line for the 144 pooled measurements intersected the (RWC=100)-axis at -0.25 MPa, and its slope

was 130% RWC per MPa. Sheathed root samples predominated in the high RWC region, since their larger volumes dried more slowly. Branch roots predominated in the low RWC region for the opposite reason.

Comparisons of RWC in the roots of transpiring plants were not satisfactory with the pot-grown plants. Though the differences between sheathed and bare roots were enhanced, the reserves of water in the soil around the crowded root mass were depleted locally and unevenly and the measured RWC values, especially those of bare roots, had large variances. The trials were therefore carried out with field-grown plants, where the reserves of soil water were spread through much larger volumes.

The effects of transpiration on the RWC of roots of field-grown plants are shown in Table 2. When root samples were collected early in the day, but after 2 to 3 h of transpiration, the values of RWC of sheathed roots were around 85% (Table 2), slightly less than their maximum of 88% (Table 1). At the same time the RWC of bare roots was much lower, just over 60%. These values were not influenced by the developmental stage of the plants from early maturity to cob-filling, nor by time from the last rainfall, nor by the depth from which the samples were collected in the soil.

The drying effect of several more hours of transpiration (sampled at 1500 h) was manifested in the root system by a further decrease in the RWC of bare roots to about 55%. The sheathed roots, however, maintained a RWC not significantly different from the early morning value (Table 2).

The comparison between the axile bare roots and their first-order branches is shown in Table 3. While the parent axes had the same RWC as was found for bare roots in Table 2, the branches they bore had very significantly lower RWCs, just over 50%. They were

thus the driest part of the root system so far found. The results were the same whatever node the parent axis came from.

DISCUSSION

Published measurements of root RWC are limited to whole root systems of a few tree species (Kandiko, Timmis and Worrall, 1980; Parker and Pallardy, 1985), and published measurements of Ψ_{root} are few (Slavíková, 1963a,b, 1967; Fiscus, 1972; Nnyamah and Black, 1977; Sharp and Davis, 1979; Blizzard and Boyer, 1980; Adeoye and Rawlins, 1981; Westgate and Boyer, 1985; Baker and van Bavel, 1986; Oosterhuis, 1987). The lines of Fig. 1 relating tissue RWC to Ψ , so common in the literature on leaves (see references in Barrs, 1968), appear to be a unique determination for roots. The curves of Ψ vs RWC in Kandiko *et al.*, (1980) and in Parker and Pallardy (1985) are for whole root systems of young trees, derived from pressure bomb measurements of balance pressure vs volume of expelled sap. They are thus averaged over a heterogeneous mixture of tissue ages and types. The principal relevance of the Ψ measurements to the current study is to validate the comparisons of RWC between the 3 types of root.

The data of Fig. 1 show that the differences observed in RWC among the 3 root types are not due to inherent differences in the responses of the tissues to changing balances of Ψ . The measured differences in RWC are real differences due to different balances of water fluxes through them. The observation that all the root types have $\Psi = -0.25$ MPa at 100% RWC does not affect the evidence that the equations of the lines are indistinguishable. It is not due to incomplete saturation of the tissue during soaking in water. Fine branches, which would equilibrate quickly, show the same intercept as thick axile roots. Progress curves of Ψ vs time of soaking showed no further increase after the

3/4 h. A similar negative water potential of fully hydrated tissues has been observed in roots by Parker and Pallardy (1985) and with leaf samples, and the cause is mysterious. For example, Yang and de Jong (1968) found that wheat leaf pieces at 100% RWC (after 4h floating on distilled water) had Ψ values of -1.0 to -1.3 MPa.

In addition to their validity value, the lines of Fig. 1 show other points of interest. The roots respond much more sensitively than leaves to negative Ψ values. Published curves of RWC vs Ψ_{leaf} commonly show a decrease of 5 to 10% RWC for a Ψ depression of -1 MPa (eg. Weatherley and Slatyer, 1957; Macklon and Weatherley, 1965; Knipling, 1967; Zur, Boote & Jones, 1981). But the slope of the line in Fig. 1 is 10 to 20 times greater. The sensitivity of RWC to changing Ψ may be the reason that the roots in wet soil had less than 100% RWC (Table 1). Small matric and osmotic potentials (ca -0.3 MPa) would be sufficient to account for this depression. The low values of RWC measured in bare roots and their branches (50 to 60%) would be fatal in leaves and many other tissues. Oppenheimer and Leshem (1966) measured the critical levels of dehydration of *Nerium* leaves by their failure to rehydrate fully after drying to various RWCs. Initial damage was found at RWCs of 75 to 70%, and increasing death of inner tissues at RWC below 70%. However, there is good evidence that root tissues are remarkably resilient to water loss. Nir, Klein & Poljakoff-Mayber (1969) found that 4-mm tips of maize roots could rehydrate and continue to grow after losing as much as 60 to 70% of their initial fresh weight for 17 h. They were killed only if the loss of water exceeded 70% of the fresh weight. In spite of this resilience to water loss, the steep slope of the line in Fig. 1 indicates that quite moderate values of Ψ_{soil} (around -0.9 MPa) may cause extensive root death. The generality of a steep slope for RWC vs Ψ curves for roots of many species is suggested by the fact that the measurements of Ψ_{root} listed in the papers at the beginning of the Discussion are in the range of 0 to -1 MPa.

The measurements confirm, with striking consistency, the qualitative impression that sheathed roots are wet and bare roots are dry. It is now apparent that sheathed roots are constantly wet at about the same level (80+%) irrespective of the extent of transpiration from the shoot. With abundant soil moisture and no transpiration bare roots have nearly the same RWC as the sheathed ones (Table 1). In contrast, when the plants are transpiring and drying the soil, the bare roots have a much lower RWC (60+%), and respond to progressive transpiration by becoming still drier (RWC 55%). The hypothesis that bare root RWC should be sensitive to the degree of transpiration because these roots are connected to the leaves by a low resistance axial path is supported. The further hypothesis that sheathed roots should be less responsive because the connection to them has a much higher axial resistance is also supported. No change in RWC was detected in sheathed roots during the day's transpiration. In its sensitivity of measurement and as a predictor of viability on re-wetting, RWC seems a more useful measure of the water status of roots than Ψ_{root} .

I thus find 2 zones of the root system, one constantly wet and insensitive to both soil moisture and progress of transpiration, the other sensitive to both. It is tempting to propose that the sensitive zone is sensitive because it depletes the soil around it of water, while the insensitive zone does not. A faster drying of the soil around the older, shallower parts of 9-day-old radish roots was measured with CAT scanning by Hainsworth and Aylmore (1986, 1989). They attribute this to the greater surface area of the tops of the roots, but it could also be a consequence of the presence there of large mature open vessels. In 10-day-old soybeans these vessels are found only in the top few centimetres of roots 20 to 30 cm long (Kevekordes, McCully & Canny, 1988). Correspondingly, there would be 2 zones of rhizosphere soil, a wet one around the sheathed roots and a dry one around the bare roots. In accord with this expectation Wullstein, Bruening & Bollen (1979) recorded

that the soil in the rhizosheath of xeric grasses had a higher moisture content (12 to ²⁵25%) than the nearby sand (3 to 8%).

It seems unproductive to speculate at this stage about what gradients of Ψ , and what radial resistances, differ between the root types. The fact that RWC in the branches is less than RWC in the bare roots they come from, does not mean that water flows from the axes to the branches. Presumably in the functioning roots Ψ in the xylem of the axes is more negative than in the xylem of their branches. But the effects of the gradients of Ψ acting across the resistances of endodermis, cortex, hypodermis and soil surface produced the measured RWCs. The details of these gradients and resistances require experimental elucidation. Shrinkage of the roots away from the soil under transpiration stress may contribute to increasing resistance at the root surface (Passioura, 1988). From the present results it is clear that negligible shrinkage would be expected for sheathed roots, and substantial shrinkage for bare roots, but the present data point the way to further experiments rather than provide answers.

The question of where water enters the root system of maize has been re-opened ever since the finding that the main xylem conduits of the sheathed roots are closed. With only early metaxylem vessels open, the negative water potential of the leaves will be rapidly attenuated in the sheathed zone, and longitudinal flow there must be relatively small (McCully and Canny, 1988). The part of the root with high axial conductance is the bare zone, and it is logical to enquire whether much water may not enter there. The suggestion that the older, more mature parts of roots take up water faster than the younger, apical regions is not new. Maertens (1971), working with 2.5-month-old maize plants, found a 6-fold greater uptake per unit length of root in the basal region, compared with the apical region. The axis of bare roots is heavily lignified and has lost its epidermis and root hairs (M.E. McCully and J. Vermeer, unpublished data), and seems an unlikely absorbing zone.

But it bears the majority of the branches (Varney *et al.*, 1991), which pierce right through the hypodermis, cortex and endodermis and connect in a complex manner directly with the large LMX elements (McCully and Canny, 1988; McCully and Mallett, 1993). The possibility that the branches may in fact be the main entry points for water has been stressed several times (McCully and Canny, 1988; Wenzel, McCully and Canny, 1989; Varney *et al.*, 1991). The measurements of the lowest RWC in the branches can be regarded as giving some support to this hypothesis.

REFERENCES

- Adeoye KB & Rawlins SL. 1981.** A split-root technique for measuring root water potential. *Plant Physiology* **68**: 44-4.
- Baker JM & van Bavel CHM. 1986.** Resistance of plant roots to water loss. *Agronomy Journal* **78**: 641-644.
- Barrs HD. 1968.** Determination of water deficits in plant tissues. - In *Water Deficits and Plant Growth* (TT Koslowski ed.) Vol 1, pp. 235-268. Academic Press, New York, NY.
- Blizzard WE & Boyer JS. 1980.** Comparative resistance of the soil and plant to water transport. *Plant Physiology* **66**: 809-814.
- Fiscus EL. 1972.** *In situ* measurement of root water potential. *Plant Physiology* **50**: 191-193.

- Gochnauer MB, McCully ME & Labbé H. 1989.** Different populations of bacteria associated with sheathed and bare regions of roots of field-grown maize. *Plant and Soil* **114**: 107-120.
- Guinel FC & McCully ME. 1986.** Some water-related physiological properties of maize root cap mucilage. *Plant, Cell and Environment* **9**: 657-666.
- Hainsworth JM & Aylmore LAG. 1986.** Water extraction by single plant roots. *Soil Science Society of America Journal* **50**: 841-848.
- Hainsworth JM & Aylmore LAG. 1989.** Non-uniform soil water extraction by plant roots. *Plant and Soil* **113**: 121-124.
- Hoppe D, McCully ME & Wenzel CL. 1986.** The nodal roots of *Zea* : their development in relation to structural features of the stem. *Canadian Journal of Botany* **64**: 2524-2527.
- Kandiko RA, Timmis R & Worrall J. 1980.** Pressure volume curves of shoots and roots of normal and drought conditioned western hemlock seedlings. *Canadian Journal of Forest Research* **10**: 10-16.
- Kevekordes K, McCully ME & Canny MJ. 1988.** Late maturation of large metaxylem vessels in soybean roots: significance for water and nutrient supply to the shoot. *Annals of Botany* **62**: 105-117.

- Knipling EB. 1967.** Effect of leaf aging on water deficit-water potential relationship of dogwood leaves growing in two environments. *Physiologia Plantarum* **20**: 656-72.
- Kramer PJ. 1983.** *Water Relations of Plants*. Academic Press, New York, NY. pp. 377-379. ISBN 0-12-425040-8.
- Macklon AES & Weatherley PE. 1965.** A vapour pressure instrument for the measurement of leaf and soil water potential. *Journal of Experimental Botany* **16**: 261-270.
- Maertens C. 1971.** Étude expérimentale de l'alimentation minérale et hydrique du Maïs. Capacité d'absorption des parties basales et apicales de racines de *Zea mais*. *Comptes Rendues del' Academie des Sciences (Paris)* **273**: 730-732.
- McCully ME & Canny MJ. 1988.** Pathways and processes of water and nutrient movement in roots *Plant and Soil* **111**: 159-170.
- McCully ME & Massett JE. 1993.** The branch roots of *Zea*: 3. Vascular connections and bridges for nutrient recycling. *Annals of Botany* **71**: 327-341.
- Nir I, Klein S & Poljakoff-Mayber A. 1969.** Effects of moisture stress on sub-microscopic structure of maize roots. *Australian Journal of Biological Sciences* **22**: 17-33.
- Nnyamah JU & Black TA. 1977.** Field performance of the dew-point hygrometer in studies of soil-root water relations. *Geoderma Journal of Soil Science* **57**: 437-444.

- Oosterhuis DM. 1987.** A technique to measure the components of root water potential using screen-caged thermocouple psychrometers. *Plant and Soil* **103**: 285-288.
- Oppenheimer HR & Leshem B. 1966.** Critical thresholds of dehydration in leaves of *Nerium oleander* L. *Protoplasma* **61**: 302-321.
- Parker WC & Pallardy SG. 1985.** Genotypic variation in tissue water relations of leaves and roots of black walnut (*Juglans nigra*) seedlings. *Physiologia Plantarum* **64**: 105-110.
- Passioura JB. 1988.** Water transport in and to roots. *Annual Review of Plant Physiology and Plant Molecular Biology* **39**: 245-265.
- Sharp RE & Davis WJ. 1979.** Solute regulation and growth by roots and shoots of water-stressed maize plants. *Planta* **147**: 43-49.
- Slavíková J. 1963a.** A critical evaluation of the determination of the root suction force. *Acta Universitatis Carolinae-Biologica* **1963**: 26-41.
- Slavíková J. 1963b.** Eine ökologische Methode zur Wurzelsaugkraftmessung. *Preslia* **35**: 241-242.
- Slavíková J. 1967.** Compensation of root suction force within a single root system. *Biologica Plantarum* **9**: 20-27.
- St. Aubin G, Canny MJ & McCully ME. 1986.** Living vessel elements in the late metaxylem of sheathed maize roots. *Annals of Botany* **58**: 577-588.

- Varney GT, Canny MJ, Wang XL and McCully ME. 1991.** The branch roots of *Zea*
I. First order branches, their number, sizes and division into classes. *Annals of Botany*
67: 357-364.
- Vermeer J & McCully ME. 1982.** The rhizosphere of *Zea*: new insight into its
structure and development. *Planta* **156**: 45-61.
- Weatherley PE & Slatyer RO. 1957.** Relationship between relative turgidity and
diffusion pressure deficit in leaves. *Nature* **179**: 1085-1086.
- Wenzel CL, McCully ME & Canny MJ. 1989.** Development of water conducting
capacity in the root systems of young plants of corn and some other C-4 grasses.
Plant Physiology **89**: 1094-1101.
- Westgate ME & Boyer JS. 1985.** Osmotic adjustment and the inhibition of leaf,
root, stem and silk growth at low water potentials. *Planta* **164**: 540-549.
- Wullstein LH, Bruening ML & Bollen WB. 1979.** Nitrogen fixation associated
with sand grain root sheath (rhizosheath) of certain xeric grasses. *Physiologia*
Plantarum **46**: 1-4.
- Yang SJ & de Jong E. 1968.** Measurements on internal water stress in wheat plants.
Canadian Journal of Plant Science **48**: 89-95.
- Zur G, Boote KJ & Jones JW. 1981.** Changes in internal water relations and
osmotic properties of leaves in maturing soybean plants. *Journal of Experimental*
Botany **32**: 1181-1191.

Table 1. Relative water contents of bare and sheathed roots from 4 pot-grown maize plants of high water status. On 4 separate days shoots were excised at evening, and the pots watered. Roots were sampled the following morning. Means $\pm$ SD (N = number of root pieces).

<u>Root Zone</u>	<u>RWC(%)</u>
Sheathed	88 $\pm$ 5 (30)
Bare	84 $\pm$ 7 (30)

Table 2. RWC of sheathed and bare zones of roots from field-grown maize plants at different ages (measured at 0900 h) and different times of day. Numbers of plants in square brackets. %, mean $\pm$ SD (N). Means with different superscripts are significantly different at $P < 0.001$.

<u>Stage</u>	<u>Sheathed</u>	<u>Bare</u>
9-10 leaves 52 days [6]	82 ± 6 (17) ^a	60 ± 5 (17) ^b
Tasseling 65 days [5]	81 ± 7 (27) ^a	66 ± 8 (12) ^b
Cob-filling 78 days [13]	86 ± 4 (15) ^a	62 ± 7 (16) ^b
<u>Time of Day</u>		
Morning 0800 h [9]	86 ± 3 (29) ^a	62 ± 6 (29) ^b
Afternoon 1500 h [8]	81 ± 7 (25) ^a	54 ± 5 (22) ^c

Table 3. RWC of the axes and first-order branches of bare roots from different nodes of field-grown maize plants measured at 0800 h. Plants aged 81 days; 17 plants for axes, 11 plants for branches. %, mean $\pm$ SD (N). Means with different superscripts are significantly different at $P < 0.0001$.

<u>Node</u>	<u>Axis</u>	<u>Branches</u>
2	62 ± 4 (11) ^a	52 ± 9 (11) ^b
3	63 ± 4 (11) ^a	51 ± 6 (11) ^b
4	63 ± 5 (10) ^a	51 ± 2 (11) ^b
5	63 ± 3 (10) ^a	49 ± 4 (11) ^b
6	-	50 ± 2 (11) ^b
Pooled means	63 ± 4 (42) ^a	51 ± 5 (55) ^b

Figure 1

Fig. 1. Plot of measured relative water content (RWC, %) against water potential (Ψ , MPa) for sheathed roots (○), bare roots (●) , and branch roots (x). Analysis of covariance shows that there is no reason to reject the null hypothesis, that all roots are samples from the same population. The regression line of the pooled samples is $RWC = 134 + 134\Psi$ (Ψ in MPa).

CHAPTER 3.
THE MATURATION AND OPENNESS OF XYLEM
CONDUITS IN FIRST-ORDER BRANCHES OF FIELD-
GROWN ROOTS ¹

1. This chapter in large part is the subject of a paper now in press (Wang, McCully and Canny, 1994).

INTRODUCTION

The discovery that the main water-conducting elements of maize axile roots are not mature and open to carry water in the zone that is strongly sheathed in soil, 20-30 cm back from the tip (St. Aubin, Canny and McCully, 1986), has prompted the suggestion that branch roots should be evaluated as sites of water uptake (McCully and Canny, 1988; Wenzel, McCully and Canny, 1989). The frequency, diameters and lengths of the first-order branches, and the diameters of their xylem elements, have been detailed by Varney *et al.* (1991). In a study of the water status of the root system, Wang, Canny and McCully (1991) have shown that the branches are the most rapidly responsive component to drying during transpiration. Measurements of water fluxes into the root system of transpiring maize plants by Varney and Canny (1993) showed that the first-order branches collected 88% of the water, and the axile roots the remaining 12%. This difference was due, not to greater average flux rates per unit surface, but to the greater surface of branches available. They also showed that the opening of the high-conductance late metaxylem (LMX) of the axile roots occurred in the zone of the axes where the young branches began to take up water. By analogy with the main axes, the branches may also contain a proportion of immature xylem, and a knowledge of the extent and duration of a low-conductance path in their xylem becomes necessary to understanding the mechanism of water uptake.

Previous studies have shown that the first-order branches are mostly short (mode 6-10 mm) (Varney *et al.*, 1991), that they grow only for an average of 2 to 3 days (Cahn, Zobel and Bouldin, 1989), and that they soon lose their apical meristems (Fusseder, 1987; McCully, 1987; Varney and McCully, 1991). The loss of their meristem coincides with loss of the soil sheath (Varney and McCully, 1991), with opening of the LMX in the parent

root (St. Aubin *et al.*, 1986), and so also with the start of water uptake by the branches (Varney and Canny, 1993).

This chapter reports the progress of maturation of LMX elements in first-order branches of field- and pot-grown maize roots. Some important features related to water uptake and transport are also reported, viz., shortening of the branches as the distal regions die and decay, plugging of mature LMX elements with mucilage and foreign material, and the changing proportions of open and closed LMX as branches mature, age and shorten.

The accumulation of mucilages and plugging of xylem vessels have long been reported as a defense response to vascular infection in a wide variety of plant species (Beckman, 1964; Beckman *et al.*, 1961; Beckman and Zaroogian, 1969; Mollenhauer and Hopkins, 1976; Phipps and Stipes, 1976; VanderMolen, Beckman and Rodehorst, 1977). These workers concluded that these polymers originate from the disintegrating cell walls of infected xylem vessels. The mucilages accumulated and finally blocked the xylem lumen. In my study, these polymers have been observed in the xylem near the broken end of the determinate branches and at the base of the branch roots inside the branch-main root junctions. My observations suggest strongly that they are synthesized in the xylem parenchyma.

MATERIALS AND METHODS

Plant material

Plants of corn, *Zea mays* L. cv. Seneca Chief were grown from seed in 12-inch pots in the greenhouse at Carleton University, and in field plots of sandy loam soil at the

Central Experimental Farm, Agriculture Canada, Ottawa, in the summers of 1990 and 1992. Samples were collected from nodal roots from tiers 1 to 4 (terminology of Hoppe, McCully and Wenzel, 1986) from plants at the stages of tasselling and early cob-filling (29 field plants, 7 pot plants, total >500 branches). Roots were carefully excavated and washed, then examined under a dissecting microscope. Samples of first-order branches were separated into the four size classes distinguished by Varney *et al.* (1991) (from the smallest, class 1, mean diameter 214 μm . to the largest, class 4, mean diameter 675 μm). The branches were also distinguished by the stage of meristem deterioration (following Varney and McCully, 1991) as indeterminate (healthy root cap and meristem), or determinate (meristem partially or completely lost). The tips classified by Varney and McCully (1991) as intermediate and broken were included here as determinate.

Anatomical study of LMX maturation

Twenty-eight indeterminate branches were processed from six field-grown plants. One- to two-millimetre-long pieces were cut in sequence along each branch. The pieces from each branch were kept separate and in order, fixed in 3% glutaraldehyde in 0.025M potassium phosphate buffer at pH 6.8, dehydrated in a methyl cellosolve, ethanol, propanol, butanol series, and embedded in glycol methacrylate (GMA). Longitudinal sections (1-2 μm thick) were cut from the pieces of each series in sequence, stained sequentially with the periodic-acid-Schiff's (PAS) procedure and toluidine blue O (pH 4.4), and viewed with bright-field optics. For details of these methods see O'Brien and McCully (1981).

Determination of openness of LMX elements

1. From thin sections of embedded material

First-order branches were collected from pot-grown plants and processed as detailed for the field-grown roots. LMX files were considered to be closed and immature if intact cross walls, cytoplasm or nuclei were present. Serial sections were examined to clarify the structure of any files that had been sectioned tangentially. The percentage of the total length of open LMX relative to the total branch length was determined for each of 68 branches.

2. Particle diffusion

Aqueous India ink suspensions have been used to determine the vessel length and vessel endings in both dicotyledons and monocotyledons such as elm, alfalfa, tomato, and banana, etc. (Beckman *et al.*, 1961; Handley, 1936; Van Alfen and Allart-Turner, 1979; Wiebe, Greer and Van Alfen, 1984). In these earlier studies the India ink fluid was pulled into vessels from the cut ends of the stems by transpiration.

Tracheary elements that were dead in the fresh branches may also be distinguished from those that had living contents by following the diffusion of particles in suspension into them. Twenty-three field-grown plants contributed branch root samples for study of the openness of xylem by this method. The branch roots were each cut under water into pieces 10-15 mm long, these pieces were kept in their original sequence, and gently evacuated under water to remove pockets of air. The pieces were then quickly transferred into a suspension of 10% aqueous India ink and left for 1-2 h. (In the study in 1992, the root pieces were 5-6 mm long, and the evacuation was done after transfer to the ink. There was no significant difference in the results from these two methods.) After a brief rinse

with distilled water, transverse hand sections of the ink-soaked root pieces were cut very gently with sharp, surgical-steel razor blades, mounted directly in 1/10,000 aqueous rhodamine B, and viewed with fluorescence optics. The rhodamine fluorescence gives a counterstain that emphasizes the spaces containing the diffused ink. The point proximal from the root tip at which the LMX opens can be determined from the position along the branch of the youngest segment into which India ink diffuses through its distal end.

Eleven branches from these field-grown plants were fixed, embedded in GMA and sectioned as above, to compare the two methods of assessing the openness of the LMX.

Progression of cell death at the tips of determinate branches

To determine if other tissues at the distal end of determinate branches were alive or dead, 43 determinate branches were selected in three age groups from the distal, middle and proximal regions of field-grown roots, and fixed in 10% formalin. Some of the younger branches from the distal group were large enough to be studied directly by hand sections cut under the dissecting microscope. The rest of these fixed roots were cleared in 80% lactic acid for 3-4 weeks and viewed whole, or cut in half. Both the hand sections and cleared roots were rinsed well with water, then mounted in 0.05% aniline blue (pH 8.6 in phosphate buffer) and viewed by fluorescence microscopy. The resultant fluorescence of walls and cytoplasm revealed the general organization of cells at the tip, thus allowing assessment of whether they had been alive prior to fixation.

The tips of some determinate branches were fixed in glutaraldehyde and embedded in GMA, sectioned and stained as explained above. These were also examined for general cytoplasmic organization.

Samples for transmission electron microscopy were post-fixed in 1% buffered osmium tetroxide, dehydrated through an acetone series and embedded in Spurr's resin. Ultrathin sections were cut with either glass or diamond knives, and stained with uranyl acetate and lead citrate and viewed with the transmission electron microscope. For more details of these techniques see O'Brien and McCully (1981).

Microscopy and photomicrography

Optical microscopy was with an Olympus Vanox system with SPlan and UVFL series objectives, or with a Zeiss Axiophot system, and electron microscopy with a Philips 420 transmission electron microscope. Photomicrographs were recorded on Kodak T-Max 100 or Ektapan film and Kodak 4489 electron microscope film.

RESULTS

Numbers and sizes of LMX conduits in branch roots

These branch roots are all smaller in diameter (Table 1), and have reduced numbers of LMX elements than those in main roots studied by Hoppe *et al.* (1986). The hair-like class 1 branches (with an average diameter of 200 μm) have only several files of very small diameter EMX elements. The LMX is absent from these branches. Branch roots of classes

2 to 4 are larger in diameter (average 283, 463 and 541 μm respectively) with usually 1 to 3 LMX conduits in the centre of each root (Figs. 7 to 10, and Figs. 1a Varney *et al.* 1991 and Varney and McCully, 1991). Branch roots with more than 3 LMX conduits are rare. Analysis shows that the number of LMX conduits in each individual root depends on its diameter ($r=0.812$, $p<0.01$, $n=46$).

The LMX elements in these branch roots are surprisingly large in comparison with those of the much wider main root axes (Table 1, Figs. 1-10). Although the largest LMX elements in these branches are about 30% smaller in diameter than those of main roots (as determined by Hoppe *et al.*, 1986), they are similar in length (Fig. 1) to those of the main roots, ranging from 600 to 1930 μm . The shortest LMX elements are found in the class 2 roots and the largest in the class 4 roots, but there is overlap in the range of lengths between class 2 and class 3 and between class 3 and class 4 roots (Table 1). The diameters of LMX elements in the branches are proportional to the diameters of the roots in which they occur ($r=0.86$, $P<0.01$, $n=70$). Even though the LMX vessels occupy a considerable portion of the volume of the branch roots, the ratio of root-surface to xylem-surface is much higher than in the nodal root axes (Table 2).

These LMX files are separated from the soil by only 3-6 cell layers: a single-layered epidermis, hypodermis and the additional layer(s) (Figs. 1, 7 to 10). In contrast to these branches, the nodal roots have 8 to 20 layers of cortical cells, all with lignified walls in their mature regions (Fig. 11 in McCully and Canny, 1988 and Figs. 40-41 in Hoppe *et al.*, 1986).

Structure of immature and mature LMX elements

The early stages of LMX development in maize branch roots have been illustrated (Figs. 10-12 and 17 of Varney and McCully, 1991). The elements begin as small derivatives (ca. 10 μm in each dimension) of the apical meristem, which extend spectacularly in length and width. We found that their mean diameters at maturity ranged from 34 μm to 67 μm . The smallest diameter LMX elements were in class 2 roots, the largest in class 4 roots (Table 1, and Varney *et al.*, 1991). LMX element lengths at maturity ranged from 0.6 mm to almost 2 mm, the longest occurring in class 4 roots (Table 1). Lignification of these cells begins with cessation of elongation, but subsequent development is slow and they remain for quite long distances proximal to the tips as enormous living cells with thick lignified lateral walls, intact end walls, nuclei and sparse protoplasm (Fig. 1). The end walls have a lignified rim of pitted secondary wall and a non-lignified primary wall centre (Figs 2-4); the lignified lateral walls have deep, wide pits (Figs 2-6). The wispy cytoplasm was hard to distinguish in fixed material, and is probably inadequately preserved by the glutaraldehyde. Cytoplasm was commonly seen adjacent to the end walls (Figs 2, 3) or around the conspicuous nucleus (Fig. 5). Short lengths of the thin cytoplasmic strands that traverse the central vacuole were occasionally included in the sections (Fig. 3).

When the LMX does mature, the protoplasm and nuclei disappear, and the central portion of the end walls disintegrates, leaving the lignified rims (Fig. 6). The empty conduits thus formed provide the major axial pathways for the transpiration stream. One such conduit occurs in class 2 branches, 1 or 2 in class 3 branches, and 3 (rarely 4) in class 4 branches (Table 1, Figs. 7 - 9, and Varney *et al.*, 1991). There are no LMX elements in class 1 branches. Here, the narrower (mean diameter 8.5 μm) early metaxylem elements form the main conduits (Table 1 and Fig. 10).

Development of openness of LMX

The measurements made on branches of field-grown roots are assembled in Table 3, sorted according to their stage of development as measured by distance from the tip of the main root. The youngest, indeterminate branches, which were found only on immature regions of the axes, had a mean length of 8 cm and the LMX conduits, as assessed by diffusive access of ink particles, were closed throughout their length. The most distal of the determinate branches (these had recently lost their tips) were the longest (mean length 16 cm), and in these about a third of their length had open LMX. The middle and proximal groups of determinate branches showed a progressive shortening, mean lengths 6 and 3 cm respectively, and the proportion of open LMX increased with branch age. But even in the oldest, shortest branches only two thirds of the length of LMX was open, and their distal regions, near the decaying tip, had closed LMX elements.

The roots of plants grown in pots had indeterminate branches on more mature regions of the axes. In these branches the xylem elements were not all still alive (Table 4). As assessed by thin sections, not particle diffusion, the smallest branches (class 1) had nearly half their xylem (all early metaxylem) open, while the other three classes had one quarter to one third of their LMX open. The remainder of the LMX in these roots was still alive, with intact cross walls and cytoplasm.

Progression of cell death in determinate branches

As shown by Varney and McCully (1991), the progressive shortening of ageing determinate branches is due to cell death. In the youngest of these branches only a few dead cells are seen, with remaining epidermal, cortical and stelar cells alive to within one or two cells of the distal end of the branch. All these living cells at the tips are immature as

judged by their short lengths compared to more proximally situated cells of the same tissues (Figs. 11, 12, 14). In some roots, wide central LMX elements are still short and have only primary walls (Fig. 12). At other determinate tips, the LMX elements have elongated and developed lignified secondary lateral walls, but still have intact end walls, nuclei and cytoplasm (Fig. 13).

In older determinate branches, cell death and decay progresses basipetally beyond the region where the LMX has matured (Figs. 15-18). Here the xylem conduits often contain bacteria, fungal hyphae and, occasionally, nematodes (Fig. 17). Unidentified mucilaginous material, staining strongly metachromatic pink or dark blue with toluidine blue, fill any remaining spaces in this xylem. Occasionally these mucilages were seen blocking the xylem even in the absence of invading organisms. Production of the mucilage appeared to precede the arrival of invading microbes (Figs. 15, 16). In the youngest branches, the decay is confined to a narrow region at the distal end, and the xylem remains mature and open for most of the length of the branch. Other tissues of both the stele and the cortex behind the decaying end remain healthy (Fig. 18). In older roots, xylem invasion and decay of surrounding tissues is often seen proximally from the tip for half of the length of branches, while xylem and other tissues closer to the nodal root axis are unaffected (Fig. 15). Few branches were seen that were infected right to their base. Surprisingly, new, healthy-looking second-order branch roots were forming (Fig. 19), even in portions of these roots where the cortical cells were dead, the xylem plugged and some other cells of the stele infected. Fusseder (1987) also observed new branches on senescent first-order branches.

The mucilages in the vessels were shown to be synthesized in the xylem parenchyma. At the beginning of xylem plugging, the xylem lumen is almost free of the polymers. But the pits are filled with the polymers and some have passed to the xylem

through the floor of the pits, and line the xylem side walls (Figs. 23 & 24) Under the transmission electron microscope, these polymers appear denser near the floor of the pits, and are thinner away from the xylem side walls (Fig. 25).

At later stages of plugging, the polymers accumulate in the lumen and finally occlude it, densest at the edge. These polymers appear fibrous and include osmiophilic granules (Figs. 23-25).

With histochemical stains, in the light microscope, at the early stage of plugging, a thin layer of polymers is deposited along the xylem lateral walls and stained metachromatically pink by toluidine blue. Later, as these polymers accumulate and become thicker in the xylem lumen, they stain dark red, purple or dark blue by the same dye (Figs. 16 & 20). All these staining reactions suggest that they are negatively charged, the dark blue colour may indicate the addition of phenolic materials (Carol Wenzel, unpublished results).

Before and during the accumulation of the polymers in the xylem elements, adjacent xylem parenchyma cells showed a number of features not seen in other parenchyma cells in the stele. These xylem parenchyma cells often exhibited structures suggestive of intensive cellular synthetic activities: They had dense protoplasm and small vacuoles, large, lobed nuclei, often with prominent nucleoli. They also contain much rough endoplasmic reticulum (ER) (Figs. 21 & 26) and many mitochondria (Fig. 22), and a notable increase in the number of Golgi bodies (Figs. 21, 24 & 27). Vesicles accumulated in the cytoplasm near the xylem-facing side (Figs. 28 & 29). These vesicles varied in size and appearance. Some contained an electron-lucent matrix, others were strongly electron-dense (Figs. 28 & 29). Periplasmic mucilage deposits accumulated next to their walls adjacent to the xylem lumen. These substances never showed up in the vacuoles of the xylem parenchyma cells,

as reported in previous studies (VanderMolen, 1978, VanderMolen *et al.*, 1977). No mucilage deposits within xylem conducting elements were ever observed where the adjacent xylem parenchyma did not show these features.

DISCUSSION

In the first phases of their maturation (i.e., while still possessing a functioning apical meristem), the branch roots show many similarities to the main axes in the development of their xylem. Their LMX are not much less wide than those of the axis, and about as long. As in the axes, these elements remain immature over much of the distal region of the branch (Tables. 3, 4). When they open to become conducting vessels in the proximal region, the longitudinal hydraulic conductance of the root xylem must increase by at least three orders of magnitude, as in the axes (McCully and Canny, 1988). In all these respects the branch is a miniature version of the axis.

The branches show a number of further developments that are not normally found in the axes. Instead of growing indefinitely, they become determinate. The apex is lost, so that growth in length ceases, and the distal regions die progressively, so that the branches become shorter and shorter (Table 3). Cahn *et al.* (1989) also found that the branch roots on the basal 10 cm of the nodal roots were 40% shorter than those further from the base. Varney and McCully (1991) and the data in Table 3 also show that the branch roots on the proximal regions of the nodal roots are the shortest in the root system. The observations in my study have confirmed that there is a sequence of dying and shortening of the branch roots basipetally along the primary and nodal roots and that this shortening is a developmental phenomenon. Branches of both field- and pot-grown plants were similar in this respect except that those of the pot-grown plants retained their indeterminate state

longer, and such branches were still present on the younger mature regions of the axes. The die-back of determinate branches is not simply death and decay, but appears to be carefully controlled and programmed (Varney and McCully, 1991). Initially, the short living cells proximal to the decaying tissue which stopped their normal extension and differentiation when the meristem was lost (Figs. 11, 12 & 14) appear to seal the remaining root from invasion by microorganisms, and must also restrict leakage of materials. The normal long LMX elements, living or dead, would be particularly vulnerable to invasion, and would offer easy passage for leakage of solutes from the root, and, when dead, of air that would embolize open vessels. As progression of the die-back moves beyond these short LMX elements, the controlled process also operates to seal them by the different mechanism of plugging with polymers and/or invading microbes (Figs. 15-17). Initially, the mucilaginous contents of the distal dead xylem elements appear sterile, but, as decay progresses proximally, they are invaded by a variety of microbes. The more proximal regions of the conduits, however, remain free of microbes and apparently open for conduction. It should be noted that the formation of tyloses in vessels by neighbouring parenchyma, a process of xylem blocking commonly found in woody plants and also found in axile roots of tomato (Bishop and Cooper 1984), and zucchini (Runions, 1991), was not observed in any of the maize branch roots studied.

The structural features observed in the xylem parenchyma cells that adjoin vessels which are being plugged by mucilage are consistent with active synthesis and secretion of this material by these parenchyma cells. Definitive evidence for this origin of the plugging materials can only be obtained by a pulse-chase autoradiographic study using a suitable radioactive precursor of the mucilage (possibly fucose). However, the 'static' evidence from my electron micrographs suggests that the mucilages are secreted from xylem parenchyma cells into the periplasm of the cells, then they penetrate through the intact walls of the pit floors into the associated vessels, where they accumulate and result in plugging. Similar secretions of polymers from parenchyma cells into the xylem vessels have been

reported by plant pathologists as plant responses to microbial infections (Bishop and Cooper, 1984; Schmitt and Liese, 1990; Shi, Mueller and Beckman, 1991). These secretions and the microbes occlude the xylem vessels and disrupt the transpiration stream and result in wilting of the shoot (see Beckman and Halmos, 1962).

Because the India ink particles are unable to pass through "pit membranes" on vessel lateral walls and endings (Handley, 1936), they were separated from the water and accumulated on the downstream side of the first intact xylem vessel end wall. Similarly, India ink particles will not diffuse through an intact vessel end wall.

From my experience in this study, the use of the India ink diffusion method can be strongly recommended as a relatively quick and easy way to determine the openness of xylem conduits. The method, however, will not distinguish between a blockage that is due to the immaturity of component elements, and a secondary blockage due to microbial invasion. Thus, in organs where tissue deterioration is occurring, as in the ends of determinate branch roots, it is also necessary to examine sectioned material to distinguish between these two possibilities.

When interpreting the results of studies of xylem openness using the India ink diffusion method, it is essential to consider whether vessels are ending within the portion of the root observed. Wiebe *et al.* (1984) showed that, when a solution of India ink was transpired up shoots of alfalfa, its passage was blocked at the pit membranes at the ends of individual vessels. Thus, in the maize branch roots, if the vessels had been short (i.e., composed of a single element or only a few elements), ink diffusion would have been stopped by vessel ends even in xylem which was mature and open for water conduction. Very short vessel elements separated from adjoining elements by intact pit membranes do occur in the maize branch roots just where the xylem conduits of branch and the main axis

join, and these walls do prevent passage of small particles (McCully and Mallett, 1993). The thin sections did not, however, reveal any vessels that were shorter than the branches examined. Thus I am confident that blockage of ink diffusion indicates either plugged or living xylem elements.

There are thus two kinds of closed LMX in the maize branch roots; LMX closed because it is still alive, and LMX that was once open vessels but is again blocked with polymers, and later also microbes (in a process that is not commonly found in the healthy nodal root axes). Both kinds of closed xylem must strongly restrict longitudinal hydraulic conductance by the xylem. Young indeterminate branches have a proportion of their length occupied by closed xylem of the first kind, and older determinate branches by closed xylem of the second kind. However, both old, and all but the very young branch roots retain an open proximal zone of xylem with high longitudinal conductance. These xylem features, open conduits protected from embolism by closed distal sections, are consistent with the major role in supply of water to the transpiration stream proposed recently for branches on mature root axes (Wang, Canny and McCully, 1991; Varney and Canny, 1993).

REFERENCES

- Beckman C H. 1964.** Host responses to vascular infection. *Annual Review of Phytopathology* 2: 231-252.

- Beckman CH and Halmos S. 1962.** Relation of vascular occluding reactions in banana roots to pathogenicity of root invading fungi. *Phytopathology* **52**: 893-897.
- Beckman CH, Mace ME, Halmos S and McGahan MW. 1961.** Physical barriers associated with resistance in fusarium wilt of banana. *Phytopathology* **51**: 507-515.
- Beckman CH and Zaroogian GE. 1969.** Origin and composition of vascular gel in infected banana roots. *Phytopathology* **57**: 11-13.
- Bishop CD and Cooper RM. 1984.** Ultrastructure of vascular colonization by fungal wilt pathogens. II. Invasion of resistant cultivars. *Physiological Plant Pathology* **24**: 277-289.
- Cahn MD, Zobel RW and Bouldin DR. 1989.** Relationship between root elongation rate and diameter and duration of growth of lateral roots of maize. *Plant and Soil* **119**: 271-279.
- Fusseder A. 1987.** The longevity and activity of the primary root of maize. *Plant and Soil* **101**: 257-265.
- Handley WRC. 1936.** Some observations on the problem of vessel length determination in woody dicotyledons. *New Phytologist* **35**: 456-471.

- Hoppe D, McCully ME and Wenzel CL. 1986.** The nodal roots of *Zea*: Their development in relation to structural features of the stem. *Canadian Journal of Botany* **64**: 2524-2537.
- McCully ME. 1987.** Selected aspects of the structure and development of field-grown roots with special reference to maize. In *Root development and function*, ed. Gregory PJ, Lake JV and Rose DA. pp. 53-70. Cambridge University Press, Cambridge, England.
- McCully ME and Canny MJ. 1988.** Pathways and processes of water and nutrient movement in roots. *Plant and Soil* **111**: 159-170.
- McCully ME, Canny MJ and Steveninck RFM. 1987.** Accumulation of potassium by differentiating metaxylem elements of maize roots. *Physiologia Plantarum* **69**: 73-80.
- McCully ME and Mallett JE. 1993.** The branch roots of *Zea*. 3. Vascular connections and bridges for nutrient recycling. *Annals of Botany* **71**: 327-341.
- Mollenhauer HH and Hopkins DL. 1976.** Xylem morphology of Pierce's disease-infected grapevines with different levels of resistance. *Physiological Plant Pathology* **9**: 95-100.
- O'Brier TP and McCully ME. 1981.** *The Study of Plant Structure. Principles and Selected Methods*. Termarcaphi Pty. Ltd., Melbourne, Australia.

- Phipps PM and Stipes RJ. 1976.** Histopathology of mimosa infected with *Fusarium oxysporum* f. sp. *perniciosum*. *Phytopathology* **66**: 839-843.
- Runions J. 1991.** *A study of the main root of Curcubita pepo L. with emphasis on the development and maturation of the secondary xylem.* Honours Thesis, Carleton University, Ottawa.
- St. Aubin G, Canny MJ and McCully ME. 1986.** Living vessel elements in the late metaxylem of maize roots. *Annals of Botany* **58**: 577-586.
- Schmitt U and Liese W. 1990.** Wound reaction of the parenchyma in Betula. *IAWA Bulletin* **11**: 413-420.
- Shi J, Mueller WC and Beckman CH. 1991.** Ultrastructural responses of vessel contact cells in cotton plants resistant and susceptible to infection by *Fusarium oxysporum* f. sp. *vasinfectum*. *Physiological and Molecular Plant Pathology* **38**: 211-222.
- Van Alfen NK and Allard-Turner V. 1972.** Susceptibility of plants to vascular disruption by macromolecules. *Plant Physiology* **63**: 1072-1075.
- VanderMolen GE. 1978.** Electron microscopy of vascular obstructions in citrus roots affected with young tree decline. *Physiological Plant Pathology* **12**: 271-274.

- VanderMolen GE, Beckman CH and Rodehorst E. 1977.** Vascular gelation: a general response phenomenon following infection. *Physiological Plant Pathology* **11**: 95-100.
- Varney GT, Canny MJ, Wang XL and McCully ME. 1991.** The branch roots of *Zea*. I. First-order branches, their number, sizes and division into classes. *Annals of Botany* **67**: 357-364.
- Varney GT and McCully ME. 1991.** The branch roots of *Zea*. II. Developmental loss of the apical meristem in field-grown roots. *New Phytologist* **118**: 535-546.
- Varney GT and Canny MJ. 1993.** Rates of water uptake into the mature root system of maize plants. *New Phytologist* **123**: 775-786.
- Wang XL, Canny MJ and McCully ME. 1991.** The water status of the roots of soil-grown maize in relation to the maturity of their xylem. *Physiologia Plantarum* **82**: 157-162.
- Wang XL, McCully ME and Canny MJ. 1994.** The branch roots of *Zea*. IV. The maturation and openness of xylem conduits in first-order branches of soil-grown roots. *New Phytologist* **126**: 000-000. (in press).
- Wenzel CL, McCully ME and Canny MJ. 1989.** Development of water conducting capacity in the root systems of young plants of corn and some other C-4 grasses. *Plant Physiology* **89**: 1094-1101.

Wiebe HH, Greer RL and Van Alfen NK. 1984. Frequency and grouping of vessel endings in alfalfa (*Medicago sativa*) shoots. *New Phytologist* **97**: 583-590.

Table 1. Sizes of late metaxylem (LMX) conduits in first-order branch roots of field-grown maize as determined from thin sections of embedded material

Class	Root diameter (μm) $\pm$ S.D. (n)	Mean LMX diameter (μm) $\pm$ SD (n)	Range of length (μm)	Number of LMX files
1	205 $\pm$ 32 (10)	8.5 $\pm$ 2 (13)*	--	0
2	283 $\pm$ 49 (12)	34 $\pm$ 12 (12)	600-1290	1
3	463 $\pm$ 88 (15)	55 $\pm$ 13 (15)	850-1780	1-2
4	541 $\pm$ 123 (18)	67 $\pm$ 19 (19)	1080-1930	1-4

* Early metaxylem (EMX) diameters, the lengths of these narrow elements could not be reliably determined from sectioned material; there are no LMX conduits in these small roots.

Table 2. Comparison of features in branches with that in nodal roots

Root	Number of LMX elements	Circumference root/ total circumference LMX	Layers of cortical cell
Nodal (tier #)*			
1	9	1.5	9
3	18	0.9	9
5	30	0.8	15
7	53	0.8	20
Branch (class #)			
4	1	8.2	-
4	2	4.1	6
4	3	2	-
3	1	7.7	-
3	2	3.8	5
2	1	9.4	4-5
1	0	-	3-4

*Data for nodal roots from Hoppe *et al.*, 1986.

Table 3. Openness of LMX conduits of first-order branch roots of field-grown maize, as determined by diffusion of India ink particles

Type and position of branch along main axis	Root length cm $\pm$ SD (N)	Length of closed LMX (cm)	Open xylem % of branch
Indeterminate tips			
distal	7.8 $\pm$ 1.6 (34)	7.8 $\pm$ 1.6	0
Determinate tips			
distal	14.7 $\pm$ 8.4 (42)	9.4 $\pm$ 5.8	39 $\pm$ 9
middle	6.2 $\pm$ 1.9 (43)	2.9 $\pm$ 1.1	53 $\pm$ 7
proximal	2.8 $\pm$ 1.7 (42)	1.0 $\pm$ 1.2	61 $\pm$ 10

Table 4. Openness of xylem of indeterminate first-order branch roots*

Class	Total length of root (cm) (N)	Length of immature xylem (cm)**	Open xylem: % of total length
1	7-9 (19)	4-5	42-44
2	8-11 (20)	6-7	25-36
3	22-26 (16)	16-18	27-31
4	26-33 (13)	18-20	31-39

*Potted corn plants, 65 days old

**Determined from embedded sections

Figure 1

Fig. 1. A montage of a median longitudinal section through the mid portion of a class 3 branch. The late metaxylem (LMX) elements are fully elongated and have thick, lignified lateral walls. They are, however, still immature with living contents, enormous vacuoles and intact cross walls (arrowheads). The upper element in the file to the right ends at a cross wall beyond the top of the micrograph. The epidermal and cortical cells were all alive but, as is common in these older regions of roots, these cells distort during embedment. Few root hairs were present. A second-order branch primordium has been sectioned (upper left). Plastic-embedded section stained with toluidine blue and the periodic acid-Schiff's procedure. Bright-field optics. X70.

Figures 2-6

Figures 2 to 6. Longitudinal sections through LMX elements. Plastic-embedded sections.

Fig. 2. Part of two immature LMX elements. The thin peripheral cytoplasm is not resolved, but a thick deposit of cytoplasm lies against the intact cross wall (arrowhead). The lateral walls are thick, and lignified. The end wall consists of a narrow rim that is also thick and lignified (arrows) and a central primary wall that stains strongly metachromatic pink with toluidine blue. Nomarski optics. X300.

Fig. 3. Section similar to that in Fig. 2, but including a piece of one of the narrow transvacuolar strands of cytoplasm (arrow) that characterize these large cells. Nomarski optics. X300.

Fig. 4. Similar section to Figures 2 & 3 but showing two strands of immature LMX. The section passes in and out of two of the narrow strands of elongated, lignified parenchyma cells which separate the LMX files. X270.

Fig. 5. A similar section to that in Figures 1 to 4, but adjacent to the base of a secondary branch which itself is sectioned longitudinally (right of micrograph). A nucleus and clump of adjacent cytoplasm (arrow) has been sectioned in the immature LMX element. This section also passes through part of an adjacent LMX and tangentially through a sieve tube (S). The long, lignified parenchyma between the LMX strands are particularly clear. Toluidine blue, PAS stain. Nomarski optics. X370.

Fig. 6. This section passes through portions of adjoining mature elements in an LMX file. The central, primary portion of the end wall has disintegrated leaving the lignified rim intact (arrows). As in Figure 5, this section also passes through the base of a secondary branch (upper right). Toluidine blue, PAS stain. Bright-field optics. X220.

Figures 7-10

Fig. 7 to 10. Thick hand-cut sections from fresh pieces of first-order branches of different classes. These were immersed in a suspension of India ink which diffused into open LMX xylem conduits but not into immature or plugged elements. Sections were counterstained with rhodamine B and viewed with fluorescence optics.

Fig. 7. In this young region of a class 4 root both LMX and EMX are immature. No ink has diffused into the stele. Air spaces (arrows) in the cortex, however, are full of ink. X180.

Fig. 8. A region of a class 4 root in which both the LMX and EMX are mature. India ink has diffused along these conduits. Some EMX in the section are empty of ink, probably because of embolisms introduced into the narrow conduits during preparation. X180.

Fig. 9. A region of a class 2 root in which the single central LMX is mature and full of ink. X180.

Fig. 10. A section of a class 1 root which lacks LMX but has mature EMX into which ink has diffused. X310.

Figures 11-14

Figures 11 to 14. Handcut longitudinal sections through the distal end of formalin-fixed determinate branches that were dying back from their tips. In each root the distal end of the branch is toward the bottom of the page.

Fig. 11. The centre of the root has rotted but the young epidermal and cortical tissue which were close to the original meristem had remained alive and had not elongated to their normal final size. Aniline blue-induced fluorescence. X160.

Fig. 12. Similar section as that in Figure 11 but showing a file of unlignified LMX elements. These elements were still alive. The most distal element adjoins rotted tissue. These distal elements were much shorter than the normal mature length of LMX (cf. Fig. 1). Aniline blue-induced fluorescence. X160.

Fig. 13. Section at the tip of branch somewhat older than that of Figure 12. LMX elements had enlarged normally and were lignified, but still alive. The most distal of these elements in which the nucleus is seen lies adjacent to a dead, rotting element. Other cells in the stele were alive. Section was cleared and mounted in lactic acid. Toluidine blue staining, bright-field optics. X420.

Fig. 14. Cortical cells adjoining the remains of deteriorated tissue at the distal end of a branch. These cells appear healthy. They have, however, not achieved their normal length. Section cleared and mounted in lactic acid. Nomarski optics. X420.

Figures 15-19

Figures 15 to 19 are longitudinal sections of plastic-embedded determinate first-order branches of field-grown roots. In each case the distal end of the root is toward the bottom of the page. Toluidine blue, PAS stain.

Fig. 15. The mid region of a determinate root that was decaying progressively toward its base. Some of the distal xylem is filled with mucilage (M) and surrounding stelar cells were dying. But more proximally (toward top of micrograph) the stelar cells were healthy. Some cortical cells were alive. Bright-field optics. X200.

Fig. 16. Close to the distal decaying end of a determinate branch. The xylem conduits are filled with mucilage (M) which stains strongly metachromatic pink with toluidine blue. Microorganisms have invaded the more distal region of this root but were not apparent in this region. More proximally the xylem conduits were open and normal. Bright-field optics. X470.

Fig. 17. Fungal hyphae invading xylem conduits at the distal end of a determinate branch were extending toward the base of the branch (arrow). A variety of organisms have invaded the large LMX element on the right. The proximal half of this root was healthy with open xylem conduits. X400.

Fig. 18. The mid region of an old determinate branch in which the xylem was mature and free of mucilaginous contents. Surrounding cells, including those of the cortex were alive. This longitudinal section lies within the stele of the branch and passes through the base of a second-order branch. Two EMX files and four sieve tubes (S) of the first-order branch are included. X400.

Fig. 19. A tangential longitudinal section through the mid-portion of an old determinate branch in which the cortical cells and many of the stelar cells were dead. A healthy, secondary root primordium has been sectioned transversely. Toluidine blue, PAS stain. Phase-contrast optics. X470.

Figures 20-25

Fig. 20. A cross section from the base of a determinate branch root. Note that all the LMX (asterisks) and some of the EMX (arrow heads) conduits are plugged by the mucilaginous polymers which are stained metachromatically pink. GMA-embedded section. PAS reaction and toluidine blue (pH 4.4). Bright field optics. X230.

Figs. 21 & 22. Cross sections from roots similar to that in Fig. 20 show the plugged LMX conduit and surrounding xylem parenchyma cells. The entire xylem lumen is occluded by the mucilaginous polymers. The xylem parenchyma cells are unusually large, with dense cytoplasm, and higher numbers of Golgi apparatus (arrowheads), mitochondria (M), rough ER (arrows), and other cell components, compared with the other parenchyma cells further away from the LMX. Periplasmic mucilage deposits (asterisks) in the xylem parenchyma adjoin the vessel walls. Fig. 21, X4,000. Fig. 22, X15,000.

Fig. 23. The beginning of xylem plugging. At this stage, the xylem lumen (LMX) is almost free of mucilages except near the pit chamber. The periplasmic space (asterisk) of the parenchyma cell and pit chambers are full of the polymers. X15,000.

Fig. 24. A later stage than that in Fig. 23. The polymers have passed through the pits in the xylem side walls and begin to accumulate in the xylem (LMX) lumen. They first line up along the cell walls (arrowheads). 15,000X.

Fig. 25. The entire xylem lumen (LMX) is full of the polymer, densest near the lateral walls. The periphery space of the surrounding xylem parenchyma cells is also filled with the same polymers (asterisks). X10,000.

Figures 26-29

Fig. 26. Xylem parenchyma cell similar to those shown in Figs. 21 & 22. This cell has dense cytoplasm, very small vacuoles, large numbers of Golgi bodies (G), mitochondria (M), very prominent rough endoplasmic reticulum (arrowheads) and numerous vesicles (arrows). X15,000.

Fig. 27. Part of a xylem parenchyma cell similar to that shown in Fig. 26. The Golgi body is showing active secretion activity and releasing vesicles (arrows) from its maturing face. M= mitochondrion. X65,000.

Figs. 28 & 29. A xylem parenchyma cell with well-preserved vesicles (arrowheads) which vary in size, shape and amount of contents. Some contain a fluffy, lightly contrasted matrix (small arrows in Fig. 29), and others have more compact, electron dense contents throughout (larger arrowheads in Fig. 29). Structurally, the contents of these vesicles look very much like the polymers in plugged xylem and in the periplasm of the xylem parenchyma cells (asterisks in Fig. 28). Fig. 28, X15,000. Fig. 29, X28,000.

2

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

CHAPTER 4.
DEVELOPMENT OF THE EPIDERMIS, SURFACE FEATURES
AND SUBERIZATION OF THE HYPODERMIS AND
ENDODERMIS IN FIRST-ORDER BRANCHES

INTRODUCTION

Miller (1938) has shown that a single corn plant transpires over 200 liters of water during its life, or 100 times of its own fresh weight. All the water transpired is taken up through the root system which consists of larger nodal roots and numerous, tiny branch roots. However, McCully and Canny (1988) found that the nodal roots of corn have living, immature late metaxylem (LMX) elements of large diameter for 20+ cm behind their tips. These living elements are accumulating ions and therefore are not likely to be involved in the transport of a large volume of water (McCully, Canny and Van Steveninck, 1987). Only small volumes of water can pass to the shoot through the narrow early metaxylem (EMX), suggesting that most of the water used by the plants must enter the mature, proximal regions, which have LMX open and bear most of the branches. This has called my attention to the branch roots. Previous studies showed that the first-order branch roots are mostly short (mode 6-10 mm), and divided into four size classes, and for most of their length have LMX open for conducting (Varney *et al.*, 1991; Wang, McCully and Canny, 1994, and Chapter 3). These branch roots differ from nodal roots in that they lose their tips and meristem at an early stage of development and become determinate, except those on the apical regions of the main roots (Fusseder, 1987; McCully, 1987; Varney and McCully, 1991; Wang *et al.*, 1994). Measurements of relative water content indicated that these branches are the most responsive part of the root system to drying during transpiration (Wang, Canny and McCully, 1991). Further, studies also showed that while the water flux through unit root surface is the same in branch and main roots, the branches collect over 80% of the water for the plant (Varney and Canny, 1993). It is, therefore, important to ask whether these branch roots have any special anatomical features related to

their role in water uptake.

The epidermis is the outermost tissue layer of roots, and epidermal cells must play a key role in controlling interchanges between the root and its environment. Extensive studies exist of the epidermis of main roots of many plant species, such as barley, *Calluna*, corn, mustard, onion, pea, and wheat, etc. (Abeysekera and McCully, 1993a & b; Clarke, McCully and Miki, 1979; Dayan, Banin and Henis, 1977; Greaves and Darbyshire, 1972; Peretto *et al.*, 1990; Smith and O'Brien, 1979). The epidermis of corn is a single cell layer with some hairless cells and others with tubular outgrowths - the root hairs. In the most apical regions, the epidermal cells are small and cytoplasmic, and columnar in shape, they may develop root hairs only after elongation to become tabular in shape (Abeysekera and McCully, 1993a; Clarke *et al.*, 1979).

● The outer surface of the main root apical regions of corn has been studied at both optical and electron microscope level. Where this surface overlies the meristematic cells of the epidermis this layer is as much as 10.5 μm thick and composed of 3 distinct sublayers. L1, the innermost layer, is the primary cell wall of the epidermis. L2, or inner pellicle, which is the thickest sublayer located immediately outside the L1, consists of fine fibrills and is usually lightly stained with uranyl-lead stains. L3, or the outer pellicle, is a coarsely fibrillar, thin cover over the L2 and positively stained with the same stains (Abeysekera and McCully, 1993a; Clarke *et al.*, 1979). All these studies have been concerned with the first few millimetres of the young, primary or nodal roots. No similar study has been done with the branch roots, and there are no studies of the surface further back into the mature zone of the roots.

Grass roots secrete mucilaginous substances and release living root cap cells at their

tips. These mixed together with living root hairs, dead deteriorated hairs, debris of other root surface cells, soil particles, and microorganisms, form a thick rhizosheath (McCully and Canny, 1988). These sheaths are tightly bound to the root surface.

Water and nutrient ions taken up by epidermal cells and root hairs from the soil must pass through the cortex into the stele and hence to the shoot. Much study of root structure which may be involved with this function has been focused on the hypodermis and endodermis, which are the outer- and innermost cell layers of the cortex respectively. Clarkson, Robards and Sanderson (1971); Haas and Carothers (1975), and Sutherland (1976) described in detail the sequence of development and fine structure of the endodermis in onion and corn primary roots. These studies agreed with the earlier literature that in most monocotyledons the endodermal cells commonly undergo a series of ontogenetic wall modifications distinguished by three distinctive developmental stages (Esau, 1965; Fahn, 1982; Krocmer, 1903). These stages are shown in Diagram 1. In the first stage (stage 1), the endodermal cells have only the primary wall, but the Casparian strip is developed in all the anticlinal (radial and transverse) walls. Later, a suberized lamella develops and covers the entire inner face of all the primary walls (stage 2). Then, a cellulose layer is deposited over the inner face of the suberin lamella. This layer becomes very thick over the inner tangential walls, thins as it extends centrifugally along the anticlinal walls, and is usually very thin in the outer tangential wall (stage 3). These late-forming walls are usually lignified; the endodermal cells are linked to each other and to adjoining cortical and pericycle cells by deep pits with plasmodesmata (Esau, 1965).

The hypodermis (also called exodermis by some authors) is similar in structure to the endodermis, in corn and many other plant species including both monocotyledons and dicotyledons (Clarkson *et al.*, 1978; Clarkson *et al.*, 1987; Enstone and Peterson, 1992a&b; Esau, 1965; Fahn, 1982; Perumalla and Peterson, 1986; Perumalla, Peterson

and Enstone, 1990; Peterson, 1987; Peterson, Emanuel and Wilson, 1982; Peterson and Lefcourt, 1990; Peterson, Murrmann and Steudle, 1993; Peterson and Perumalla, 1990). Because the hypodermal wall modifications develop in the same three stages as those of the endodermis, I am going to apply the same terminology to both tissues. Perumalla and Peterson (1986) have described the development of stage 1 and stage 2 endodermis and hypodermis in main roots of corn. They found that the stage 2 endodermis matures at about 14 cm, and stage 2 hypodermis at 16 cm behind the root tip in normal growing plants. But, in another study with the primary roots of corn, Clarkson *et al.* (1987) showed that stage 2 hypodermis was mature closer to the root apex (about 5.0-5.5 cm from the root tip), and they found no sign of the earlier Casparian strip development in this tissue. They also reported that stage 3 is absent or little developed in the hypodermal cells.

On the one hand, a series of physiological experiments revealed that there were some correlations between the development of suberized lamellae and Casparian strips in both hypodermis and endodermis and a marked decline in the rate of water uptake and transport into the xylem (Clarkson *et al.*, 1971; Sanderson, 1983). Thus, these two layers of cells have been considered as two distinct barriers (and the only barriers) to water movement into the stele through the cell wall apoplast. On the other hand, the view that cell walls are open porous structures in which water may flow along the plane of the wall has been denied by Canny (1990, 1993), and Canny and Huang (1993). They have shown that movement of dyes in the cell wall apoplast is by diffusion at rates about 1/100 of that in water, and that this movement is unaffected by transpiration. Thus, water probably does not flow through anticlinal cell walls of root tissues, even when the walls contain no Casparian strips or suberized lamellae. The presence of such deposits, therefore, may be irrelevant to water uptake. Varney, McCully and Canny (1994) have shown that water uptake into maize roots is into the symplast at the root periphery. Water movement across the cortex would then be through the symplast, either through plasmodesmata or through

cell walls and plasmalemma from cell to cell (Boyer, 1985). Since it is still unclear which way water moves through the symplast (i.e. the plasmodesmata or cell to cell pathway), I have assumed that water transports through the plasmodesmata and have studied the pits and plasmodesmata in the two barriers. Because the plasmodesmata are confined to the pits, especially in cells with thick secondary walls (Robards, 1976), the best way to find the frequency of plasmodesmata is first to count the number of pits, and then to find how many plasmodesmata occur in a pit (Kuo, O'Brien and Canny, 1974).

Water (and nutrients) collected by the epidermis and root hairs moves through the cortex and stelar cells in the symplast and finally gets into the xylem where upward water transport occurs as the transpiration stream. Therefore, it is important to know whether and where the xylem gets mature, and whether the maturation of the xylem relates to the suberization of the hypodermis and endodermis.

Bell and McCully (1970); Karas and McCully (1973) described in detail the initiation and early development of the epidermis in corn branch roots during development of the pre-emergent branch primordium. In contrast, my attention is focused on the growing and functioning branch roots after they emerge. My observations concern the development of the epidermis and the surface features in the first-order branch roots. Since no such study has been done since Kroemer (1903) in branch roots, the comparison has to be made with main roots. I report a study of the development of Casparian strips and suberization, at both optical and electron microscopic level, of both the hypodermis and endodermis of branch roots, and their relationship to the maturation of the large metaxylem elements.

MATERIALS AND METHODS

Plant materials

Corn plants, *Zea mays* L. (cv. Seneca Chief) were grown either in a greenhouse at Carleton University, or in a field plot of well-drained, sandy loam soil at the Central Experimental Farm, Agriculture Canada, Ottawa. Root systems of plants aged 36-69 days were carefully excavated and carried to the laboratory in a plastic bag covered with wet soil. The roots were washed clean and the first-order branch roots were collected and sampled randomly from tiers 3-4 of nodal roots (terminology of Hoppe, McCully and Wenzel, 1986). Measurements and investigations were made with these first-order branch roots.

Development of the epidermis

Preparation of epidermal strips

Branch roots with a length of 10-50 mm from potted plants were washed clean and sampled. Under a dissecting microscope, the root cap (about 0.5 to 1 mm) was cut off and 1-2 mm long segments were cut at distances 0.5, 5, 10 and 15 mm from the tip, and kept in their original sequence. A small drop of cyanoacrylic glue (Krazy Glue, Itasca III, USA) was placed on a glass slide. Root segments were then attached to the glue and allowed to polymerize for 20-30 seconds. The epidermal peels were carefully pulled off in thin strips using a pair of fine forceps, and kept in distilled water. Then these epidermal strips were stained either by the periodic acid-Schiff's (PAS) reaction (in Schiff's reagent for 10 seconds) or toluidine blue O pH 4.4 for 20-30 seconds, and viewed with bright-field

optics.

Sizes of epidermal cells were determined from the epidermal strips and transverse hand sections. Measurements were made on negatives and prints. At least 10 cells were measured from each root, and measurements from at least 100 cells (except those at 15 mm from root tip) were pooled to take the means.

Embedded materials

Pieces of field-grown branch roots 1-2 mm long were cut in sequence along each root from the tip, kept in their original order and fixed in 3% glutaraldehyde with 0.5% alcian blue 8GX (Aldrich Chemical Company, Inc., Milwaukee, Wis. USA) in 0.025M potassium phosphate buffer (pH 6.8) overnight on ice, dehydrated in a methyl cellosolve-ethanol-propanol-butanol series and infiltrated with glycol methacrylate monomer mixture (GMA). After UV polymerization, 1-2 μ m-thick median longitudinal sections were cut and stained sequentially with either the PAS procedure, or PAS counter stained with toluidine blue O (pH 4.4), or toluidine blue O (pH 4.4) followed by 0.3% alcian blue 8GX in 0.1 N sodium acetate buffer (pH 5.6), and viewed with bright-field optics. For more details of these techniques see O'Brien and McCully (1981).

Preparation of whole mounts

To assess whether epidermal cells were living or dead, seventy eight freshly sampled branch roots of field-grown corn were washed clean. Root pieces 5-10 mm long were cut from distal and basal regions of each root, and separated into the size classes (1-4) distinguished by Varney *et al.* (1991) with a dissecting microscope and eye piece micrometer, stained with the PAS procedure and mounted directly in diamidino phenyl

indole (DAPI), pH 8.0, and viewed with the fluorescence microscope (O'Brien and McCully, 1981).

Over 40 formalin-fixed branch roots similar to the above, and the main root segments from which these branches came, were washed, stained sequentially with the PAS procedure, counter stained with light green, mounted in acetic acid and viewed with bright-field or Nomarski optics

Autofluorescence of the epidermal cell walls

Eight first-order branch roots were taken from freshly excavated field-grown plants. After washing clean, cross hand sections were cut at about 2-3, 5-7, 10 and 15 mm from the root tip, mounted in distilled water and viewed with UV light under fluorescence optics.

Anatomical study of the surface of the branches

Seventeen branches from field-grown plants were fixed, embedded in GMA, and sectioned transversely or longitudinally as described above. Sections were stained with the PAS procedure and toluidine blue O pH 4.4, and viewed with bright field optics. Over 50 branches from the same plants were post-fixed in 1% buffered osmium tetroxide, dehydrated through an acetone series and embedded in Spurr's resin. Ultrathin sections were cut, stained with uranyl acetate and lead citrate, and viewed with the transmission electron microscope. Thicker sections from the same blocks were stained with toluidine blue O, pH 11, and viewed with bright-field optics.

The suberization of the hypodermis and endodermis

Twenty five indeterminate first-order branch roots were sampled from 12 field-grown plants. Serial transverse hand sections were cut starting from about 5 mm behind the root tip. Sections were stained with the PAS procedure for 5-10 min, washed and then stained with 0.01% fluorol yellow 088 (Sigma Chemical Co., P.O. Box 14508 St. Louis, MO. 63178 USA, Lot 70H3272) in polyethylene glycol (PEG) 400 (Brundrett, Kendrick and Peterson, 1991) for about 1 hour at room temperature, washed for about 5 seconds and mounted in 75% glycerol, observed with UV light under fluorescence optics for suberin lamellae and Casparian strips. The PAS reaction blocks the non-specific autofluorescence of the epidermal cell walls and emphasizes the contrast of the Casparian strips and suberized lamellae.

Six determinate branch roots from two of the plants mentioned above were processed in the same way as described for indeterminate branch roots, to study suberin development in the endodermis and hypodermis.

Eleven indeterminate branch roots from two field-grown plants were sampled, fixed, dehydrated and embedded in GMA as described. One- to two- μ m thick transverse sections were cut, stained with the PAS procedure for about 30 min, washed and stained with 0.01% fluorol yellow (Brundrett *et al.*, 1991) for about 2 h at room temperature. After brief washing, sections were mounted in 75% glycerol and viewed with the fluorescence optics.

Relationship between suberization in the cortex and LMX maturation

Twenty seven determinate first-order branch roots were taken from the apical, middle and basal regions, and nine indeterminate branch roots from the apical regions of 13 field-grown plants. The same roots were used to study the suberization of the hypodermis and endodermis and the maturation of the LMX.

Suberization of hypodermis and endodermis

Serial hand sections from these 27 determinate and 9 indeterminate branch roots were cut transversely, stained and observed as described.

The maturation of the LMX

The maturation or openness of the LMX in these 27 determinate and 9 indeterminate branch roots was gauged by the diffusion of India ink particles into the vessels as described in Wang *et al.* (1994) (see Chapter 3 in this thesis).

Pit frequencies on the outer tangential cell walls of hypodermis and endodermis

Kuo *et al.* (1974) have found that in the mestome sheath cells of wheat leaves most pits (and therefore the plasmodesmata) occur where the cells abut the metaphloem, and the pits are absent from the part of the same cell adjacent to the xylem. The same phenomenon, by which numbers of pits in any given cell vary where its faces abut different cells, can also be found in macerated LMX vessels in corn branch roots (X.L. Wang unpublished

observations); here most of the pits appear on those lateral walls which face the root periphery, and few are in the walls that are adjacent to another LMX vessel and none are in the wall facing the central parenchyma. These large variations in pit frequency within and between cells suggest that estimates of plasmodesmatal frequency by direct counts of the number of plasmodesmata from ultrathin sections would be erroneous, because, in secondary walls, the plasmodesmata are confined to the pits (Robards, 1976). The only way to get a reliable estimate of the plasmodesmatal frequency is to start by counting pits, then estimate plasmodesmatal frequency from the pit frequency and number of plasmodesmata per pit.

Macerated materials

Partially macerated root segments from regions of main and branch roots where the endodermal and hypodermal cells were mature were used to explore the frequency of the pits in hypodermal and endodermal outer tangential walls. I chose the outer tangential walls of the hypodermis and endodermis because it is technically easier. Freshly excavated main roots and their branches were washed clean and fixed separately in 10% formalin for more than 24 h. After washing for about 1 h with 0.025 M phosphate buffer, segments 10 mm long were cut from the mature region, two pieces from each root. One piece was used to study pits in the hypodermis and the other in the endodermis. For the hypodermis, pieces were macerated in equal amounts of 5% nitric acid and 5% chromic acid for about 3 days at room temperature (modified from O'Brien and McCully, 1981). After washing, partially macerated root segments were stained with toluidine blue O, pH 11, and washed. The epidermal cells stain strongly pink, and so can be recognized and carefully removed with sharpened, fine insect pins under a dissecting microscope, to expose the outer tangential walls of hypodermal cells. These lightly stained, naked walls of the hypodermis were then viewed with phase contrast optics.

For the endodermis, similar root pieces were put under a dissecting microscope, the cell layers outside the endodermis (epidermis + remaining cortex) were peeled off with forceps to expose the outer tangential walls of the endodermal cells. The remaining pieces with endodermis and stele were soaked in the acid for about 2 days at room temperature. Partially macerated root pieces were then washed briefly and then the single layer of endodermal cells was carefully taken off, mounted in water and viewed with phase contrast optics.

The frequency of the pits in the outer tangential walls of hypodermal and endodermal cells was measured from negatives and prints of the preparations. Measurements from the same types of cell wall were pooled to take the means.

Embedded root tissues

Pieces, approximately 1 mm long, from mature regions of at least 50 branch roots were cut, fixed, dehydrated, and embedded in Spurr's resin (O'Brien and McCully, 1981). Longitudinal, transverse and paradermal sections were cut, stained with the uranyl-lead procedure, and observed with TEM. Thicker sections of 0.5-1 μm from the same blocks were stained with toluidine blue O (pH 11), and viewed with bright-field optics.

Microscopy and photomicrography

All epidermal peels, macerated materials, whole mounts and thick embedded sections were examined with an Olympus Vanox or CH2 compound microscope equipped with bright-field, phase-contrast, Nomarski and fluorescence optics. The UV exciter (UG

1, 350 nm) and UV barrier filter (420 nm and up) combination was used for DAPI- and fluorescein yellow-induced fluorescence, and also for wall autofluorescence. Ultrathin sections were examined with a Philips 420 transmission electron microscope. Images were recorded on Kodak T-Max 35mm film and Kodak 4489 electron microscope sheet film.

RESULTS

Branch roots retain a living epidermis

Branch roots differ from the main roots of corn in that most of the epidermal cells along the branch remain alive, although a few or a cluster of epidermal cells can occasionally be seen to be dead and sometimes shed. The living, mature cells have very large central vacuoles. When stained with PAS and DAPI, and examined with the fluorescence microscope, most of the epidermal cells showed strong bright blue fluorescence of the nuclei all along the branch roots, from the apical regions to the base (Figs. 6 & 7). Careful examination of branch roots of classes 1, 3 and 4 from old, basal regions of the nodal roots showed that about two thirds of the hair-like class 1 roots retain a living epidermis. The larger, thicker classes 3 and 4 roots each have a higher percentage of roots with living epidermis (Fig. 4, Table 1). These living cells contain the usual complement of organelles. In contrast, most of the epidermal cells in main roots from which the branch roots were sampled are dead and broken, with the empty remains of the root hairs. Only a few epidermal cells in these main roots are intact, with stained nuclei and cytoplasm (Fig. 5). The hypodermal cells of both main and branch roots all contain nuclei and cytoplasm and appear to be alive.

My observations also showed that in branch roots of both field-grown and potted plants, most epidermal cells bear root hairs, but some of them do not develop until after epidermal cells are mature. That is to say, they may initiate and develop root hairs at any part of the entire length of the root, from root apical regions to the base; these late-developing hairs are then interspersed among the older hairs developed normally at the proximal end of the elongation zone. The density of new root hairs varies considerably from 11 to 157 new root hairs per centimetre of root segment. A survey of over 160 branch roots from nodal roots of tiers 1-4 of more than 10 mature plants of both greenhouse- and field-grown corn shows that there are more new hairs on branch roots from younger, higher tiers of nodal roots, and from younger plants, as well as on roots grown in the greenhouse. I interpret these root hairs as newly developed because they are of varying length, from those just emerging to those of full length (Figs. 1 & 2), and each contains a nucleus and cytoplasm (Fig. 3). They share the same feature of aniline-blue-induced fluorescence in their tips with the young, developing root hairs in the root hair zone closer to the root tip.

All these results suggest that most of the branch roots retain a living epidermis throughout most of their life.

Development of the epidermis

The developmental sequence of the epidermis in indeterminate first-order branch roots appears identical to that shown for the primary root by Clarke *et al.* (1979); Moore and Smith (1990), and for primary and nodal roots by Abeysekera and McCully (1993 a&b). During development, epidermal cells change their shape from columnar (Figs. 11-13) in the active cell dividing zone (area 3 as defined by Abeysekera and McCully, 1993a) to cuboidal when cells begin to elongate, then to tabular (Fig. 19) in the mature zone (area

8, Abeysekera and McCully, 1993a) and beyond (Fig. 4). As these cells finally reach their full size and produce root hairs, soil particles stick to their surface and form a wet, thick rhizosheath (Fig. 21).

The elongation of epidermal cells is accompanied by a dramatic increase in cell size. As can be seen in Figs. 11-13 & Table 2, at a distance about 0.5 mm from the root pole, the cells are small and columnar in shape. During elongation, they expand in the direction of the root axis from an average length of 8.5, to an average of 140 μm in mature cells, while the width stays the same and the depth decreases slightly. This is approximately a 17-times increase in cell length and a 14.5-times increase in volume (Table 2).

These mature epidermal cells are of different shapes and sizes: a few short cells and many more long cells. The shorter cells have an average length of 80 μm ; and longer ones, an average length of 160 μm . Long and short cells are interspersed together in no defined pattern (Figs. 18 & 19). Compared with the long cells, the short epidermal cells, especially in young epidermis, have denser cytoplasm, thicker cell walls, and both their cell contents and walls are stained more strongly with toluidine blue (Fig. 18). The short epidermal cells also have a higher percentage (72%) of cells producing root hairs than do the long cells (49%, Table 3).

During the investigation I noted that the indeterminate branch roots appear to be growing at different rates. The fast growing ones which are distributed on the apical regions of the nodal roots, are normal elongating roots, while the slow growing ones have already stopped growing or will stop and become determinate soon. The developmental sequences in these two types of root are mostly similar, but differ in that the latter show a short cell division zone (about 150 μm compared with 550 μm in fast growing branches), and a much shorter elongation zone as indicated by the distances at which the root hairs

begin to emerge behind the root tip (4-10 mm in slow growing branches and 25-50 mm in fast growing ones).

In determinate branch roots which lose their root tips and meristem during development, with a more or less dead end at their tips (Fusseder, 1987; McCully, 1987; Varney and McCully, 1991), the developmental stage of the epidermal cells behind determinate tips depends on how far the roots have progressed in terminating growth. Most of them have mature tissues including epidermis, often with root hairs right to the dead ends (Figs. 20, 25 & 26 in Varney and McCully, 1991), some of them have dead tips, but their distal epidermal cells are either just starting to elongate, or elongating but not yet at their final size (Figs. 11, 12 and 14 in Wang *et al.*, 1994, and in Chapter 3)

Changes in the epidermal cell outer surface during root elongation

As in the main roots, the surface of branch root epidermis reaches its maximum thickness over the epidermis on the actively dividing zone of the branch roots. This surface is so strongly PAS positive throughout that it is difficult to distinguish the pellicle from the outer walls of the epidermis (Fig. 12). However, when stained with ionically-binding dyes such as toluidine blue, or alcian blue, etc., the pellicle is distinguished and usually seen to be composed of two sublayers (Fig. 13 in this chapter and Fig. 13 in Varney and McCully, 1991) as described by Abeysekera and McCully (1993a) and Clarke *et al.* (1979) for main roots. During epidermal cell elongation, the thickness of the pellicle is reduced to a very thin layer where epidermal cells are elongating most rapidly (i.e., becoming tabular in shape).

Under the transmission electron microscope, the epidermal outer surface over the columnar cells in branch roots appears as shown in Fig. 16. The two distinct layers of the

pellicle seen in the same portion in main roots (Abeysekera & McCully, 1993a) are also clear in branch roots; L2 is fluffy in texture with loosely organized microfibrils. Cavities in this layer that are free of microfibrils are frequent especially over elongating cells. In contrast with the main roots, this layer stains strongly with uranyl-lead over its full length along the root. L3 is very thin and coarsely fibrillar. As the epidermal cells elongate, the L2 and the cell wall (L1) on the outer surface thin, and may be indistinguishable as the epidermal cells become fully elongated, and L3 mostly disappears. The L1 is the primary cell wall composed of microfibrils as in the main roots. This layer is also stained strongly with the uranyl-lead procedure through the entire root length.

The grooves on the epidermis overlie the radial wall junctions of neighboring epidermal cells and are formed as the pellicle disintegrates more proximally. In fine structure, the material in the bottom of the grooves is similar to the pellicle over the columnar epidermal cells in that it consists of open, fibrillar materials. These grooves have a more loose, open texture than any other part of the epidermal cell wall (Fig. 17 compared with Figs. 14, 15 & 16).

Epidermal cell outer surface in the mature region

The maturation of the epidermis is indicated by the formation of root hairs. In mature epidermal cells, the L2 disappears, and the L1 also thins as this layer stretches. Infrequently, some coarsely fibrillar materials typical of L3 in Abeysekera and McCully (1993a) can be seen as a discontinuous thin layer on the outer surface of elongated epidermal cells (Figs. 14 & 15). At the resolution of the TEM, the hair-bearing cells showed some structural changes compared with non-hair-bearing cells. In addition to the hair outgrowth, a thin, electron dense layer of cell wall forms adjacent to the plasma

membrane, from near the base of the hair to half or two thirds of the distance to the tip. This layer persists in old, mature hairs.

Autofluorescence of the epidermal cell walls

Another feature that can be easily seen in fresh hand sections during epidermal cell development is the increasing intensity of autofluorescence of the walls, from the root apex basipetally. At a distance of 2-3 mm behind the root tip, the epidermal cells show almost no autofluorescence (Fig. 8), though the cytoplasm of all the root cells is strongly autofluorescent. Faint fluorescence first appears in the outer tangential walls of the epidermis at about 5-7 mm behind the tip. Here the thick pellicle is weakly yellowish autofluorescent, in contrast to the bright blue fluorescence of the underlying outer cell walls. By this stage all the cytoplasmic autofluorescence has disappeared, and the anticlinal walls also begin to fluoresce blue (Fig. 9). At about 10-15 mm from the root tip, the outer tangential wall of the epidermis fluoresces strongly blue (Fig. 10). At increasing distances behind the root tip, the bright blue autofluorescence in these walls increases in intensity until all the cell walls of the epidermis (even the hypodermis) show strongly blue autofluorescence in the region where the hypodermal suberization starts, as will be described later in this chapter.

The bright blue autofluorescence of the epidermal cell walls is similar in colour to that of the Casparian strips in both hypodermis and endodermis. However, there must be chemical differences because the blue autofluorescence of the epidermal cell walls can be completely blocked by staining with toluidine blue or the PAS reaction, while the fluorescence of the Casparian strips is unaffected. When stained with fluorol yellow the

suberized lamellae in the hypodermal and endodermal cells show bright yellow fluorescence, however, the epidermal outer tangential and anticlinal walls are not normally stained by fluoro yellow, either with or without a pretreatment with the PAS procedure.

Other surface features in branch roots

Soil sheaths

Like the main roots of corn and many other grasses, the apical regions of all the indeterminate branch roots arising from the sheathed main roots develop a thick, coherent soil rhizosheath for a certain distance along the root axis, except at the growing tip. Sheathed branches occur only on sheathed regions of main roots, not on bare regions. Though the lengths of the branch roots with rhizosheaths vary from 10 to 50 mm, the percentage of the root length with soil sheath is relatively stable (45-65%, average 58%, $n=37$). The thickness of the sheath is variable, but it approximately doubles the root diameter. It persists, even after repeated washing during fixation, dehydration, and embedding procedures (Fig. 21). When viewed with the electron microscope, the sheaths are seen to be complex conglomerates as in main roots (see Introduction). In branch roots, as in main roots, the loss of the rhizosheaths usually corresponds to the opening of the LMX, but in the branches the epidermal cells and root hairs mostly do not die when this loss occurs.

Detached root cap cells

As in the main roots, detached root cap cells remain attached to the surface of the epidermal cells for a considerable distance behind the growing root tip. Occasionally, they can persist in the sheath or even in the basal regions among the root hairs (Fig. 20),

depending on the growth conditions. Under favourable moisture conditions, these detached root cap cells may be still alive, and have healthy-looking cytoplasm with ER, dictyosomes, mitochondria, and nuclei (Fig. 20). They also have alcian blue-positive, fuzzy, fibrillar mucilages on their outer surface, which resemble the mucilages produced by cells on the surface of the root cap. The detached cap cells can be distinguished from profiles of sectioned root hairs, and epidermal cells by their distinct shape and extremely loose cell walls and the distinctive surrounding mucilage (Fig. 20).

Microorganisms on root surface and suberization of epidermal cell wall

Microorganisms in the rhizosphere of branch roots are either embedded in the soil sheath, or attached to the root surface in sheathed regions, or associated with the epidermal cells and root hairs on bare regions (Figs. 21, 22 & 23). Most of the bacteria are aggregated into colonies. In general, very few fungi can be found. Most of the fungi appear individually (the species and sizes of population vary greatly between years). Mycorrhizal fungi can only occasionally be seen invading the root (possibly due to high phosphorous content in the soil).

Electron microscopy showed that the colonization of root surface by microbes is not uniform. Some of them are located on the convex curvature of the epidermal cell walls. But most of them are near or within the grooves above the junctions of two adjacent epidermal cell walls. These are the most preferred sites for microbial colonization of the root (Figs. 22 & 23). Sometimes, bacteria and fungal hyphae have been observed inside the grooves between epidermal cells or inside the dead epidermal cells. Occasionally, fungal hyphae can be seen in the intercellular spaces between the epidermis and hypodermis.

Bacteria are frequently surrounded by an electron translucent shell, and others show electron translucent deposits within the cells. The types and sizes of the bacterial populations associated with epidermal surface have been detailed by Gochnauer, McCully and Labbé (1989).

Suberized epidermal cell walls have been reported in corn and other plants by Wilson and Peterson (1983) and sand sedge (*Carex arenaria* L.) and some other plants by Robards, Clarkson and Sanderson (1979). In corn branch roots the lamellate suberins typical of the hypodermis and endodermis were not found in epidermal cell walls unless they were being invaded by microorganisms (Fig. 23). Here the TEM micrograph shows the invasion of a fungus and the suberized cell wall of the host epidermal cell. Apparently as a defense response, when the fungal hypha broke the epidermal cell wall either chemically or mechanically, the epidermal cell produced wall papillae, and also developed a suberin lamella (Fig. 23). Sometimes, no broken wall was observed in the epidermal cells with such suberized cell walls, but these cells always had microbes on their outer surface. Presumably, I failed to find the microbial entry point into these cells because the epidermal cells are long and tabular in shape, and my sections cut through only a narrow region of the cell. Deposition of suberins in the cell walls has been considered as a wound-induced response; these suberins can either be in a lamellate form or diffuse form (see review in Moon, Peterson and Peterson, 1984).

Suberization of the hypodermis and endodermis

Serial sections showed that in branch roots, the development of both Casparian strips and suberized lamellae in hypodermal and endodermal cells was similar to that in primary roots of the same plant as described in Clarkson *et al.* (1987); Ferguson and

Clarkson (1975); Hass and Carothers (1975); Perumalla and Peterson (1986). In both cases, the development of the endodermis always begins closer to the root tip than does that of the hypodermis. And proximally, the various stages of development of these tissues appear first in the endodermis (Diagram 2). The three developmental stages have been explained in the Introduction and as shown in Diagram 1.

As I described earlier, branches are either determinate or indeterminate and the latter are of two types: those that seem to be fast growing and those that may be slow growing. In fast growing branches, the Casparian strips in the endodermis begin to develop at about 1.5-2.0 cm from the root tips. They are first seen in cross sections of the roots as pin-points in the middle of the radial walls. These narrow Casparian strips later expand to fill the full radial wall (stage 1). Suberized lamellae in these cells start to differentiate at about 2.5 cm from the tips. At about 4.0 cm, these lamellae are fully differentiated and surround completely all the cells of the endodermis (stage 2) (Fig. 24). In slow growing branches, the stage 1 development in the endodermis occurs close to the root tip (about 0.5 cm from the root tips), and stage 2 starts at 1.0 cm and is complete at about 2.0 cm. The stage 3 endodermis (Fig. 28) in both types of root differentiates at variable distances behind the tips, and there is no clear distinction between the completion of stage 2 and the initiation of stage 3.

In the hypodermis of fast growing branches, the Casparian strips (stage 1) starts to develop at 2.0-2.5 cm behind the root tips, and stage 2 begins about 2 cm more proximal and is complete at 5.0-5.5 cm from the tip. In slow growing branches, stage 1 of the hypodermal development begins 0.5-1.0 cm from the root tip, and stage 2, 1.5-2.0 cm. Stage 2 is complete at about 3.0-3.5 cm.

In determinate branch roots, stage 2 endodermis and hypodermis extend to the broken ends (Fig. 29). Stage 3 also develops and matures closer to these ends (Diagram 2).

The sequence of development of stage 2 in the inner and outer tangential walls is different in the endodermis from that in the hypodermis (see Diagram 2 and Figs. 24-26). In the endodermis, the suberized lamellae develop first adjacent to the inner tangential walls, then on the outer tangential walls, and finally on the anticlinal walls. In contrast, in the hypodermis, stage 2 starts on the outer tangential walls, and progresses next to anticlinal walls, finally to the inner tangential walls.

It is very common in corn branch roots to find in the same cross section that the development of the wall structures distinguishing the different stages of differentiation in both the endodermis and hypodermis does not occur simultaneously in the same tissue: some cells are in one stage and some in another. This is especially the case in the endodermal cells. Here it is easy to find that one or more cells opposite the LMX strands (more often the EMX poles) may not complete stage 2 until later (Figs. 25 & 30), and they never develop to stage 3 (Fig. 32). These are passage cells (terminology in Esau, 1965). No such passage cells have been reported in main roots of corn. These passage cells were also observed in the young hypodermis (Fig. 31). In older regions of the branches endodermal and hypodermal passage cells had all developed to stage 2 (Fig. 26). As in the endodermis, it is unclear where the stage 3 hypodermis (Fig. 33, 34) in branch roots starts to differentiate, and there is no clear distinction between the completion of stage 2 and the initiation of stage 3.

Under the TEM, substructure of the suberized lamella can be resolved. It is a 3-layered band, 30 to 40 nm thick, which surrounds the cell completely on the inner face of

the primary walls. These bands appear as a thin, electron translucent layer beside a dark, electron dense, thick layer; these two layers are separated by a dark membrane-like structure (Fig. 34). The three-layers are more obvious near the plasmodesmata in the pit floor. Not infrequently, the suberized lamella is discontinuous near the plasmodesmata in the tangential walls and beside the Casparian strips in the anticlinal walls (Figs. 35 & 36) in both endodermis and hypodermis. I have counted all the endodermal cells in some sections in the mature regions of several branch roots, and found that about 68% (n=25) of them have gaps in the suberized lamellae beside the Casparian strips. These gaps are variable in length up to as much as 0.9 μm , and all 3 layers of the lamellae are missing in these areas (Figs. 35 & 36). Gaps in suberized lamellae have also been observed in the endodermis of primary roots of corn by Sutherland (1976) (see Fig. 49 in her Thesis).

LMX maturation in relation to suberization of the endodermis and hypodermis

The same branch roots were used to investigate the relationship between suberization of the endodermis and hypodermis and the maturation of the LMX, using the India ink diffusion technique and the PAS-fluorol yellow staining procedures.

In fast elongating branch roots, the Casparian strips and suberized lamellae have developed in both tissues throughout the proximal half of all the roots. Most of these roots have living, immature LMX throughout their length, and some have living EMX proximal to stage 2 endodermis and hypodermis. In determinate branch roots, although stage 2 endodermis and hypodermis always are present right to the broken tips, as indicated in Chapter 3, the LMX in the distal part of the root may be immature, or open but blocked by mucilages, bacteria, and fungal hyphae. Therefore, in all branch roots, stage 2 hypodermis

and endodermis are present distal to the open, conducting LMX, and distal even to mature EMX in some roots.

Pits and plasmodesmata in the outer tangential walls of hypodermis and endodermis

Under the light microscope, most of the pits in the hypodermis and endodermis are oval in face view, and some of them are elongated oval with length 2-3 times the width. The long axis ranges from 2 to 6 μm . Large and small pits are mixed together (Fig. 38). I noticed that pits are larger in cross section if there are only a few pits in the cell wall and relatively smaller in walls with more pits. This correlation has also been observed in the cortex of *Laminaria hyperborea* and *L. saccharina* by Schmitz and Kühn (1982).

In longitudinal or transverse sections, at the resolution of the electron microscope, the pits in outer tangential walls of both the endodermis and the hypodermis appear as "tunnels" in the secondary cell wall. In the floor of the pits, protoplasts connect from one cell to the next by plasmodesmata which traverse the thin primary walls and suberized lamellae. The pit tunnels or cavities vary in depth (depending on the thickness of the secondary wall), and in the numbers of plasmodesmata seen in sections that pass transversely through portions of the pit floor. The pit cavities are filled with cytoplasm often containing ER, mitochondria and dictyosomes. The pit floor is mainly only primary wall and suberized lamella and occasionally are overlaid by very thin secondary walls. Total thickness of the pit floor (0.5-0.6 μm) is greatly less than that of the surrounding walls (Fig. 40). The suberized lamellae do not occlude the plasmodesmata (Fig. 36). In mature endodermal and hypodermal cells plasmodesmata are confined to the floor of pits, and do not occur elsewhere in the walls.

The distribution of pits on outer tangential walls of the mature cells of these two tissues can be readily studied in macerated materials (Figs. 38 & 39). Pits were counted as numbers per cell, and expressed as the number per $100\text{ }\mu\text{m}^2$ of cell surface, as shown in Table 4. The large standard deviation indicates that pits are unevenly distributed in these walls in the hypodermal and endodermal cells of main and branch roots. I have counted 1.1 pits in one sample of $100\text{ }\mu\text{m}^2$ cell surface in a tangential wall of a hypodermal cell of a branch root, which is about 2-3 times the average for the same type of cell. There are significantly ($P<0.01$) more pits per $100\text{ }\mu\text{m}^2$ surface area of the endodermis than the hypodermis in both main and branch roots. The highest frequency of pits in the outer tangential walls is in the endodermis of main and branch roots, the lowest in the hypodermis of both roots. The numbers of pits in the outer tangential walls in the hypodermis are not significantly different ($P>0.05$) in main and branch roots.

There are about twice as many pits per unit area in the tangential walls of the endodermis as in the hypodermis (Table 4). The radius of the endodermis (and therefore its surface area) was about half that of the hypodermis, so the total numbers of pits through the two surfaces are approximately equal.

The frequency of pits in anticlinal and inner tangential walls of hypodermis and endodermis was not investigated in depth, but from my observations of whole mounts of main and branch roots stained with light-green, there was a marked difference in the density of pits between the transverse and radial walls of the hypodermis, with many more per unit length in transverse walls than in the radial walls (Fig. 37).

The frequency of plasmodesmata per pit in hypodermal and endodermal cell walls was not determined, but some of my paradermal and transverse sections through pit floors

showed that there are many plasmodesmata in each pit (Figs. 40-42). These plasmodesmata are unevenly distributed in the pit floors in the tangential walls of the hypodermis and endodermis (Fig. 42). Figs. 41 & 42 show two oblique sections through parts of two pit floors in the outer tangential wall of the branch root hypodermis, in which there are 20 visible plasmodesmata in Fig. 41, and 33 in Fig. 42. On the basis of such observations of parts of pit floors, it seems that an estimate of at least 20 plasmodesmata per pit is justified.

DISCUSSION

Branch roots retain a living epidermis

McCully and Canny (1988) have found that the epidermis and root hairs of the main roots of corn and some other grasses (McCully, 1987; Wenzel, McCully and Canny, 1989), die and are shed in a zone about 30 cm behind the root tip, leaving a heavily lignified and suberized hypodermis at the root surface. This has been shown to be coincident with the loss of soil sheath and the maturation of the LMX elements. In contrast to these main roots, my observations show that most branch roots retain a living epidermis throughout most of their life, indicated by the production of new root hairs along the entire root length, and the presence of nuclei and cytoplasm in epidermal cells from not only the young, distal regions (Fig. 6), but also the old, basal regions (Fig. 7 and Table 1).

Mauseth (1988) has reviewed root hair formation, fine structure, number, size and longevity, and concluded that root hairs are never produced in the old regions of roots proximal to the "primary" root hair production zone. This view has been supported by research done with maize primary root (Jaunin and Hofer, 1986) in which they concluded

that the formation of root hairs always occurred in the elongation zone of the root, and that the epidermal cells which do not produce hairs before cessation of epidermal cell elongation cannot subsequently produce hairs. In the present study, new root hairs were formed not only in the root hair zone just proximal to the elongation zone, but also in the basal regions of the branch roots. Although these newly-forming root hairs are small in number, and they *per se* may not play an important role in water and nutrient uptake, they are indicators that these mature epidermal cells are still alive and active in metabolism.

A living epidermis has been claimed to be extremely important in water and ion uptake and transport into the stele through the symplast, especially in plants which develop Casparian strips and suberized lamellae in the hypodermis (Peterson, 1987). This would be so if the hypodermal cells are not able to take over the uptake of water and ions after the death of the epidermis. A living epidermis may also be important in plant defence against invading microorganisms.

Branch roots have a loose, open outer surface

The epidermal outer surface in the branch roots studied showed some special features. First, the L2 layer over the meristematic zone stained differently from, and was more open and porous than this layer in main roots. It may well have a different chemical composition and permeability. Second, the epidermal outer surface in the mature zone looks more loose and open than in main roots (compare Fig. 14 in this thesis with Figs 21-23 in Vermeer-Macleod, 1982). Third, the material in the grooves at the ends of epidermal radial walls is looser and more fluffy in texture than other regions of the wall (compare Fig. 17 with Figs. 14, 15 & 16). All three of these features may turn out to be related to the uptake of solutes by providing an enhanced diffusion path, and more permeability than the corresponding walls measured by Canny (1990) and Canny and Huang (1993).

The longitudinal grooves between mature root epidermal cells have long been thought to be outlets for exudates from the root, and preferred foci for microbial colonization (Bowen and Rovira, 1976), and entrance to the root (Fig. 23). There are over 50 times more bacteria in these regions than on the adjacent epidermal cell surface, and fungi and actinomycetes tend also to grow along these grooves (Bowen and Rovira, 1976). The most recent study of water uptake in maize roots showed also that these grooves in branches are probably regions of rapid water flux (Varney, McCully and Canny, 1994).

Other features of the branch epidermis

Early study (Clarke *et al.*, 1979) of corn primary roots has shown that epidermal cells in the root-hair forming zone are of two types: long and short. These short cells produce most of the root hairs though some long cells produce root hairs and many short cells do not. Row and Reeder (1957) found that in some panicoid plants (like corn), which normally have uniformly long epidermal cells, there are small areas in which the hair-bearing cells seem somewhat shorter than the others. My observations show that the epidermal cells of the branches are also mixed long and short, and that these are not distributed in any defined pattern (Figs. 18 & 19). Both long and short cells of the branches produce root hairs, but the short cells have a higher percentage of hair-bearing cells (Table 2).

McCully and Canny (1988) have shown that the formation of a rhizosheath on the apical region of the main roots of corn under appropriate soil conditions is a developmental phenomenon, and that these sheaths only occur on immature regions of the roots. The underlying root tissues have living, immature LMX, and a higher relative water content irrespective of transpiration rate (Wang *et al.*, 1991). The sheaths slough off the main

roots and they become bare in the mature regions, which has been shown to coincide with the maturation of the LMX elements, and with death and shedding of the epidermis and root hairs (St. Aubin, Canny and McCully, 1986). In contrast, although the loss of the soil sheath in branch roots usually corresponds to the opening of the LMX, the epidermal cells and root hairs mostly do not die. Presumably, the binding mechanism of the soil sheath to the branch root surface differs from that described for main roots by McCully and Canny (1988), and Watt, McCully, and Jeffree (1993). Further, more detailed study is needed to clarify these differences.

Development of the epidermal autofluorescence in corn branches is similar to that in *Carex arenaria* (Robards *et al.* 1979) and onion (Peterson, Peterson and Robards, 1978). It may be due to the compounds found by Wilson and Peterson (1983) in the epidermal cell walls of corn axial roots, namely lipids, phenols, suberin and lignin. My observations with the TEM show that the lamellate suberin typical of the hypodermis and endodermis is absent from the epidermis unless it is invaded by microbes.

The frequency of pits in hypodermis and endodermis

My observations show that the outer tangential walls of corn hypodermal and endodermal cells in main and branch roots have many pits. Each pit probably contains large numbers of plasmodesmata. But I did not investigate in detail the frequency of these plasmodesmata. Clarkson *et al.* (1971) investigated the pits in the inner tangential walls of barley root endodermis, and found that they occurred at a frequency of 1.25 per 100 μm^2 and occupied about 2.5% of the total surface of the cells. Seagull (1983) measured the pits in the cortical cell walls of apical regions of young, elongating roots of clover, corn, radish and sorghum. Most cell walls in the cortex of these roots would be primary so that his counts would be mainly of primary pit-fields. He reported frequencies of the 'pits' in the

range of 80 to 180/100 μm^2 of wall in mature cells. The hypodermis or other specific cell layers of the cortex were not distinguished. According to Clarkson *et al.* (1978), pits are absent from hypodermal cell walls in onion roots.

My data indicate that the pit frequency in the outer tangential walls of the endodermis of the corn branch roots is twice that in the hypodermal walls. However, the numbers of pits per unit area are low (0.3 and 0.6 per 100 μm^2) compared with Clarkson's value for the inner tangential wall of the endodermis (1.25 per 100 μm^2). My results are also low compared with the frequency found by Kuo *et al.* (1974) for the inner tangential walls of the mestome sheath cells next to the phloem in wheat leaf veins. Their values range from 8 per 100 μm^2 on the flank of the small intermediate veins to 0.5 at the abaxial side of the midrib. I found significantly more pits in the transverse walls than in the anticlinal walls in the hypodermis, but comparatively few pits across Casparian strips in the radial walls of hypodermis and endodermis in the branches. This is in good agreement with the findings that there are very few pits in the radial walls in the hypodermis in Clarkson *et al.* (1971); Hass and Carothers (1975), and Juniper and Barlow (1969). Bonnett (1968) did not find any pits in the radial walls in the endodermis.

The frequency of plasmodesmata in various plant cells has been studied extensively (see review papers by Robards, 1975; 1976; Robards and Lucas, 1990; Tyree, 1970), but there are surprisingly few publications reporting on the pit frequency in root cells. As shown in Fig. 41, my estimate that there are at least (see Results section) 20 plasmodesmata in a single pit floor (33 in Fig. 42) in the outer tangential walls of the hypodermis and endodermis, is almost certainly a great underestimate. Single sections were seen with more plasmodesmata included from a single pit (33 in Fig. 42); each pit likely contains many more since my sections are somewhat less than 0.1 μm thick, so would include <5% of the pit floor, and plasmodesmata are almost 0.1 μm wide. But

using the pit frequency for the hypodermis from Table 4, there would be about 6 plasmodesmata per $100\ \mu\text{m}^2$ cell surface in the outer tangential walls of the hypodermal cells. Thus my figure is much lower than that in Seagull (1983) (about $700/100\ \mu\text{m}^2$), and Clarkson *et al.* (1987) (54 plasmodesmata per $100\ \mu\text{m}^2$ cell surface in the hypodermal outer tangential wall). Kuo *et al.* (1974) have also reported over 100 plasmodesmata per pit and about 770 plasmodesmata per $100\ \mu\text{m}^2$ cell surface in mestome sheath cells of the wheat leaf. These plasmodesmata in the wheat are also unevenly distributed through the pit floor.

Water flux through each pit

As explained in the Introduction, water taken up from soil solution probably moves in the symplast from the epidermis to the stele. One possible path is through the pits (i.e., plasmodesmata) in the hypodermal and endodermal tangential walls. The measurements by Varney and Canny (1993) show that the water flux through each unit cell surface area is the same in both branch and main roots. If I use their measured water flux ($4\ \mu\text{l h}^{-1}\ \text{cm}^{-2}\ \text{root}$) that enters the root surface and the number of pits from Table 4, then, the rate of water flux through each pit can be easily estimated. The results are given in Table 5. The water fluxes per pit in the tangential walls of the hypodermis and endodermis are both about 11-14 pl/h/pit. My calculated flows of water through pits are of the same order as those estimated by Clarkson *et al.* (1971) across the barley endodermis (7-16 pl/h/pit).

A picolitre is a cube standing on a square $10\ \mu\text{m}$ on a side, and about the volume of a large nucleus in a plant cell. My estimate of water fluxes through the pits of hypodermis and endodermis imply the passage of such a volume through a pit every 4 to 6 min. This seems fast, but not impossibly so.

Comparison of suberin development between branch and main roots

My observations show that all the branch roots developed Casparian strips and suberized lamellae in the hypodermis and endodermis. It is not surprising that these structures developed right to the end in determinate branch roots. The developmental sequence of suberization is the same in indeterminate branch roots, as in young primary roots described by Perumalla and Peterson (1986) (They used roots from the same corn variety, but grown under Laboratory conditions). However, the distances at which suberization started in these two root types are quite different. My results are closer to those of Clarkson *et al.* (1987) (also for main roots). Perumalla and Peterson (1986) found that the stage 2 hypodermis matured at about 16 cm behind the root tip in normal growing plants, but Clarkson *et al.* (1987) showed that stage 2 was mature closer to the root apex (about 5.0-5.5 cm from the root tip), and they also found no sign of the Casparian strip development in the hypodermal cell walls. These differences in suberization may be due to the different growing conditions and types of root. Robards *et al.*, (1973) have observed differences in distances at which endodermal suberin lamella development in different types of root from the same barley plant. Studies have shown that environmental and nutritional factors may affect suberin deposition; other affecting factors include low temperature (Clarkson *et al.*, 1987), mechanical impedance (Wilson and Robards, 1978), osmotic stress (Perumalla and Peterson, 1986), magnesium deficiency (Pozuelo, Espelie and Kolattukudy, 1984), etc. In the present study, the development of suberization in determinate branches might be regarded as another example, but it is still unclear what is causing the progressive senescence and decay of the apical meristem in these branches.

In branch roots of corn, the formation of suberized lamellae in epidermal cell walls can occasionally be seen to be associated with microorganism invasion. Presumably, it is a

defense response of the root epidermal cells to this invasion, but further investigation is needed to confirm this.

Relationship between suberization and LMX maturation and water uptake

The present study shows clearly that the Casparian strips and suberized lamellae in cell walls of the hypodermis and endodermis develop and mature distal to the maturation of the LMX and even the EMX in some branches. A main object of this study was to see whether the branch roots (known to take up most of the water for the plant) either had any special reduction of those barriers believed to limit apoplastic transport (the suberized and lignified layers of the hypodermis and endodermis), or had rapidly maturing xylem elements for the longitudinal transport of water. My study has shown that they have neither. The LMX differentiates proportionately to about the same degree as in main roots from those branches with indeterminate tips. For those with determinate tips it differentiates right to the end but is plugged there. The apoplastic barriers show their full range of development in the branches, in both hypodermis and endodermis. Open LMX is found only inside the developed barriers. This is consistent with the view that water uptake and transport from the soil to the xylem is symplastic, and thus independent of barriers in the apoplast. Their main function must be to further reduce the diffusion of solutes in the apoplast. They may also impede the progress into the root of pathogens.

REFERENCES

- Abeysekera RM and McCully ME. 1993a.** The epidermal surface of the maize root tip I. Development in normal roots. *New Phytologist* **125**: 413-429.
- Abeysekera RM and McCully ME. 1993b.** The epidermal surface of the maize root tip II. Abnormalities in a mutant which grows crookedly through soil. *New Phytologist* (in press).
- Bell JK and McCully ME. 1970.** A histological study of lateral root initiation and development in *Zea mays*. *Protoplasma* **70**: 179-205.
- Bonnett HT. 1968.** The root endodermis: Fine structure and function. *Journal of Cell Biology* **37**: 199-205.
- Bowen GD and Rovira AD. 1976.** Microbial colonization of plant roots. *Annual Review of Phytopathology* **14**: 122-144.
- Boyer JS. 1985.** Water transport. *Annual Review of Plant Physiology* **36**: 473-516.
- Brundrett MC, Kendrick B and Peterson CA. 1991.** Efficient lipid staining in plant material with Sudan red 7B or fluorol yellow 088 in polyethylene glycol-glycerol. *Biotechnic and Histochemistry* **66**: 111-116.

- Canny MJ. 1990.** Rates of apoplastic diffusion in wheat leaves. *New Phytologist* **116**: 263-268.
- Canny MJ. 1993.** The transpiration stream in the leaf apoplast: water and solutes. *Philosophical Transactions of the Royal Society of London*. **341**: 87-100.
- Canny MJ and Huang CX. 1993.** Rates of diffusion into roots of maize. *New Phytologist* **125**: 000-000. (in press).
- Clarke KJ, McCully ME and Miki NK. 1979.** A developmental study of the epidermis of young roots of *Zea mays* L. *Protoplasma* **98**: 283-309.
- Clarkson DT, Robards and AW Sanderson J. 1971.** The tertiary endodermis in barley roots: fine structure in relation to radial transport of ions and water. *Planta* **96**: 292-305.
- Clarkson DT, Robards AW, Sanderson J and Peterson CA. 1978.** Permeability studies on epidermal-hypodermal sleeves isolated from roots of *Allium* (onion). *Canadian Journal of Botany* **56**: 1526-1532.
- Clarkson DT, Robards AW, Stephens JE and Stark M. 1987.** Suberin lamellae in the hypodermis of maize (*Zea mays*) roots; development and factors affecting the permeability of hypodermal layers. *Plant, Cell and Environment* **10**: 83-93.

- Dayan E, Banin A and Henis Y. 1977.** Studies of the mucilaginous layer of barley (*Hordeum vulgare*) roots. *Plant and Soil* **47**: 171-191.
- Enstone DE and Peterson CA. 1992a.** A rapid fluorescence technique to probe the permeability of the root apoplast. *Canadian Journal of Botany* **70**: 1493-1501.
- Enstone DE and Peterson CA. 1992b.** The apoplastic permeability of root apices. *Canadian Journal of Botany* **70**: 1502-1512.
- Esau K. 1965.** *Plant Anatomy*. Second ed. John Wiley and Sons, Inc., New York.
- Fahn A. 1982.** *Plant Anatomy*. Third ed. Pergamon Press, Oxford, New York, Toronto, Sydney, Paris, Frankfurt.
- Ferguson IB and Clarkson DT. 1975.** Ion transport and endodermal suberization in the roots of *Zea mays*. *New Phytologist* **75**: 69-79.
- Fusseder A. 1987.** The longevity and activity of the primary root of maize. *Plant and Soil* **101**: 257-265.
- Gochnauer MB, McCully ME and Labbé H. 1989.** Different populations of bacteria associated with sheathed and bare regions of roots of field-grown maize. *Plant and Soil* **114**: 107-120.

Greaves MP and Darbyshire JF. 1972. The ultrastructure of the mucilaginous layer on plant roots. *Soil Biology and Biochemistry* **4**: 443-449.

Haas DL and Carothers ZB. 1975. Some ultrastructural observations on endodermal cell development in *Zea mays* roots. *American Journal of Botany* **62**: 336-348.

Hoppe D, McCully ME, Wenzel CL. 1986. The nodal roots of *Zea*: their development in relation to structural features of the stem. *Canadian Journal of Botany* **64**: 2524-2537.

Jaunin F and Hofer R-M. 1986. Root hair formation and elongation of primary maize roots. *Physiologia Plantarum* **68**: 653-656.

Juniper BE and Barlow PW. 1969. The distribution of plasmodesmata in the root tip of maize. *Planta* **89**: 352-360.

Karas I and McCully ME. 1973. Further studies of the histology of lateral root development in *Zea mays*. *Protoplasma* **77**: 243-269.

Kroemer K. 1903. Wurzelhaut, hypodermis and endodermis der Angiospermenwurzel. *Bibliotheca Botanica* **12**: 1-159.

- Kuo J, O'Brien TP and Canny MJ. 1974.** Pit-field distribution, plasmodesmatal frequency, and assimilate flux in the mestome sheath cells of wheat leaves. *Planta* **121**: 97-118.
- Mauseth JD. 1988.** *Plant Anatomy*. The Benjamin/Cummings Publishing Company, Inc., Menlo Park, California.
- McCully ME. 1987.** Selected aspects of the structure and development of field-grown roots with special reference to maize. In *Root Development and Function*, ed. Gregory PJ, Lake JV and Rose DA, pp.53-70. Cambridge University Press, Cambridge.
- McCully ME and Canny MJ. 1988.** Pathways and processes of water and nutrient movement in roots. *Plant and Soil* **111**: 159-170.
- McCully ME, Canny MJ and Van Steveninck RFM. 1987.** Accumulation of potassium by differentiating metaxylem elements of maize roots. *Physiologia Plantarum* **69**: 73-80.
- Miller EC. 1938.** *Plant Physiology*. Second Ed. McGraw-Hill, New York.

- Moon GJ, Peterson CA and Peterson RL. 1984.** Structural, chemical, and permeability changes following wounding in onion roots. *Canadian Journal of Botany* **62**: 2253-2259.
- Moore R and Smith HS. 1990.** Morphometric analysis of epidermal differentiation in primary roots of *Zea mays*. *American Journal of Botany* **77**: 727-735.
- O'Brien TP and McCully ME. 1981.** *The Study of Plant Structure. Principles and Selected Methods.* Termarcaphi Pty. Ltd., Melbourne, Australia.
- Peretto R, Peretto S, Faccio A and Bonfante-Fasolo P. 1990.** Cell surface in *Calluna vulgaris* L. hair roots. *In situ* localization of polysaccharidic components. *Protoplasma* **155**: 1-18.
- Perumalla CJ and Peterson CA. 1986.** Deposition of Casparian bands and suberin lamellae in the exodermis and endodermis of young corn and onion roots. *Canadian Journal of Botany* **64**: 1873-1878.
- Perumalla C, Peterson CA, and Enstone DE. 1990.** A survey of angiosperm species to detect hypodermal Casparian bands. I. Roots with a uniseriate hypodermis and epidermis. *Botanical Journal of the Linnean Society* **103**: 93-112.

- Peterson CA. 1987.** The exodermal Casparian band of onion roots blocks the apoplastic movement of sulphate ions. *Journal of Experimental Botany* **38**: 2068-2081.
- Peterson CA, Emanuel ME and Wilson C. 1982.** Identification of a Casparian band in the hypodermis of onion and corn roots. *Canadian Journal of Botany* **60**: 1529-1535.
- Peterson CA and Lefcourt BEM. 1990.** Development of endodermal Casparian bands and xylem in lateral roots of broad bean. *Canadian Journal of Botany* **68**: 2729-2735.
- Peterson CA, Murrmann M and E Steudle. 1993.** Location of the major barriers to water and ion movement in young roots of *Zea mays* L. *Planta* **190**: 127-136.
- Peterson CA and Perumalla CJ. 1990.** A survey of angiosperm species to detect hypodermal Casparian bands. II. Roots with a multiseriate hypodermis or epidermis. *Botanical Journal of the Linnean Society* **103**: 113-125.
- Peterson CA, Peterson RL and Robards AW. 1978.** A correlated histochemical and ultrastructural study of the epidermis and hypodermis of onion roots. *Protoplasma* **96**: 1-21.
- Pozuelo JM, Espelie KE and Kolattukudy PE. 1984.** Magnesium deficiency results in increased suberization in endodermis and hypodermis of corn roots. *Plant Physiology* **74**: 256-260.

- Robards AW. 1975.** Plasmodesmata. *Annual Review of Plant Physiology* **26**: 13-29.
- Robards AW. 1976.** Plasmodesmata in higher plants. In *Intercellular Communication in Plants: Studies on Plasmodesmata* . Ed. by Gunning B. E. and Robards AW. Springer-Verlag, Heidelberg.
- Robards AW, Clarkson DT and Sanderson J. 1979.** Structure and permeability of the epidermal/hypodermal layers of the sand sedge (*Carex arenaria*, L.). *Protoplasma* **101**: 331-347.
- Robards AW, Jackson SM, Clarkson DT and Sanderson J. 1973.** The structure of barley roots in relation to the transport of ions into the stele. *Protoplasma* **77**: 291-312.
- Robards AW and Lucas WJ. 1990.** Plasmodesmata. *Annual Review of Plant Physiology and Plant Molecular Biology* **41**: 369-419.
- Sanderson J. 1983.** Water uptake by different regions of the barley root. Pathways of radial flow in relation to development of the endodermis. *Journal of Experimental Botany* **34**: 240-253.
- Schmitz K and Kühn R. 1982.** Fine structure, distribution and frequency of plasmodesmata and pits in the cortex of *Laminaria hyperborea* and *L. saccharina*. *Planta* **154**: 385-392.

- Seagull RW. 1983.** Differences in the frequency and disposition of plasmodesmata resulting from root cell elongation. *Planta* **159**: 497-504.
- Smith MM and O'Brien TP. 1979.** Distribution of autofluorescence and esterase and peroxidase activities in the epidermis of wheat roots. *Australian Journal of Plant Physiology* **6**: 201-219.
- St. Aubin G, Canny MJ and McCully ME. 1986.** Living vessel elements in the late metaxylem of maize roots. *Annals of Botany* **58**: 577-586.
- Sutherland JM. 1976.** *Observations on the Development of the Endodermis in Zea mays Roots*. MSc. Thesis. Carleton University, Ottawa, Canada
- Tyree MT. 1970.** The symplast concept: A general theory of symplast transport according to the thermodynamics of irreversible processes. *Journal of Theoretical Biology* **26**: 181-214.
- Varney GT and Canny MJ. 1993.** Rates of water uptake into the mature root system of maize plants. *New Phytologist* **123**: 775-786.
- Varney GT, Canny MJ, Wang XL and McCully ME. 1991.** The branch roots of maize. I. First-order branches, their number, sizes and divisions into classes. *Annals of Botany* **67**: 357-364.
- Varney GT and ME McCully. 1991.** The branch roots of *Zea*. II. Developmental loss of the apical meristem in field-grown roots. *New Phytologist* **118**: 535-546.

- Varney GT, McCully ME and Canny MJ. 1994.** Sites of entry of water into the symplast of maize roots. *New Phytologist* **126**: 000-000. (in press).
- Vermeer-Macleod J. 1982.** *Histochemical, histological and structural study of field-grown corn roots.* M. Sc. Thesis, Carleton University, Ottawa, Canada.
- Wang XL, Canny MJ and McCully ME. 1991.** The water status of the roots of soil-grown maize in relation to the maturity of their xylem. *Physiologia Plantarum* **82**: 157-162.
- Wang XL, McCully ME and Canny MJ. 1994.** The branch roots of *Zea*. IV. The maturation and openness of xylem conduits in first-order branches of soil-grown roots. *New Phytologist* **126**: 000-000. (in press).
- Watt M, McCully ME and Jeffree CE. 1993.** Plant and bacterial mucilages of the maize rhizosphere: Comparison of their soil binding properties and histochemistry in a model system. *Plant and Soil* **151**: 151-165.
- Wenzel CL, McCully ME and Canny MJ. 1989.** Development of water conducting capacity in the root systems of young plants of corn and some other C-4 grasses. *Plant Physiology* **89**: 1094-1111.
- Wilson AJ and Rowards A*** 1978.** The ultrastructural development of mechanically impeded barley roots. Effects on the endodermis and pericycle. *Protoplasma* **95**: 225-265.

Wilson CA and Peterson CA. 1983. Chemical composition of the epidermal, hypodermal, endodermal and intervening cortical cell walls of various plant roots. *Annals of Botany* **51**: 759-769.

Table 1. Percentage of branch roots with living epidermis*

Branch Class	Mean Diam. (mm)	Branches with living epidermis (%)	Sample size
1	0.2	62	23
3	0.46	93	14
4	0.55	98	22

* Field-grown corn roots, 56-69 days old.

Table 2. Epidermal cell size at different distances from the tips of branches.
Means $\pm$ SD(N).

Distance mm	Length μ m	Width μ m	Depth μ m	Volume μ m ³
0.5	8.5 $\pm$ 3(109)A	19 $\pm$ 3(109)A	33 $\pm$ 4(82)a	5 000
5	44 $\pm$ 19(140)B	19 $\pm$ 3(140)A	28 $\pm$ 4(109)b	23 000
10	96 $\pm$ 60(100)C	22 $\pm$ 4(100)B	26 $\pm$ 4(95)b	53 000
15	142 $\pm$ 54(114)D	22 $\pm$ 4(114)B	25 $\pm$ 5(137)b	77 000

Figures in the same column followed by different letters are significantly different, upper case letters denote ($P < 0.001$), lower case denote ($P < 0.05$).

Table 3. Sizes of branch epidermal cells and the proportions of them bearing root hairs. Means $\pm$ SD(N).

Cell type	Length	Width	Number with hairs	Total	% with hairs
Long	160 $\pm$ 94(94)a	21 $\pm$ 4(94)a	107	221	49
Short	80 $\pm$ 15(22)b	25 $\pm$ 3(22)b	52	72	72

Figures in the same column with different letters are significantly different ($P < 0.001$)

Table 4. Frequency of pits in hypodermis and endodermis of corn roots, in outer tangential walls. Number of pits per 100 μm^2 of cell surface: Mean $\pm$ SD(N).

	Pit Frequency	
	Main root	Branch root
Hypodermis	0.30 $\pm$ 0.09(24)	0.37 $\pm$ 0.16(33)
Endodermis	0.63 $\pm$ 0.46(18)	0.58 $\pm$ 0.24(11)

Table 5. Calculated water flux through a single pit.

	Main roots		Branch roots	
	Hypodermis	Endodermis ^b	Hypodermis	Endodermis ^c
Number of pits per 100 μm^2	0.3	0.63	0.37	0.58
Water flux ^a pl/h per 100 μm^2 surface	4	7.2	4	8
Water flux pl/h per pit	13	11	11	14

^a Data from Varney and Canny, 1993.

^b Assuming radius of the endodermis is 1/1.8 of the root radius.

^c Assuming radius of the endodermis is 1/2 of the root radius.

Diagram 1

Diagram 1: The Three Stages of Endodermal Development

Legend

Casparian Strip

Cellulose Wall

Suberized Lamellae

Passage Cell

Diagram 2

Key for Diagram 2:

A. Fast growing indeterminate branches. Ep. Epidermis.

B. Slow growing indeterminate branches. Hy. Hypodermis.

C. Determinate branches. En. Endodermis.

X. Xylem.

Developing stage 1 endodermis and hypodermis.

Mature stage 1 endodermis and hypodermis.

Developing stage 2 endodermis and hypodermis.

Mature stage 2 endodermis and hypodermis.

Mature stage 3 endodermis.

Mature stage 3 hypodermis.

Closed, or immature xylem.

Open, conducting xylem.

Diagram 2. Sequence of hypodermal and endodermal suberization and the maturation of the LMX in branch roots of corn

Figures 1-5

Fig. 1. A whole mount of a mature portion of a field-grown first-order branch root, with a second-order branch at the lower right (arrowhead). In the upper left corner, a newly-emerged root hair (arrow) is developing among the long, old hairs; a mature hair overlies it. Toluidine blue/aniline blue stain. X150.

Fig. 2. Detail of the new root hair (arrow) with overlying mature hair (arrowhead) indicated in Fig. 1. X430.

Fig. 3. A transverse hand-cut section from the old region of a field-grown branch root, including an immature root hair with dense cytoplasm and nucleus (arrow). The cell walls of underlying hypodermal (H) and cortical cells are thick. Unstained section, Nomarski optics. 430X.

Fig. 4. Surface view of an old region of a branch root. In contrast to the main roots, an intact, living, turgid epidermis is present. At the bottom of the micrograph there is a developing root hair, broken during preparation. PAS reaction followed by light green staining. Bright-field optics. X430.

Fig. 5. Surface of an old region of a main root. Root hairs and most epidermal cells have died and detached, leaving the hypodermis (H) exposed. Occasionally there are groups of a few epidermal cells (E) still attached, some still alive (arrow indicates a nucleus in one of these cells). PAS/light green stain. Bright-field optics. X220.

Figures 6-10

Fig. 6. Whole mount of the apical portion of an old branch root stained with DAPI after the autofluorescence of the epidermal cell walls was blocked by the PAS procedure. The nuclei in these epidermal cells fluoresce strongly. The blurred underlying fluorescence is from out-of-focus nuclei of the hypodermis. Fluorescence optics. X440.

Fig. 7. Similar preparation as in Fig. 6 but at the base of an old branch root. X440.

Figs. 8-10 Transverse hand-cut sections from 2-3 (Fig. 8), 10 (Fig. 9) and 15 (Fig. 10) mm from the root tip of a field-grown branch root, showing autofluorescence when mounted in distilled water. Fluorescence optics. Equivalent exposures on negatives and prints. Epidermis = E; Hypodermis = H. All prints are at the same magnification. X230.

Figures 11-17

Fig. 11. A longitudinal section in the meristematic region of a branch tip showing the columnar epidermal cells (E) and the thick complex surface layer. In this surface the epidermal walls (arrowheads) and the pellicle (asterisks) can be distinguished. Arrow indicates a metaphase figure. GMA-embedded material. Toluidine blue/alcian blue staining. Bright-field optics. X1,300.

Fig. 12. A similar section to that in Fig. 11 but stained by the PAS procedure. The epidermal surface (asterisks) is so strongly PAS positive throughout that it is difficult to distinguish the epidermal walls from the pellicle. E = epidermal cell. GMA-embedded section. Bright-field optics. X300.

Fig. 13. Similar section to that in Figs. 11 and 12, but stained with toluidine blue. The pellicle can be seen to be composed of an outer, thin, strongly-staining layer (L3)(arrows), and an inner, unstained, thick layer (L2). The epidermal outer wall (arrowheads) can also be distinguished. Bright-field optics. X430.

Fig. 14. Outer root surface in the region where epidermal cells (E) are fully elongated. The pellicle has thinned and is loose in texture. X30,000.

Fig. 15. A more tangential view of an epidermal surface in the mid-region of the elongation zone, somewhat older than that in Fig. 16. X20,000.

Fig. 16. Surface of an epidermal cell in the distal region of the elongation zone. Two pellicle layers (L2) and (L3) and the epidermal wall (L1) can be distinguished. The pellicle is very open and fluffy in texture. X20,000.

Fig. 17. A cross-section cut through the groove in the epidermis over the junction of adjacent epidermal cells (E). The material in the bottom of the groove has a more loose and open texture than any other part of the epidermal cell wall. X15,000.

Figures 18-19

Fig. 18. An epidermal peel made at 10 mm from the tip of an indeterminate branch root. Short (S) and longer (L) cells can be distinguished. Some cells have been damaged during the preparation. Toluidine blue stain. X430.

Fig. 19. An epidermal strip as in Fig. 18 but made at the base of a mature branch root. Short (S) and longer (L) cells are still present. X430.

Figures 20-23

Fig. 20. A transverse section through a detached root-cap cell stuck to the base of a branch root. This cell was alive. The cell wall is loose and surrounded by coarse aggregates of root-cap mucilage that has been bound by the alcian blue in the fixative. X9,000.

Fig. 21. A portion of rhizosheath adhering to the surface of a mature epidermal cell of a branch root. This sheath includes clay particles (C), bacteria (arrows) and mucilages of bacterial and root cap origin (asterisks). X10,000.

Figs. 22&23. Transverse sections of mature regions of branches. Fungi (F) have challenged these epidermal cells (E) and the cells have responded by forming suberized lamellae (arrowheads) on their outer tangential walls. In Fig. 23, a fungal hypha has entered one of the epidermal cells into a thick wall papilla (P). These fungi are located in the grooves that overlie the radial walls of these epidermal cells. Fig. 22, X5,000; Fig. 23, X10,000.

Figures 24-29

Figs. 24-28 are all from transverse, hand-cut sections of indeterminate, first-order branch roots. The sections have been stained with PAS followed by fluorol yellow and viewed with fluorescence optics.

Fig.24. Three centimetres from the root tip. The endodermis is in stage 3 development and has mature Casparian strips and suberized lamellae. Some secondary wall has been deposited in the endodermis. The hypodermis (H) is in stage 1, with only the Casparian strips mature. X230.

Fig. 25. Section 4.5 to 5 cm from the tip. Early part of stage 2 in the hypodermis (H); the inner tangential walls are the last to suberize. Note that one of the endodermal cells (arrow) just opposite an EMX poles remains unsuberized; it is thus a passage cell. X440.

Fig. 26. Section 5-5.5 cm from the tip (a little proximal to the section in Fig. 25). All the cell walls in the hypodermis (H) and endodermis are now suberized. X240.

Figs. 27&28 are from the same section of an old, mature region. The hypodermal cells (H) are fully suberized (Fig. 27), and endodermal cell walls are also fully suberized and have developed thick cellulose inner tangential walls (stage 3, asterisks in Fig. 28). Both Figs. X440.

Fig. 29. A transverse hand-cut section cut about 2 mm from the distal end of a determinate branch root. Here the hypodermis (H) (and endodermis also- not shown) is fully suberized. PAS followed by fluorol yellow stain. Fluorescence optics. X440.

Figures 30-32

Figs. 30-32. Transverse, hand-cut sections of branches. Passage cells are present in the endodermis (arrows in Fig. 30) and hypodermis (arrow in Fig. 31). In Figs. 30 & 31, the passage cells are in stage 1 development. In Fig. 32, all endodermal cells, except the passage cells (arrows), have developed to stage 3 with thick cellulose inner tangential walls. The passage cells are now in stage 2, with a complete suberized lamella. H= Hypodermis. Fig. 30, X440; Fig. 31, X250; and Fig. 32, X230.

Figures 33-36

Fig. 33. Transverse section of old, mature region of a branch root. The hypodermal cells (H) have progressed to stage 3 development so that all the cell walls are thickened (also those of other cortical cells). Arrowheads indicate adjacent radial walls as shown in Fig. 34. GMA-embedded section, stained with PAS and fluorol yellow. Fluorescence optics. X440.

Fig. 34. A region of adjoining radial walls between 2 hypodermal cells as indicated by arrow heads in Fig. 33. Each suberized lamella (arrowheads) consists of a wide electron-translucent band with a membrane-like electron-dense layer on each side. This is similar in structure to suberized lamellae in the endodermis. These hypodermal cells have developed thick secondary walls (SW). X42, 000.

Figs. 35&36. The suberized lamellae (arrowheads) have discontinuities in radial walls of the endodermis beside the Casparian strip (Fig. 35), and in the hypodermis within the pit floors near the plasmodesmata (Fig. 36). Fig. 35, X28,000; Fig. 36, X65,000.

Figures 37-42

Fig. 37. Pits (arrowheads) in the radial and transverse walls of the hypodermis in a main root. Whole mount of a paradermal section. Stained with PAS/light green. Partial Nomarski optics. X290.

Fig. 38. Macerated tissue from a main root showing pits (arrowheads) in the outer tangential face of hypodermal cells. Phase contrast optics. X430.

Fig. 39. Hypodermal cell in macerate of branch root showing pits in the outer tangential wall. Phase contrast optics. X670.

Figs. 40-42. Oblique sections through parts of individual pits from the outer tangential walls of the hypodermis in branch roots. In the portions of the pit floors in Figs. 41 and 42, there are respectively about 20 and 30 plasmodesmata that can be distinguished. The plasmodesmata are unevenly distributed in the pit floors. E= epidermis, H= hypodermis. Fig. 40, X22,000; Fig. 41, X28,000; and Fig. 42, X30,000.

CHAPTER 5.
GENERAL DISCUSSION AND CONCLUSIONS

The question was raised in the Introduction whether branch roots show any unique differences in structure and activity from the main roots. Given the fact observed by so many, that root systems in the soil consist predominantly of fine branches, and the recent demonstration by Varney and Canny (1993) that the branches collect most of the water, the question had become a pressing one. My work provides a preliminary answer. Briefly, the structure and activity of first-order branches are broadly similar to those of the main roots, but there are several conspicuous differences. I will discuss first the similarities, and then the differences.

Similarities of branches to main roots

The branches are small-scale copies of main roots (on a smaller scale of distance and over a shorter interval of time) in three respects. These are:

1. Maturation of late metaxylem

The mature indeterminate branches of class 2 and larger have their LMX dead, open and available for conduction for much of their proximal length, and, like the main roots, a living, closed section distally. The open LMX provides a low-resistance path for water flow to the xylem of the parent main root. It is probable that the living, distal LMX elements accumulate ions, as do those of the main roots (McCully, Canny and Van Steveninck, 1987).

2. Maturation of apoplastic barriers

The branches exhibit a development of the the three stages of cell wall deposition in both endodermis and hypodermis closely parallel to that of the main roots, but compressed into shorter distances. Whatever limitations these apoplastic barriers provide to the main roots will also apply in the branches. It has been argued in Chapter 4 that these apoplastic barriers are not relevant to water movement, which is symplastic across the epidermis/hypodermis and the endodermis, but will further restrict solute diffusion in these cell walls. The diffusivity of solutes across these barriers is reduced from its usual level of 1/100 to 1/1000 of the value of their diffusivity in water to nearly zero. This has been demonstrated for sulphorhodamine in branches by Canny and Huang (1993), and in main roots for berberine sulphate by Enstone and Peterson (1992), and for sulphate ions by Peterson (1987).

3. Water flux

The measurements of water flux into corn main roots and branches by Varney and Canny (1993) showed that proximal to the opening of the LMX in main roots, the fluxes into main roots and branches were approximately equal. At 25 cm back from the main root apex, where the LMX opened, the fluxes into both sorts of root were about $4 \mu\text{l h}^{-1} \text{cm}^{-2}$, but in older regions both fluxes declined to less than $1 \mu\text{l h}^{-1} \text{cm}^{-2}$.

Differences of branches from main roots

The differences highlighted by my study of the branches are six:

1. Further stages of maturation

As shown in Chapter 2, branch roots mature beyond the stage normally achieved by main roots. After the cessation of meristematic activity and the loss of the apex, branch roots decay backwards from their distal ends, becoming shorter and shorter (Varney and McCully, 1991). As this happens, the LMX, which has been open and conducting, is plugged by polymers secreted from the adjacent parenchyma cells, and again becomes closed and non-conducting. This process can proceed right back into the region of the branch roots still enclosed in the cortex of the parent root. There is thus likely to be a proportion of the branch root population which is inactive in collecting water, because it has no means of transferring the water to the parent main root. It is still not known whether such roots can still take up and transfer nutrient ions through their phloem.

2. More intense response to transpiration

As shown in Chapter 2, the branches are especially liable to drying out at the time of day when water loss by transpiration exceeds water uptake by the roots. It is remarkable that their RWC can be reduced to around 50% without their dying. Linked with the last section, this is fully consistent with the low-resistance connection of the branches to the pull of transpiration through their open LMX. It is also consistent with the major role of the branches in water collection already discussed.

3. Retention of epidermis and root hairs

It is possible that Peterson (1988) is correct in claiming that the presence of a living epidermis is essential for the uptake of water. It is also possible that, with the loss of the epidermis, the hypodermal layer is able to take up water just as well. Evidence for this latter possibility is provided by two observations. First, as mentioned above, Varney and Canny (1993) showed that water fluxes into branches and main roots were the same in the zones 50 to 100 cm from the tip. In this zone of field roots the epidermis is certainly dead. They did not thoroughly investigate the state of the epidermis in their mist-grown main roots, but the illustrations in the companion paper (Varney, McCully and Canny, 1994) provide evidence of dead epidermis, and also of the second observation. This evidence is the formation of the accumulations of dye (sumps in their terminology), by which they measured the water fluxes, inside a dead epidermis, and on the outer tangential wall of the hypodermis.

A unique role for the epidermis could well be in the uptake of nutrient ions, and it may be this activity of branches which is preserved by the retention of the living epidermis, and which is lost in main roots. The retention of living root hairs on the branches (Chapter 4) may assist also in ion collection.

4. Open texture of outer epidermal cell walls

The indications in my work that the outer epidermal cell walls of branches may differ both in chemical composition and physical porosity from those of main roots need further investigation. The differences in image density and in staining shown in Chapter 4

could be the visible sign that these walls are more open for solute diffusion. Canny (1990) and Canny and Huang (1993) have measured a very large range of diffusivities in cell walls (from 1/50 to 1/10,000 of the values in water). Such low rates of diffusion must impose limitations on the possible rates of acquisition and transport of solutes in the apoplast. It is quite possible that some plant cell walls have evolved a looser structure which extends this range upwards towards the values in water, and the outer wall of the branch root epidermis is surely a likely place to look for such walls.

The unique structure I found in the grooves above the branch epidermal radial walls may be another example of special permeability at the root surface. Varney *et al.* (1994) show evidence (in the especially dense formation of dye sumps) of especially rapid water fluxes at these grooves in the branches. The main roots did not show the same strong accumulations in their grooves. The presence of especially abundant bacterial colonization of these same grooves (Bowen and Rovira, 1976, and Chapter 4) suggests the possibility of enhanced outward diffusion of solutes at these sites.

5. High proportion of high resistance roots

The class 1 branches of field-grown corn roots were separated from the rest by Varney *et al.* (1991) both because they were the smallest in diameter, and because they had no LMX. Their only tracheary elements are a few (around six) very narrow vessels with a lumen diameter of about 6 μm . Figure 7 of Varney *et al.* (1991) emphasizes that their calculated conductance for water flow is about 1/10 to 1/100 that of the class 2 branches. In the same study it is shown that these high resistance roots constitute about 35% of the total number of branches. My study has confirmed these facts and dimensions, and shown that class 1 branches seem to have no other unique features. A working hypothesis for

investigation could be that they are less involved in the collection of water, and more specialized for the collection of nutrient ions.

6. *Consistent numbers of pits through apoplastic barriers*

The measurements of pit frequency in the outer tangential walls of the hypodermis and epidermis given in Table 4 of Chapter 4 reveal a striking constancy in the total number of pits through the two barriers. Whatever the detailed pathway of water through the cortex (symplast/plasmodesmata, or cell-to-cell), it is clear that at the hypodermis and endodermis the water must pass through the pits. The suberized lamellae enclosing all the cells in the barriers would surely block the cell-to-cell path. It could be argued that some water might bypass the barriers in the apoplast by means of the secondary wall of the suberized tissue, if the gaps observed in my study are functional. However, until this is determined we assume a complete apoplastic barrier.

The numbers of pits observed seem small in comparison with the values already published. At 0.3 and 0.6 pits per $100\ \mu\text{m}^2$ through hypodermis and endodermis, they are at the lower end of the range of those counted by Kuo, O'Brien and Canny (1974) on the sugar pathway through the inner tangential wall of the mestome sheath of wheat leaves. They are typical of those mestome sheath cells at the bottom of the mestome sheath which are probably not carrying much of the traffic of sugar. My value for the outer tangential wall of the endodermis is just half that found by Clarkson *et al.* (1987) for the inner tangential wall of the barley endodermis. But if the constancy of pit number already found through the outside of the two barriers is carried on through the inner wall of the endodermis, it is likely that the same number of pits, concentrated into the restricted space of this heavily thickened wall, might well be at twice the density.

Conclusions

My study has disclosed several fruitful lines for further investigation, and provided working hypotheses for testing. The main lines are:

1. Significance of living epidermis

As argued above, it seems more likely that the root activity unique to the epidermis, and which may be lost in main roots whose epidermis dies, is likely to be the uptake of ions rather than the uptake of water. A test of this hypothesis might reveal, on the contrary, that the hypodermis is fully competent for both activities, and that the epidermis has no unique property except the ability to make root hairs.

2. Diffusivity in epidermal cell walls

The hypothesis is that high diffusivities of solutes are expected. Measurements like those of Canny and Huang (1993) might be extended to measure diffusivities of dyes in the epidermal cell walls of branch roots, but special refinements of the method would be necessary. Because the distances over which the solutes would move are so small (μm), and the expected values are higher, the time scale of the experiments would necessarily be extremely short (fractions of a second).

3 of/de 3

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

3. Structure and activity of the grooves

The diffusivities of solutes into the grooves over the radial epidermal walls could be measured by the refined method developed for the last experiments. Local exudation of organic materials out of the grooves on branch roots might be revealed by the tropic movements of motile bacteria, following the methods originally devised by Pfeffer (Bünning, 1989).

4. Role of class 1 roots

The hypothesis is that class 1 roots take up nutrient ions rather than water. Varney and Canny (1993) did not find any structural correlation with rates of water flux, but the root system in their mist-culture had very uniform branches, not the mixture of branch classes developed in field-grown roots. Experiments to test this hypothesis will need to be done with more heterogeneous, naturally-grown root systems.

5. Plugged determinate roots

The alternative hypotheses are: i) that such roots have minimal activities and are not contributing water or nutrients; or ii) that such roots take up nutrient ions but not water. Again, experiments must be done on mature, field-grown root systems.

REFERENCES

- Bowen GD and Rovira AD. 1976.** Microbial colonization of plant roots. *Annual Review of Phytopathology* **14**: 122-144.
- Bünning, E. 1989.** Transl. by HW Pfeffer, *Ahead of His Time: Wilhelm Pfeffer, Early Advances in Plant Biology*. Carleton University Press, Ottawa.
- Canny MJ. 1990.** Rates of apoplastic diffusion in wheat leaves. *New Phytologist* **116**: 263-268.
- Canny MJ and Huang CX. 1993.** Rates of diffusion into roots of maize. *New Phytologist* **125**: 000-000. (in press).
- Clarkson DT, Robards AW, Stephens JE and Stark M. 1987.** Suberin lamellae in the hypodermis of maize (*Zea mays*) roots; development and factors affecting the permeability of hypodermal layers. *Plant, Cell and Environment* **10**: 83-93.
- Enstone DE and Peterson CA. 1992.** The apoplastic permeability of root apices. *Canadian Journal of Botany* **70**: 1502-1512.

- Kuo J, O'Brien TP and Canny MJ. 1974.** Pit-field distribution, plasmodesmatal frequency, and assimilate flux in the mesophyll sheath cells of wheat leaves. *Planta* **121**: 97-118.
- McCully ME, Canny MJ and Steveninck RFM. 1987.** Accumulation of potassium by differentiating metaxylem elements of maize roots. *Physiologia Plantarum* **69**: 73-80.
- Peterson CA. 1987.** The exodermal Casparian band of onion roots blocks the apoplastic movement of sulphate ions. *Journal of Experimental Botany* **38**: 2068-2081.
- Peterson CA. 1988.** Exodermal Casparian bands: their significance for ion uptake by roots. *Physiologia Plantarum* **72**: 204-208.
- Varney GT and Canny MJ. 1993.** Rates of water uptake into the mature root system of maize plants. *New Phytologist* **123**: 775-786.
- Varney GT, Canny MJ, Wang XL and McCully ME. 1991.** The branch roots of maize. I. First-order branches, their number, sizes and divisions into classes. *Annals of Botany* **67**: 357-364.
- Varney GT and ME McCully. 1991.** The branch roots of *Zea*. II. Developmental loss of the apical meristem in field-grown roots. *New Phytologist* **118**: 535-546.

Varney GT, McCully ME and Canny MJ. 1994. Sites of entry of water into the symplast of maize roots. *New Phytologist* **126**: 000-000. (in press).

END

1 9-07-94

FIN