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**WATER UPTAKE INTO THE MATURE ROOT SYSTEM OF
AEROPONICALLY-GROWN MAIZE**

by

GREGORY T. VARNEY, B. Sc. (Hons.)

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

Master of Science

Department of Biology

Carleton University

Ottawa, Ontario

January 29, 1993.

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Thesis Supervisors

Chair, Department of Biology

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ABSTRACT

A method was developed to measure the rates of water uptake throughout the mature root system of plants of *Zea mays* (L.) cv Seneca Chief 125 grown by nutrient-mist culture (aeroponics). The root system of the aeroponically-grown plants was similar in (i) morphology; (ii) maturation of late metaxylem vessels (LMX) and (iii) suberization of the hypodermis and endodermis to plants grown under field conditions. Rates of water uptake (water fluxes per unit length J_L and surface J_S) were measured by the rate of accumulation of a marker solute (sulphorhodamine-G, abbreviated SR) on the root surface from a mist of 0.5 mmole l⁻¹ SR in water. The mean water fluxes (J_S) into branches and main roots were similar at approximately $5 \pm 5 \mu\text{l cm}^{-2}_{\text{root}} \text{h}^{-1}$; maximum fluxes into each were 40 and 16 $\mu\text{l cm}^{-2}_{\text{root}} \text{h}^{-1}$ respectively. In a systematic survey of main roots and branches throughout the root system, highest fluxes occurred in the apical 25 cm of the main roots; highest fluxes into branches were measured in branches 25 - 70 cm from the apex of the main root (25 cm is *ca* the point of maturation of the LMX of the main roots). The volume of water collected per hour on a 1 cm segment of main root was eight times less than the volume collected by the branches on that segment (which had 8 times more surface). The site of accumulation of SR was investigated by retaining the dye *in situ* by freeze-substitution and anhydrous embedding in resin. Observation by light microscopy revealed dense accumulations of SR on the surface of the epidermis or (in some roots) the hypodermis. Accumulation of SR on the cell surface resulting from the separation of SR from the water by osmotic filtration by the plasma membrane, indicates the sites of entry of water into the root symplast at the epidermis and hypodermis. SR diffused inwards through the cell wall apoplast up to the anticlinal walls of the hypodermis. SR did not diffuse beyond the hypodermis into cortical cell walls unless the surface layers were damaged.

ACKNOWLEDGEMENTS

I wish to express my thanks to Drs M. E. McCully and M. J. Canny for their time, patience and encouragement in helping me complete this thesis, and for providing me with a first class education. This project would not have been possible without the skillful technical assistance of Cliff Wilson in building and maintaining the mist boxes, and the dedicated support of Ed Bruggink, Art Goodenough and Henk Datema in operation of the aeroponic culture system. Thanks to Dr. H. Damman for always having time to answer MacIntosh and statistical questions. I would like to thank the Ontario Government for an Ontario Graduate Scholarship. I also thank Kathleen Ismond, Niamh O'Dowd and Michelle Watt for their help with the finishing touches. Special thanks to Nori Hubbs for helping type many parts of this thesis and for her patience, and also to my parents for their support and encouragement.

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CHAPTER 1

GENERAL INTRODUCTION

Water is essential to all forms of life. Life itself evolved in an aquatic environment. Although plants are among the oldest organisms in the fossil record, they have been on land for only a relatively short time (the last 10% of the age of the earth). This delay must have been caused by the complexity of evolving structures capable of collecting and distributing water throughout the plant body, as well as conserving it in an inherently dry environment (Boyer, 1985). Water plays a crucial role in almost every process which occurs in plants, "ranging from photolysis during photosynthesis to hydrophobic bonding of macromolecules to the maintenance of form in non-woody tissue" (Hsiao, 1973).

The forces which drive water movement are understood, and we can determine these forces and the resulting volume flows through plants. However, the pathway of water movement through a plant is only partially understood, and viewed by many as a "black box" (Boyer, 1985). This gap in our knowledge is the result of our inability to label and follow the movement of water through the plant. The most simple question Where does water enter the plant? is far from understood. There are several reasons why we do not clearly understand where water enters a root.

Roots are hidden underground, which makes them difficult to observe and very inaccessible. Roots of *Prosopis juliflora* are an extreme example, since they have been found alive at a depth of 53 m during the development of an open pit mine (Phillips, 1963, in Nobel, 1991)! Unearthing a root system of a mature plant of maize *Zea mays* L. (which has 50-70 roots which can each be a metre or more long) is a difficult task; this usually results in the destruction of many of these roots. It is therefore, usually not possible to study roots while they are still on the plant under normal field or pot conditions. Thus most researchers resort to studying water uptake into primary roots of small seedlings in a soil-free situation. For example, the results of Graham *et al.* (1974) and Sanderson (1983) obtained using such seedlings, which have been accepted by most physiologists, showed that the highest fluxes of water occurs in the apical 10 cm of the primary root of barley (*Hordeum vulgare*) and marrow (*Cucurbita pepo*). These studies

have led to the generally accepted view that water uptake occurs only in the apical regions of roots. Although this is probably true for small seedlings, the results of studies of the roots of mature plants of maize grown under field conditions contradict this (St. Aubin *et al.*, 1986). The contradiction is that the apical regions of the main roots (i.e., the primary roots and nodal roots) have low axial conductance for water in the apical *ca* 20 cm, while the more proximal regions have much higher conductance.

The main roots of mature plants of maize appear to be dimorphic when grown in the field. The apical 20 - 30 cm of the roots are covered by a coherent soil sheath (termed sheathed roots). The sheath is shed from the proximal regions of the main roots near the base of the plants and the roots develop many fine branches (bare roots, McCully and Canny, 1985). These two regions are different, both structurally and physiologically. The late metaxylem vessels (LMX) are immature in the sheathed regions, with intact end walls and living protoplasts in the apical 20+ cm. Even though there are numerous protoxylem (PX) and early metaxylem (EMX) vessels which are mature and thus open to conduct xylem sap in the sheathed regions, the amount of xylem sap which can be carried is very low because of the small diameter of these vessels. (Since flow through a tube varies with the fourth power of tube radius, one LMX vessel carries 4000 times more than an PX or EMX vessel, see McCully and Canny, 1988). Consequently, these sheathed regions are characterised by their low axial conductance. The end walls and protoplasts of the LMX are lost in the bare regions and the LMX become true vessel elements. The bare regions are characterised by their high axial conductance through their open LMX vessel elements (St. Aubin *et al.*, 1986).

The low axial conductance of the apical regions of the field-grown roots of maize is surprising, and this suggested that the bare regions and especially the many branches found there must play an important role in the water uptake of maize roots (St. Aubin *et al.*, 1986; McCully and Canny, 1988). The need for studies of the structure and function

of the first order branches has been expressed in several studies (Hoppe *et al.* , 1986; McCully and Canny, 1985, 1988; Wenzel, *et al.*, 1989).

Several aspects of the first order branches on maize roots have been studied, beginning with their initiation and development (Bell and McCully, 1970), followed by studies of their frequency, size and distribution (Varney *et al.*, 1991), development of determinate apices (Varney and McCully, 1991) and their response to transpiration demands of the shoot (Wang *et al.*, 1991). However, nobody has attempted to study uptake of water by branches.

In this thesis I will describe my development of a new method of measuring water fluxes into roots in order to explore the uptake of water throughout the root system including both main roots and branches. This method derives from the recent work on water movement through leaves (Canny, 1990a, b, c). Canny showed that dye tracers cannot show the path of the transpiration water beyond the xylem, but they can be used to show where water crosses cell membranes from the apoplast to the symplast. At this point the dye is separated from solution and accumulates at a high concentration. Canny (1990a) has called these sites of dye/water separation *sumps*. My new method applies this idea at the other end of the plant. It tests the hypothesis that a similar separation of dye from water would occur in the roots. If so, then the accumulation of dye can be used to determine both where water enters and how much water enters at each place.

Plants were grown for 30 - 70 days in a nutrient mist culture system (aeroponics, see Chapter 2). Water uptake was measured by the rate of accumulation of the apoplastic tracer Sulphorhodamine-G (abbreviated SR) from a mist of SR-water. The rate of water uptake into branches and main roots was studied throughout the root system. The sites of accumulation of the marker were observed by light microscopy to determine the site of entry of water into the root symplast. The morphology of the mature root system on plants grown aeroponically was compared with that of field-grown plants. Several

structural features were studied including the development of mature LMX and suberin lamellae in the hypodermis and endodermis.

CHAPTER 2

GENERAL METHODS FOR CULTURING PLANTS OF MAIZE AEROPONICALLY

INTRODUCTION

Plants were first grown in nutrient mist culture (aeroponically) by Carter (1942) to study the effect of mealy bug infestation on pineapple plants. This method was developed to allow for repeated and critical examination of roots without causing the damage which would result from the examination of roots grown in sand or water culture. Carter (1942) also proposed that this method would be useful for studying other aspects of roots and several researchers have used this technique for this purpose. There have only been a few studies which have used aeroponics since that time.

Aeroponics was then used to study the effect of *Phytophthora* infection and the toxicity of nitrite and other ions on citrus and avocado roots (Klotz, 1944). Aeroponics has also been used to inoculate the roots of a variety of plants with nodule-forming rhizobia (Zobel *et al.*, 1976). Hubick *et al.* (1982) compared aeroponically-grown *Helianthus annuus* (sunflower) with plants grown by a continuous flow culture method and by traditional sand culture techniques. Zobel (1989) has recently reviewed aeroponic culture systems used to grow plants of maize and other species. However few morphological and physiological measurements of these plants were included in this review.

Since there are a variety of systems available to culture plants aeroponically, this chapter is intended to provide a detailed description of the materials and methods used to grow the plants of maize aeroponically. All plants used in this project were cultured by these methods. The general morphology and size of the roots of aeroponically-grown plants of maize are presented in Chapter 3.

MATERIALS AND METHODS

I. Plant Material

All experiments were performed with plants of *Zea mays* cv. Seneca Chief (Stokes Seed Co., St. Catherine, Ont., Lot. No. 9156-T), grown in the Environmental Laboratories Biology Annex Greenhouse at Carleton University (Ottawa, Ont.). The temperature in the greenhouse ranged from 17 to 36 °C, relative humidity 20 - 90%. Plants were grown under sunlight and supplemented by 400W HIO Optimarc Super Metal Halide lamps (Average intensity $250 \mu\text{mole m}^{-2} \text{s}^{-1}$; 18h photoperiod; spectral emission see figure 1). These plants were grown from seeds which were germinated and grown in several ways.

1. Pot-grown plants

Seeds were germinated and grown in large (30 cm) peat pots in 'Pea Mixture' soil (1:1:1 mixture of a sandy loam soil, peat, perlite; this mixture was then pasteurised for 30 min at 180 °C). Plants were watered twice-daily and fertilized weekly with a solution of 20:20:20 N:P:K fertilizer in dechlorinated water (*ca* 7 g l⁻¹).

2. Aeroponically-grown plants

Seeds were either (i) germinated in 'Pea Mixture' soil in 12-cm peat pots or (ii) germinated in vermiculite then transferred to 12-cm peat pots of soil. Prior to transfer to the aeroponic chambers (hereafter referred to as mist boxes), plants were watered twice-daily and fertilized like the pot-grown plants. Once the primary root and several main roots had pierced the pots, the plants were prepared for transfer to the mist boxes. To reduce fungal growth which originated from the peat pots, the seedlings were watered with a solution of No-Damp (15 ml l⁻¹; Plant Product Co. Ltd., Bramalea, Ont., L6G 1G1). The plants were watered with this fungicide 24 and 12 h prior to transfer to the mist boxes, and at the time of transfer. The seedlings were transferred to the mist boxes by placing the peat

pots in which the plants were growing into 12 cm diameter holes in the styrofoam lids so that their roots hung downwards inside the mist box (see figure 2).

Figure 1: Emission spectrum of 250W Otimarc HIO Super Metal Halide Lamp (from Optimarc, Inc.).

II. Aeroponic Culture System

The mist boxes used were simplified versions of the aeroponic culture system described in Zobel (1989). The mist boxes were constructed of plywood and sealed on the inside with plastic resin to prevent leakage and deterioration of the plywood. The chambers of the boxes were approximately 0.65 x 0.8 m square and 1.25 m deep. One box could accommodate eight plants (box # 1), while the other could hold four plants (box # 2). The central hole in the lid was left open to improve circulation of mist in the upper regions of the boxes. Plants were grown in the boxes for up to 70 days. The roots of the plants were accessed through a door built into one side of each box. Their roots were continually wetted with mist, which was generated by spraying a stream of nutrient solution onto a spinning disk at the bottom of the boxes (figure 2). These rotating discs were driven by motors recycled from Warburg manometers (Motors and stands by Georgii Kobold Electro-Motoren Apparatebau model KOD-444-S52a, 1400-1700 rpm, Leinfelden,

Stuttgart; Disk drive systems by B. Braun Apparatebau Melsungen, model UVL13). The endurance and reliability of these motors was invaluable since all other motors used could not withstand continual use for such long periods of time. To prevent vibration of the plants neither the spinning disks nor the motors were attached to the boxes. To prevent corrosion of the motors by the solution the motors did not come in contact with the nutrient solution.

The nutrient solution was circulated by Magnetic Drive Pumps (March Mfg. Inc., Fisher Sci. Ltd.). The solution was pumped from the reservoir onto the spinning disk, then drained back into the reservoir to be recirculated. The reservoir for box # 1 held approximately 45 l, and that for box # 2 had a 35 l capacity. The reservoirs were kept dark under black plastic to reduce algal growth. The essential elements were provided by a nutrient solution diluted with dechlorinated water to produce mixtures ranging from 75% to 25% strength solution. The levels of nutrients in this solution are listed in table 1. Solution pH ranged from 7.2-7.8. Fresh solution was added as required (1-2 times weekly), or replaced when the plants appeared deficient or the reservoir became dirty.

Figure 2: Illustration of the mist box apparatus used to culture maize plants aeropically. Plants were grown with their roots continually wetted by a nutrient-mist inside the chamber (A). The mist was produced from a nutrient solution (B) which was pumped (C) onto a rapidly-spinning disk (D). The disk was driven by a motor (E) which was isolated from the box and the nutrient solution as indicated. Control plant covered or detopped (F).

Table 1: Concentration of elements in the stock nutrient solution, and diluted solutions applied as a mist to the maize root systems.

Percent of nutrient solution	CONCENTRATION ($\mu\text{moles}\cdot\text{l}^{-1}$)		
	100%	75%	25%
NUTRIENT			
KNO₃	5 X 10³	3.8 X 10³	1.3 X 10³
Ca(NO₃)₂·4H₂O	2 X 10³	1.5 X 10³	5 X 10³
MgSO₄·7H₂O	1 X 10⁴	7.5 X 10³	2.5 X 10³
K₂HPO₄	1 X 10³	7.5 X 10²	2.5 X 10²
Fe(NH₄)SO₄·6H₂O	18.5	13.9	4.6
Versenol (Fe-EDTA)	6.1	4.6	1.5
H₃BO₃	46.2	34.6	11.5
MnCl₂·4H₂O	9.1	6.8	2.3
ZnSO₄·7H₂O	7.7 X 10⁻¹	5.7 X 10⁻¹	1.9 X 10⁻¹
MoO₃	4.9 X 10⁻¹	3.6 X 10⁻¹	1.2 X 10⁻¹
CuSO₄·5H₂O	3.2 X 10⁻¹	2.4 X 10⁻¹	8.0 X 10⁻²

The essential elements provided by each form of nutrient are in bold typeface. Formulation modified from Hoagland and Arnon (1938).

The vertical distribution of mist inside the boxes was measured by suspending Eppendorf tubes (mouth area 0.5 cm²) at five different heights and two positions in each box. The tubes were suspended on strings at a 45° angle. The volume of nutrient solution collected in a given time was measured gravimetrically (assuming 1 g = 1 ml), and expressed as $\mu\text{l h}^{-1} \text{mm}^{-2}$. The distribution profile of mist in the boxes is shown in figure 3.

Figure 3: Vertical distribution of mist in the mist boxes

After each experiment, remaining plants were either discarded or their roots collected and stored in 30% ethanol, and the mist boxes were cleaned with Javex or detergent. The boxes were rinsed several times with tap water, then tap water was circulated in the boxes for several days. During this rinsing period, the water was replaced each day to remove any residual SR or detergent so that the boxes were clean for subsequent use.

CHAPTER 3

MORPHOMETRY OF AEROPONICALLY-GROWN PLANTS OF MAIZE

INTRODUCTION

It is not surprising that the methods used to grow plant materials for experimental studies may influence the morphology and physiology of the plant material. For example, it has recently been shown that plants of honey locust (*Gleditsia triacanthos*) cultured hydroponically or by sand culture have morphological differences in their root systems (Graves, 1992). Roots of plants grown by sand culture are shorter and more extensively branched than the roots of hydroponically-grown plants (which also lack root hairs). The dry weight and hydraulic conductivity (L_p) of the roots of plants cultured by these two methods were similar (Graves, 1992). Therefore it was necessary to study the morphology of the roots of aeroponically-grown plants of maize to determine if they are comparable to the roots of field-grown maize.

Weaver *et al.* (1922) and Weaver (1926) conducted extensive studies on the size and distribution of the root systems of various cultivars of *Zea mays* grown in soil at many different sites in the United States. Hoppe *et al.* (1986) studied the development and structure of nodal roots (main roots) of maize plants which were grown in the field at the Central Experimental Farm, Ottawa. Varney, Canny, Wang and McCully (1991) have also studied the size, length and distribution of first order branches on the main roots of field-grown maize.

There are various methods available for studying roots grown in the field. Early researchers such as Weaver (1926) and others like Dittmer (1938) and Pavylchenko (1937) excavated the root system of field-grown plants by digging a 1-3 m deep trench on one side of the plant then carefully excavating along the trench wall towards the plant. The size and position of the exposed roots were recorded by making detailed illustrations as the roots were unearthed. Rather than measure the entire root systems, reasonable estimates of the dimensions and structure of maize roots can be determined by collecting samples systematically at various positions on the root system (Varney *et al.*, 1991, and Varney and McCully, 1991). Morphological studies of aeroponically-grown plants have

not previously been conducted, therefore it was necessary to determine the size of these roots.

Presented in this chapter are the methods used in this thesis to determine the size and morphology of the root systems of plants which have been grown-aeroponically, and the results of these studies. Many of these measurements were conducted during the studies of water uptake, therefore the methods for mensuration of the roots were common to all experiments in chapter 5. The results of these morphological studies are necessary for calculating water uptake.

MATERIALS AND METHODS

I. General Morphology of the Root System

The architecture of the root system was observed on plants which were grown in the mist boxes for 50 to 70 days. The root systems of some plants were collected and observed fresh. Others were grown, then detopped and their roots stored in 30% ethanol until they were studied (n=10 plants). The node of origin of the main roots (also referred to as main axes), was observed by removing all the main roots and then bisecting the stem base longitudinally with a knife. The number and length of main roots on these plants were also noted. Finally, the size, frequency and distribution of the first order branches on the main axes were determined. Note that only first order branches were studied.

II. Measurement of the Root Systems

1. Dimensions of individual roots

The size of individual roots was measured from main roots and branches collected during several experiments, including the measurement of SR elution rates, "Water Free Space" and during the preliminary SR accumulation experiments (see Chapter 5). Branch diameter (d) was measured either from negatives of

photomicrographs of hand-cut transverse sections, or by placing the branch on a micrometer in a Petri dish filled with tap water and observing with a dissecting microscope. Diameter of main roots was also measured with a micrometer observed through a dissecting microscope. Mean diameter of branches and main roots was calculated from these data. The lengths (L) of branches and main roots were measured with a ruler. Root surface area was calculated by the formula for surface area of a cylinder ($S.A. = \pi d L$), from the diameter (d) and length (L) of the root. The dimensions of the entire root system of the aeroponically-grown plants were also determined.

2. Dimensions of the root systems

The length of the main roots was determined during the morphological studies (above). The frequency, length and distribution of first order branches found along the main roots were surveyed using a systematic sampling procedure similar to that used by Varney *et al.* (1991). One main root was collected from the root system of each of five plants (ages 40-60 days), which had been stored in 30 % ethanol. Centimetre-long segments of main root were collected from each fifth centimetre along the entire length of the root. The number and length of all the branches on each 1-cm segment were measured. These measurements were repeated on all the segments from the entire length of main root. This procedure was repeated on all five main roots. These measurements were used to calculate the mean number and length of branches at each position on the main roots. An overall mean length and frequency of branches on the main roots, as well as the total length and surface area of all branches and main roots were calculated from this data.

III. Measurement of Shoot Size

The surface area of the shoots of plants used in experiments 2, 4, 1.3, and 1.4 (Ch. 5) were estimated from photocopies of the shoots. This was done by first determining the

mass of 1 cm² of paper by weighing 2 x 5 cm pieces of paper and dividing the mass (g) by the surface area as follows:

$$\frac{\text{Mass of piece of paper}}{20 \text{ cm}^2} = \text{mass of 1 cm}^2 \text{ paper.}$$

The mean mass of 1 cm² of paper was 0.0042 g cm⁻² (from three samples). The photocopied images of each shoot were cut out and weighed. Surface area was estimated by dividing the mass of the shoot photocopy by the mass of 1 cm² of paper.

$$\frac{\text{Mass of the photocopy of shoot (g)}}{\text{Mass of 1 cm}^2 \text{ paper (g cm}^{-2}\text{)}} = \text{surface area of shoot (cm}^2\text{)}$$

RESULTS

I. General Morphology of the Root System

The numbering system used to describe the nodal roots of maize derives from the study by Hoppe *et al.* (1986). The root system was made up of one root from the seed (seminal or primary root) and successive tiers of roots from the first six stem nodes. (The primary and roots emerging from the stem nodes will be referred to as main roots or main axes.) After the seminal root emerges from the seed and elongates, the main roots develop successively from each stem node starting at node 1 (the coleoptilar node), followed by node 2, then node 3 and continuing to nodes 7 or 8. The seminal root and roots from the first five stem nodes penetrate the peat pots and develop inside the mist boxes. The number of main roots from each stem node was observed on plants at a stage of maturity

similar to those used in each experiment. There were approximately four main roots from node 1; nodes 2 through 5 had five to eight main roots, node 6 had up to 16 main roots.

After 50 -70 days growth in the mist boxes, main roots from stem nodes 1 to 3 were longer and more numerous than the younger roots from higher stem nodes 4 - 6. These roots from nodes 1-3 were similar in diameter and were so entangled that it was difficult to follow the roots and determine the length of main roots from each stem node. There were 7 to 20 main roots which were very long (ca 100 cm) on each plant (Mean 11 ± 4 roots, $n=10$ plants, 50-70 days).

II. Measurement of the Root System

1. Dimensions of individual roots

(i) Main root diameter and length

Diameter measurements were made from the main roots collected for the 'Water Free Space' experiments (see Ch.5). (All measurements and the variation of these values are presented as the mean $\pm$ standard deviations.) The younger, apical regions (apical 20-30 cm) were 1.3 ± 0.3 mm in diameter (modal diameter = 1.3 mm, $n=24$). Older, basal regions of the main roots were 0.9 ± 0.2 mm in diameter (modal diameter 1mm, figure 1). Root diameter ranged from 0.5-1.4 mm in diameter, however the diameter of most roots was 1-1.1 mm (figure 1).

Figure 1: Histogram of main root diameter (n = 67, basal regions).

The length of the main roots used in each experiment varied depending on the length of time the plants were grown in the boxes. The roots of seven plants (50 days old) from one experiment were 80-90 cm long. The roots from three 70-day old plants ranged from 50-190 cm long (mean length 88.2 ± 41 cm long, $n=32$ roots). Roots from the higher nodes (5 and 6) rarely exceeded 30-40 cm in length.

(ii) Branch diameter and length

The diameters of branches were measured during the 'Water Free Space' and water uptake experiments (Ch.5). These measurements were pooled (288 branches collected from 27 plants); the mean diameter calculated from this data was 0.34 ± 0.1 mm (figure 2).

Figure 2: Diameter of first order branches from the WFS and Water Uptake Experiments (n = 288).

Branch diameter ranged from 0.15-0.75 mm, however 77% of these branches have a diameter of 0.25 - 0.45 mm. The mean diameter (0.34 mm), modal class (0.3-0.35 mm) and median are all similar.

The length of the branches in this collection was also measured (figure 3). The length of branches in figures 3 and 4 are not normally distributed, and means are not very useful for comparison of such data. It is therefore more rational to compare the ranges and distributions of branch length.

Figure 3: Length of branches used in the WFS and Water Uptake Experiments (n = 288).

Figure 4: Branch length from a survey of root system (n=787, 50-70 days)

Several long branches raised the mean length of these branches to 45 ± 30 mm, ($n=288$). This mean was slightly larger than the modal length (37 mm) and the median (30 mm). The branches from this collection were randomly selected from the main roots during the WFS and water uptake experiments. They were therefore carefully excised and measured, then stored individually in tubes of 30% ethanol. When branch length was measured during a survey of the whole root system, the mean length was shorter (26 ± 18 mm, $n=787$; 5 plants, 40-60 d). Most of the branches measured during this survey were very short (1-5 mm, figure 4). The modal length of these roots was 0-5 mm, median = 10 mm. The total length and surface of first order branches are calculated from the length measurements and the frequency and distribution of branches on each 1-cm segment of main roots (see below).

II. Dimensions of the Root System

The number (7-20 main roots per plant, mean 11 ± 4 roots) and length of main roots (50-150 cm) were determined. It was then necessary to determine the frequency, distribution and length of the first order branches on these roots. The number of branches on each fifth centimetre of main root was constant along the length of the main roots (figure 5, except the apical 10 cm which had not yet developed branches). The mean number of branches/cm main root was 9.4 ± 2.6 branches/cm ($n=5$ roots; 1 root from each of 5 plants).

The mean length of branches on each fifth centimetre of main root (figure 6) shows a barely perceptible dependence with distance from the root apex. Many branches on the basal regions of the main roots get extremely long (see large standard deviation at 75 cm, figure 6). Only a few of these long branches were collected in each fifth centimetre during the systematic sample collection. Therefore the branches appear only

slightly longer in the basal 50 cm than branches in the apical parts of the main roots. The overall mean length of all the branches in this survey was 2.6 ± 1.8 cm ($n=787$).

Figure 5: Frequency of first order branches along the main roots (Mean 9.4 ± 2.6 branches/cm; error bars=SD)

Figure 6: Mean length of first order branches at each position along the main roots (Overall mean length 26 ± 18 mm; $n=787$).

These figures have been summarized in table 1. The branches have 8 to 14 times more surface area than the main roots.

Table 1: Summary of morphometric measurements of the roots of aeroponically-grown plants of maize.

1. Number of main roots:	11 $\pm$ 4 roots	
2. Length of main roots:	88 cm	
3. Diameter of main roots	0.1 cm	
4. Surface area of 1 main root	27.7 cm ²	
5. Total length of main roots	968 cm	
6. Total surface area of main roots	304 cm ²	
1. Branch frequency	9 $\pm$ 3 branches/cm main axis	
2. Branch length	(from survey) 2.5 cm	(from fig.6) 4.5 cm
3. Branch diameter	0.34 $\pm$ 0.1 mm	
4. Total length branches	21 780 cm	39 204 cm
5. Branch surface area/ cm axis	2.4 cm ²	4.3 cm ²
6. Total branch surface per main axis	211 cm ²	380 cm ²
7. Total branch surface area	2323 cm ²	4175 cm ²
8. Surface branches/surface main roots	8 times	14 times

III. Shoot Size and Condition

The shoots were 0.5-1.25 m tall and the mean surface area of the leaves was 3200 $\pm$ 1200 cm² (table 2). Surface area of the leaves of seven plants ranged from 1630 cm² to 5500 cm². If the plants remained in peat pots on the bench for more than 3 or 4 weeks prior to transfer to the mist boxes, the resulting plants remained very short even though they had developed extensive root systems in the mist boxes. This diminutive stature was due to reduced stem elongation, and such plants had large leaves and would

eventually tassel even though they were short. Such 'Bonsai' plants were not included in these studies.

The leaves sometimes had a striped appearance due to a slight chlorosis in the mesophyll between the lateral veins. It was thought that this was due to an iron deficiency, and iron was supplemented by addition of Fe-HEDTA to the nutrient solution to a final concentration of $8 \mu\text{mole l}^{-1}$ Fe (from the recipe of Chaney and Bell, 1987). This had little effect on the striped appearance of the leaves.

Table 2: Surface area of the shoots of plants grown in the mist boxes.

Experiment	Age (days)	Length (cm)	Surface Area of Shoot (cm²)
2	58-65	120	1633
2	58-65	121	2808
2	58-65	130	2783
4	70	92	3512
5	50	145	2647
1.3	50	216	5513
1.4	38	145	3149

DISCUSSION

I. General Morphology of the Root System.

The root system of maize consists of a seminal root and tiers of main roots each of which originates slightly above a stem node. These tiers of roots develop sequentially from the first to the seventh or eighth lowest nodes beginning at the coleoptilar node (Hoppe *et al.*, 1986). Under field conditions, main roots from the first 5 nodes developed below the soil surface from the crown (stem base); main roots developing from sixth and seventh nodes were initiated above the soil surface and later grew down into the soil (tier 7 roots grow slowly). The root system of maize plants which were grown aeroponically for *ca* 50 days consisted of a primary root and 10 - 20 main roots from the first 3 or 4 stem nodes. After 70 days growth in the mist boxes, main roots from stem nodes 4, 5 and 6 also penetrated the peat pots and developed inside the mist chambers, however these roots did not normally exceed *ca* 50 cm. The framework of main roots which make up the root system of aeroponically-grown plants was similar to those grown in the greenhouse in pots or in the field (with the exception that in the field 6 tiers of root developed underground, Hoppe *et al.*, 1986).

There were 4 main roots from node 1, 5 - 8 main roots from nodes 2 - 5 and *ca* 16 main roots from node 6 of the aeroponically-grown plants of maize. This was similar to the number of main roots produced on the lower stem nodes of field-grown and pot-grown plants (Hoppe *et al.*, 1986). Hoppe *et al.* (1986) found that there was a strong environmental influence on the number and distribution of main roots above node 5. There were fewer main roots from the higher nodes (nodes 5 - 7) and these were less developed on the aeroponically-grown plants. Of the approximately 70 main roots which develop on maize under field conditions, over half are from stem nodes 6 and 7 (Hoppe *et al.*, 1986). Greenhouse-grown plants of maize tend to be more spindly and have smaller diameter stems than those of field-grown plants (0.3X diameter of field-grown plants).

According to Hoppe *et al.* (1986) who cite Martin and Hershey (1935), more main roots of larger diameter were found on plants with larger diameter stems. Stem diameter was not measured, however, it was noted that in general stem diameters of aeroponically-grown plants of *Zea* appeared smaller than those of pot-grown and field-grown plants. This may account for the reduction in numbers of main axes from nodes 5 through 7 on the aeroponically-grown plants.

II. Measurements of the Root System

1. Dimensions of individual roots

(i) Main roots

Under field conditions the mean diameter of main roots increased with node of origin, where node 1 roots had smaller diameters than node 2 roots and which were in turn smaller than node three roots. Root diameter on field-grown plants ranged from 0.3 mm (node 1) to 2.1 mm (node 4) (Hoppe *et al.*, 1986, table 2). Unlike the main roots from the different stem nodes of field-grown plants, which can easily be differentiated by their diameter, the main roots from the seed and stem nodes of plants grown in the mist boxes were difficult to distinguish because they were uniform in diameter. The diameter of main roots from the stem nodes 1 - 4 from the aeroponically-grown plants ranged from 0.5 - 1.4 mm (mean diameter 0.9 ± 0.2 mm, node 1 - 1.1 mm). The apical regions of the main roots had larger diameters (1.3 mm). (Weaver (1926) reported diameters of 3 - 5 mm at the tip of roots in the field.)

The length of main roots on plants grown in the mist boxes increased over time. Main roots from the seed and stem nodes 1 - 3 of 70-day old plants were normally 88 ± 40 cm (nodes 4 - 6 were 30 - 50 cm), but sometimes reached 120 - 150 cm in length. Roots from stem nodes 4 - 6 were 30 - 50 cm in length. This was well within the range of main roots on field-grown plants of maize. Weaver *et al.* (1922) and Weaver (1926) report main roots at depths of 135 - 140 cm (55 days) to 180 - 250 cm (116 days).

(ii) Branches

The first order branches on the main roots of aeroponically-grown plants ranged from 0.15 - 0.75 mm in diameter, however most branches were 0.3 - 0.35 mm (mode) similar to the mean diameter 0.34 ± 0.1 mm (figure 2). The range of diameter of these branches was similar to that of branches on maize plants under field conditions (0.1 - 0.8 mm diameter), but the 4 classes of branches found on the roots of field-grown plants (Varney *et al.*, 1991) could not be distinguished on roots from the mist boxes (figure 2). Miller (1981) found 12 classes of branches on the primary roots of maize seedlings which were grown hydroponically. The range of diameter of branches was greater in this study (0.05 - 1 mm, Miller, 1981), however he did not determine if these were primary, secondary or tertiary branches.

The mean length of first order branches on the roots of plants grown in the mist boxes varied depending on the method of sample collection (see figures 3 and 4). Branches collected during the WFS and water uptake experiments (figure 3) were selected randomly from various positions along the main roots. Most of the branches collected by this method were 20 - 40 mm long (mode 37 mm), however a few long branches raised the mean to 45 mm (figure 3, $n = 288$ roots). From the survey of the entire root system, branches on each fifth centimetre of main root of aeroponically-grown plants were mostly shorter than 10 mm (figure 4). The mean length (26 ± 18 mm) was greater than the mode (0 - 5 mm), and median (10 mm). Roots used for the survey were collected from the root systems of five plants which had been stored in a bucket of 30 % ethanol. It was necessary to untangle the roots and separate one main root from many others on the plant. Centimetre-long segments were excised from each fifth centimetre from the entire length of each main root. Therefore some branches may have been broken or cut during this process. The segments of main roots with their attached

branches were used to determine the frequency and distribution of first order branches along the entire length of the main roots (see below).

The mean length of branches from the survey was similar to that obtained in a survey of the length of branches on field-grown plants (mean 30 mm, mode 6 - 10 mm, Varney *et al.*, 1991). The illustrations published by Weaver (1926), also show that the branches on main roots of maize grown under a variety of conditions were mostly short (50 mm) when the plants were fully irrigated (50 mm) or longer under dry soil conditions.

2. Dimensions of the root system

The number of branches on each fifth centimetre of main root was similar along the entire length of the main roots on the aeroponically-grown plants (mean 9 ± 2 branches/cm axis) except for the apical 10 cm which did not have branches (figure 5). This agrees with Varney *et al.* (1991), who found there was no variation in the linear density of branches on the tier 2 roots of pot-grown plants. The overall number of branches per centimetre of main root on field-grown maize was 12 ± 4 branches/cm (Varney *et al.*, 1991). Weaver (1926) found 4 - 13 branches/cm main root on field-grown maize.

Branches were slightly longer on the basal regions of the main roots (60 - 100 cm from the tip, figure 6). Varney *et al.* (1991) did not measure the length of branches along the main roots of field-grown maize. Branches on regions of main roots in the first 25 cm of soil were very much longer than branches on main roots in deeper soil horizons (Weaver *et al.*, 1922, Weaver, 1926).

III. Leaf Surface Area and Shoot Condition

The surface areas of the plants used in these studies (table 2) were much larger than the values for aeroponically-grown plants studied by Wenzel *et al.* (1989). The plants used in this thesis were much older and therefore much larger than those of Wenzel *et al.*,

(1989). The surface area of pot-grown plants was not measured. However, the group of largest plants from the mist-boxes (table 2, experiment 1.3) were similar to pot-grown plants of similar age.

Unsuccessful attempts were made to eliminate the striped appearance of the leaves by addition of iron supplements in a form which were more available to maize roots (Fe chelated to HEDTA rather than EDTA). This suggests that perhaps there was a deficiency of some other element under these conditions. Iron chelated to EDTA is unavailable to maize; this deficiency reduces growth of the main root which increases branch growth, and causes chlorosis in leaves of maize plants even when iron is supplied at high levels (Chaney and Bell, 1987). The iron chelated to HEDTA was simply added to the nutrient solution which contained EDTA, which may have made the supplemented iron unavailable also. Therefore, the iron supplement (FeHEDTA) should be added to nutrient solution which does not contain EDTA. An investigation of the mineral requirements of aeroponically-grown maize plants could require a study similar in size to this thesis. This was the only difference between the plants grown in the mist boxes and those grown by other methods, and therefore should have little effect on the results of this thesis.

CHAPTER 4

STRUCTURAL DETAILS OF AEROPONICALLY-GROWN PLANTS OF MAIZE

INTRODUCTION

The development of mature LMX vessels plays an important role in the capacity of a root to conduct xylem sap (discussed in the first chapter). It is now clear that the LMX vessels are living, with intact end walls and protoplasts in the apical 20 -30 cm of the main roots of field-grown maize. The xylem vessels mature in the more proximal regions of the main roots, losing their end walls and protoplasts to become true vessel elements which are thus conduits open for the movement of xylem sap (St. Aubin *et al.*, 1986). This delayed development, not recognized earlier, was subsequently also observed in other C4 grasses (Wenzel *et al.*, 1989), soybean (Kevekordes *et al.*, 1988), barley (Sanderson *et al.*, 1988), common bean (Jaramillo *et al.*, 1992) and sorghum (Cruz *et al.*, 1992).

Xylem maturation is influenced by soil compaction, whereby increased soil bulk density causes maturation of LMX to occur closer to the root apex in common bean (Jaramillo *et al.*, 1992). Soil moisture deficits also influence xylem maturation. Roots of sorghum which were growing through vermiculite at very low moisture contents ($\theta = 6.5\%$) developed only mature EMX (PX and LMX remained immature as determined by a dye perfusion technique). Low water supply slightly reduced the diameter of the LMX in primary roots of tropical maize (Weerathaworn *et al.*, 1992). Until now, openness of LMX has not been considered in relation to water uptake studies.

Potometric studies of water uptake revealed higher water fluxes into the apical regions of roots of barley (*Hordeum vulgare*) and marrow (*Cucurbita pepo*) (Robards *et al.*, 1973). Reduced rates of water uptake in the basal regions were correlated with modifications which occur in the walls of the endodermis (see figure 1). These modifications include: (1) the incrustation of a band of suberin and lignin-like compounds in the primary anticlinal walls (termed Casparian bands); (2) the subsequent adcrustation of a layer(s) of similar compounds on the inner surface of all primary walls (termed suberin lamella(e)); and (3) subsequent deposition of layers of cellulosic wall overlying

the suberin lamellae on the inner tangential and anticlinal walls (Esau, 1977). These modifications to the endodermal cells were thought to block movement of water and solutes through the wall apoplast; any movement across this layer would be *via* the cell symplast (Robards *et al.*, 1973; Clarkson and Robards, 1975). These conclusions led many to assume that regions of main roots with mature endodermis no longer play an important role in the absorption of water and solutes by the root system (even though Clarkson and Robards (1975) admitted that the unsuberized regions make up only a small proportion of the total root length).

The hypodermis, a cell layer between the epidermis and cortex, was overlooked by physiologists until it was found to contain Casparian bands and suberin lamellae in its walls. The formation of suberin lamellae in the hypodermis of the basal regions of root of sand sedge (*Carex arenaria*. L.) was correlated with decreased permeability to water and phosphate (Robards *et al.*, 1979). It was concluded from these studies that the formation of a suberized hypodermis led to reduced water and solute movement through walls. Suberization of the hypodermis reduced the permeability of maize roots to phosphate (Ferguson and Clarkson, 1976) and apoplastic dyes (Peterson *et al.*, 1982). Casparian bands were identified in the walls of the hypodermal cells of corn and onion (Peterson and Perumalla, 1986). (Since Casparian band development precedes the formation of suberin lamellae, the term suberization will be used to describe both the formation of Casparian bands and suberin lamellae.)

There is evidence which contradicts this view: Clarkson *et al.* (1987) found that roots of maize grown at a lower temperature (13 °C) had higher rates of water uptake than roots grown at a higher temperature (20 °C), even though the roots grown at 13 °C were more heavily suberized. Cruz *et al.* (1992) found that roots of sorghum grown in vermiculite at extreme moisture deficit developed extremely lignified and suberized hypodermis and endodermis, in comparison to unstressed roots. This stress reduced the permeability of these roots to water (Cruz *et al.*, 1992).

These studies suggest that both the maturation of the LMX and development of a suberized endodermis and hypodermis are influenced by the culture method used to grow the plants. There is no previous study of these features in aeroponically-grown plants. Therefore the maturation of LMX and suberization of the hypodermis/endodermis of the roots of aeroponically grown plants of maize were surveyed throughout the root system. The results of these studies were also necessary for correlation with rates of water uptake measured in the subsequent chapter.

METHODS AND MATERIALS

I. Plant Material

Plant material used for structural observation was grown aeroponically following the method outlined in chapter 2. Root tissue was collected from 9 plants of maize (1 root/plant), ranging in age from 40 to 60 days. Fresh root tissue was collected by excising main roots at the stem base. Roots were transported to the laboratory in tap water. This fresh material was either sectioned and observed on the day of collection, or stored at room temperature in 30% ethanol in distilled water. Ethanol-fixed tissue is more rigid than fresh material and therefore easier to section by hand. These sections were rinsed in distilled water before they were stained (see section III).

II. Survey Procedures

1. Openness of late metaxylem (LMX)

LMX maturity of nine main roots was assessed from longitudinal sections (LS) of fresh tissue and preserved root tissue collected at various distances from the root apex (0, 5, 15, 30, and 45 cm from the apex). The openness of LMX in main roots was also studied by excising segments of main root (5 - 10 cm long), placing one end of the segment in water

and blowing air into the other. If air bubbles emerged from the root the LMX were judged open. This test was verified by hand sections of adjacent tissue and by blowing air through roots which had their cortex removed to ensure that air was moving through the LMX.

Maturity of LMX in the branches was studied by cutting LS at each second centimetre from the apex to the base of the branch. Four to six branches at each position of the main root (10-15, 30, and 45 cm from apex of main root; collected from 5 main roots one from each of 5 additional plants) were sectioned and observed.

2. Suberization of main roots and branches

The extent of suberization of the main roots and branches was surveyed with hand sections of 5 main roots (1 from each of the five plants used in the LMX experiment, part 1.) which were grown in the mist boxes and stored in 30 % ethanol for several weeks. Sections were hand-cut (TS), rinsed in water for several minutes, then stained with the suberin stains Fat Red 7B and Fluorol Yellow 088 (see section III) and observed. Sections of main root were made at 0, 5, 15, 25, and 45 cm from the root apex. Branches were removed from the segments of main roots collected at 10 - 15, 30 and 45 cm from the root apex. TS were cut at each second centimetre from the apex to the base of each branch as in the survey of LMX maturity. The sections were pooled at each position along the branches, then stained with the suberin stains and observed. This was repeated with the branches collected at each position on the main root.

III. Sectioning

Transverse and longitudinal sections (TS and LS respectively) were hand-cut using sharp surgical steel razor blades. Sections (TS and LS) of main roots were hand-cut following methods of O'Brien and McCu'ly (1981). Branches were hand-sectioned while observing the process through a dissecting microscope. Branches were submerged in a droplet of water on a piece of dental wax, immobilized with a fingertip or pair of fine

forceps, and hand-cut with a razor blade so that the sections floated off into the droplet of water. The floating sections (thickness 30-100 μm) were transferred to stains and slides by drawing the water droplet with the sections into the end of a Pasteur pipet.

All sections of fresh material were stained (section III) , mounted under a coverslip in distilled water (unless stated otherwise) and observed. Sections of branches were mounted in water under coverslips which were supported by shards of broken coverslips to prevent the sections from being squashed under the weight of an unsupported coverslip. (All hand-cut sections of branches were mounted this way.)

IV. Staining

1. Toluidine Blue O

Hand sections of fresh tissue and tissue which was fixed in ethanol (30% v/v) were rinsed in tap water, then stained for 30 - 60 sec in 0.05% (w/v) toluidine blue O in sodium benzoate buffer (pH 4.4), then rinsed for 1 - 2 min in 2 changes of distilled water. Sections were mounted on clean glass slides under a coverslip in distilled water and observed.

2. Suberin Stains - Fat Red 7B, Fluorol Yellow 088.

Hand-sections were stained in fat red 7B and fluorol yellow 088 following Brundrett, Kendrick and Peterson (1991). Staining solutions were filtered (0.2 μm pore diameter) before use. Sections were stained for 1 - 12 h in 0.1% (w/v) fat red 7B (Sigma Co., C.I. 26050 lot 40H0556) in a mixture of polyethylene glycol (400 M.W.) : glycerol: water (50:45:5 v/v/v), rinsed briefly in distilled water, then mounted in 75% glycerol (aqueous) and observed (brightfield illumination).

Hand-cut sections of fresh material and material stored in 30% ethanol to be stained with fluorol yellow 088 (Sigma Co., no C. I. #, lot 70H3272), were first stained with the periodic acid-Schiff's reaction to block autofluorescence. Sections were oxidized

with 1% periodic acid (w/v in distilled water) for 1 - 10 min, rinsed for several minutes in tap water and then stained in Schiff's reagent for 20 - 30 min. Sections were rinsed three times in distilled water (1 - 2 min each rinse), then stained for 1 - 12 h in 0.01% fluorol yellow in a mixture of polyethylene glycol (400 M.W.) : glycerol: water (50:45:5 v/v/v). Sections were briefly rinsed in water, then mounted in 75% glycerol (v/v, aqueous). Stained sections were observed by epifluorescence microscopy.

V. Microscopy

Observations were made on a variety of light microscopes including Olympus models CH-2, BH-2, and Vanox, and a Zeiss Axiophot photomicroscope. These microscopes were equipped with brightfield illumination and epifluorescence optics. Epifluorescence microscopy was with ultraviolet-induced fluorescence (ultraviolet excitation, UG-1 exciter filter, DM 400 dichroic mirror and L-420 barrier filter).

RESULTS

I. Openness of Late Metaxylem (LMX)

The results of the survey of the maturation of the LMX in the main roots and branches are presented in table 1. The LMX of most main roots opened between 15 - 30 cm from the root apex. One root had open LMX at 7 cm from the tip, however the apex of this root was damaged and branches had developed down to the root tip (branches normally develop only by 10 - 15 cm from the root apex).

Branches were collected from segments of main roots at the positions 10 - 15, 30 and 45 cm from the apex of the main root. At each of these distances the data for the main root and branches are shown in the rows. Only 2 of the five branches collected 10 - 15 cm from apex of the main root had open LMX at their base, 4 - 5 cm from the branch

apex. The LMX remained closed until 8 - 9 cm from the apex of the branches collected 30 cm behind the apex of the main roots. In branches collected 45 cm from the apex of the main root, the LMX opened 4 - 5 cm behind the branch tip (see table 1).

II. Suberization of the hypodermis and endodermis.

Results of the survey of the extent to which the roots were suberized are presented in table 2. The development of the suberization of the hypodermis and endodermis is shown in figure 1. The hypodermis and endodermis of the main roots were completely suberized 5 cm from the root apex. Suberization of the branches collected from 10 - 15 cm behind the root apex was incomplete. The radial walls of the hypodermis and endodermis were stained with the suberin stains at 2 - 3 cm from the apex of the branches. The hypodermis was completely suberized in the basal regions of these branches (5 cm long), but there were endodermal cells which had not yet become fully suberized (see fig. 1B). At 30 cm the branches were longer than those collected at 10 - 15 cm from the apex of the main root. Their apical pattern of suberization was similar to branches at 10 - 15 cm, but the incomplete suberization extended for 5 cm instead of 2 - 3, and they had 6 - 11 cm where the endodermal and hypodermal cell layers were completely suberized. The older branches, collected 45 cm from the apex of the main root, were completely suberized down to their apices (with the exception of a few endodermal cells).

Table 1: Development of open late metaxylem vessels in the roots of aeroponically-grown plants of maize.

Distance from apex of main root (cm)	LMX Openness						
	Main Roots ^{1,2,3}	Branches ^{1,4,5}					
		Distance from branch apex (cm)					
		10-11	8-9	6-7	4-5	2-3	0-1
45	9/9	3/3	3/3	3/3	3/3	0/4	0/4
30	8/9	1/1	1/1	0/2	0/3	0/4	0/4
10-15	1/9				2/5	0/5	0/5
5	0/9						
0	0/9						

¹. Each entry is the number of roots with open LMX over the number of roots observed.

². Openness of LMX in main root determined by hand-cut LS and by blowing air through roots. ³. One root from each of 5 plants. ⁴. LMX openness in branches determined by hand-cut LS. ⁵. Branches collected from 5 main roots, 1 main root from each of 5 plants.

Table 2: Suberization of the roots of aeroponically-grown plants.

Distance from apex of main root (cm)	Suberization of hypodermis and endodermis ¹ .						
	Main Roots	Branches					
		Distance from branch apex (cm)					
		10-11	8-9	6-7	4-5	2-3	0-1
45	+++	+++	+++	+++	+++	+++	++
30	+++	+++	+++	+++	++	+	o
10-15	+++				++	+	o
5	+++						
0	o						

¹. o - no suberin in hypodermis or endodermis; + - partial suberization of hypodermis and endodermis (radial walls, see fig. 1A); ++ - partial suberization of hypodermis and endodermis (gaps in endodermis, see fig. 1B); +++ - complete suberization of the hypodermis and endodermis (see fig. 1C).

Figure 1: Development of Casparian bands and suberin lamellae in the hypodermis (h) and endodermis (n) of a branch. **Fig. A.** Casparian bands (red) form in the anticlinal walls of the hypodermis and endodermis. **Fig. B.** Suberin lamellae (red) form on the primary walls of the hypodermis and in some of the endodermal cells. **Fig. C.** Endodermis is completely suberized. Gaps form between some epidermal cells (e) separate or become distorted. A tertiary wall forms on the inner tangential and anticlinal walls of the endodermis (blue). Figures A, B, and C represent symbols +, ++, and +++ in table 2. c = cortex, LMX = late metaxylem, e = epidermis.

DISCUSSION

I. Openness of LMX.

The maturation sequence of the LMX in the main roots of aeroponically-grown plants of maize is similar to that in the main root of plants grown under field conditions. In aeroponically-grown plants the LMX open at 15 - 30 cm from the apex, while under field conditions St. Aubin *et al.* (1986) reported the persistence of immature LMX in the distal 20 - 30 cm of the main roots. This zone of opening was also noted in several other C4 grasses (*Zea mexicana*, *Sorghum bicolor*, and *Sorghum sudanese*) grown in the field (Wenzel *et al.*, 1989). The method of culturing plants of maize aeroponically, therefore did not alter LMX maturation.

In contrast, Cruz *et al.* (1992) found that moisture deficit delayed LMX maturation and reduced the number and diameter of open protoxylem and EMX in the roots of sorghum. The roots exposed to extreme moisture deficit were only 15 cm long and did not reach the layer of moist vermiculite because root growth was inhibited by the water deficit. Open LMX in the apical 15 cm of sorghum roots would not occur under normal conditions. Non-conductive protoxylem in these stressed roots was unexpected, since under normal conditions the protoxylem mature 1 cm from the root apex and well before the EMX and LMX (Cruz *et al.*, 1992).

In branches of aeroponically-grown roots, maturation of LMX occurred in the basal 1 - 3 cm for branches from 10 - 15 cm and 30 cm from the apex of the main root. Older branches (45 cm) had a greater proportion of open LMX; only the apical 3 cm of the 10 - 11 cm long roots had closed LMX (table 1). Compared with field-grown determinate branches which lack a growing root apex (Varney and McCully, 1991), branches on the apical regions of the main roots had a lower proportion of their length with open xylem (35 %), while older branches collected from the basal regions of the main root had 60 % of their length with open LMX (Wang *et al.*, in prep).

II. Suberization of the hypodermis and endodermis

The main roots had completely suberized endodermal and hypodermal cell layers at 5 cm behind the root apex. Only the apical regions of branches found 10 -15 and 30 cm from the apex of the main roots were not yet completely suberized. Branches collected 45 cm from the root apex were almost completely suberized (except a few endodermal cells at the branch apex). Since the main roots often reached lengths of 100 - 120 cm, most of the the roots of plants grown aeroponically had a completely suberized endodermis and hypodermis.

The formation of Casparian bands and suberin lamellae in the endodermis and hypodermis of corn (*Zea mays*) and onion (*Allium cepa*) was studied in the primary roots of corn grown in vermiculite and hydroponically, and in the roots of hydroponically-grown onion (Perumalla and Peterson, 1986). Formation of Casparian bands preceded deposition of suberin lamellae in both corn and onion. These wall modifications were complete 12 and 4 cm from the apex of corn grown hydroponically and in vermiculite respectively. Onion roots developed Casparian bands and suberin lamellae 12 - 16 cm behind the apex. Development of Casparian bands and suberin lamellae occurred closer to the root apex if root growth was inhibited with polyethylene glycol (Perumalla and Peterson, 1986).

The development of Casparian bands and suberin lamellae has not been previously studied in branches. These results show that most of the root system was suberized, and it will be interesting to determine if there is any correlation between water uptake and xylem maturation and suberization of the hypodermis and endodermis.

CHAPTER 5

THE RATES OF WATER UPTAKE IN THE MATURE ROOT SYSTEM OF AEROPONICALLY-GROWN PLANTS OF MAIZE

INTRODUCTION

The question, "Where does water enter a root?" has been studied extensively since 1675, when Malpighi first reported that roots absorbed solutions in the root hair zone (Popesco, 1926). In the forward to his review of the early literature and presentation of the results of his own studies, Popesco (1926) wrote "The many theories proposed to explain the phenomenon (of root function) proves the uncertainty which reigns on the subject of the exact location of the absorbing regions of root." (translated from Popesco, 1926). Seventy years later this view remains surprisingly true. Even though Popesco (1926) goes on to show that there was considerable conflict in the views presented by the early researchers (sometimes without data), he begins by stating two important views: i) Root hairs are the principal organs of absorption and ii) Roots absorb solutions through their tips.

These early studies of water uptake into roots (reviewed below, from Popesco, 1926) involved placing different regions of roots in containers of water, followed by observation of the plant condition. Other studies also used stains or salts to follow water uptake. About half of the authors reviewed by Popesco (1926) concluded that water entered roots in the apical regions which were covered by numerous root hairs, approximately one third hypothesized that most water entered the tips, while a few others speculated that water entered the epidermis or basal regions. For example, Senebier (1800) found that when the basal regions of roots were submerged the plants deteriorated; however Ohlert (1837) found that plants remained alive and well when only the basal regions of the roots were in water (from Popesco, 1926). More recently, Höhn (1934) found that the presence or absence of root hairs had little effect on water uptake in the roots of *Zea mays*, *Vicia faba*, or *Tradescantia*. The view that water uptake was into either the root apex and root hairs was favoured in spite of these obvious uncertainties.

Physiologists and agronomists have long since been trying to develop methods to determine the sites of water uptake into roots and the rates at which water is absorbed.

There is an enormous literature on water uptake into roots; several critical and sound reviews have been presented by Newman (1976), Kramer (1983), Boyer (1985) and Passioura (1988); water uptake by roots from soil has been reviewed by Tinker (1976), and Taylor and Klepper (1978). Several of these methods for studying water uptake will be reviewed. This will be followed by an explanation of the theory of the method developed in this project to study water uptake by the roots of maize.

I. Potometers

The simple containers used by the first researchers were modified into the classic potometer so that the amount of water absorbed by the roots could be measured volumetrically or gravimetrically. Regions of roots could be sealed in the potometer chamber to determine the volume of water extracted by that part of the root (Sierp and Brewig, 1935; Brewig, 1937). Various forms of potometers have been developed to measure water fluxes into root systems (Maertens, 1971; Navara, 1987), whole roots (Gregory and Woodford, 1939; Hayward *et al.*, 1942; Graham, *et al.*, 1974 and most recently by Sanderson, 1983), root hairs (Rosene, 1943, 1950, 1954, 1955) and even isolated regions of root hairs (Cailloux, 1972). This method has been successfully used to measure water flux into roots of many plant species, however this is a technically-demanding method which has several disadvantages.

It is very difficult to insert the roots into the potometers without damaging the roots and equally difficult to obtain an effective seal around the root. This limits the application of potometers to unbranched regions of roots (as stated by those using potometers) since only the immature, apical regions of the roots are unbranched. Sanderson (1983) removed the branches from the mature regions of roots to seal them in the potometers (Sanderson states that the branch stumps become blocked by air embolisms, and resulting fluxes were through the main roots). Consequently, potometers have been primarily used to measure water flux into the seminal roots of small seedlings which are developmentally immature

and have low transpiration rates and water fluxes. Only Sanderson (1983) has studied water uptake into small branches. Maertens (1971) and Navara (1987) measured water flux into whole roots of large plants of maize (*Zea mays*), however the fluxes of branches and main roots could not be distinguished. The time required to install roots into potometers also limits the number of them which can be used. This is a major disadvantage when trying to survey a heterogeneous phase space such as a root system. In spite of these difficulties, many useful studies have been done; the results of these studies will be compared with the fluxes into maize roots measured in this project.

The answers provided by these potometric studies to the question, "Where does water enter a root (and at what rate)?" are as heterogeneous as the answers provided by the early studies reviewed by Popesco (1926). The pattern of water uptake into the secondary roots of *Vicia faba* was very peculiar. At low transpiration rates water flux was highest near the apex of the 12 cm long secondary roots. At high transpiration rates water fluxes were highest in the basal regions of the roots (Brewig, 1937; Brouwer 1953 a and b). Hayward *et al.* (1942) and Maertens (1971) also found higher fluxes in the basal parts of main roots of large plants of maize. This contradicts Graham *et al.* (1974) who found that water fluxes were highest in the apical 10 cm of the main roots of *Cucurbita pepo* (marrow) and *Hordeum vulgare* (barley); water fluxes in the proximal parts of the main roots fell to very low values. High fluxes into the apical 10 cm of the main roots of barley seedlings were also measured by Sanderson (1983).

Even though high water fluxes were measured in apical and basal regions, most physiologists adopted the view that high fluxes of water into roots were limited to the root hair zone (apical 10 cm). This pattern was extended to explain water uptake into roots of most plants. The acceptance of this theory is reflected by its appearance in many plant physiology textbooks (eg. Salisbury and Ross, 1985). The high fluxes in the apical parts of the main roots were confidently associated with the root hair zone, an endodermis which was not yet heavily suberized or lignified, and mature xylem elements capable of carrying

fluxes (Clarkson and Robards, 1975; Lauchli *et al.*, 1978), but this was recently shown to be incorrect by St. Aubin *et al.*, (1986).

II. Measurement of Water Extraction from Soil by Roots

Agronomists have generally discounted the view taken by the physiologists, assuming that the whole length of roots absorb water (Barber *et al.*, 1988). Just as physiologists have measured the rate at which roots absorb water from a potometer chamber, agronomists have measured the rates at which roots absorb water from a volume of soil. Tensiometers placed at different depths and positions in the soil around a plant are used to monitor changes in soil water content (θ). Careful excavation of the root system or core samples taken at various depths and positions around the base of the plant were used to estimate root density. The volume of water extracted by the length (or surface) of roots in the volume of soil was then calculated and expressed as water fluxes. It should also be noted that soil water can move vertically and horizontally in response to local variations in water content caused by evaporation or absorption by roots (gravity will also cause soil water to move downwards; Brady 1990). Estimates of rooting density or root length from core samples may also be inaccurate; this can markedly influence calculated water fluxes (Passioura, 1988; and also see Discussion). These studies also fail to identify what proportion of the total root length is actually involved in water uptake (Passioura, 1988).

Changes in water content of soil surrounding roots have been studied with nuclear magnetic resonance imaging (MacFall *et al.*, 1991) and by computer assisted tomography applied to X-ray attenuation measurements (Cat-scanning, Hainsworth and Aylmore, 1986). Qualitative differences in the rates at which soil water content is reduced have shown that small lateral roots of *Pinus taeda*, loblolly pine, are more efficient at collecting water than larger diameter roots per unit volume (MacFall *et al.*, 1991). Hamza and Aylmore (1992) used computer assisted tomography applied to gamma and X-ray

measurements to study water uptake by radish (*Raphanus sativus*) and lupin (*Lupinus angustifolius*) roots from soils at a range of NaCl concentrations. They found that water uptake was faster in the basal regions of the radish roots due to increased surface area per unit length root (caused by increased diameter); water uptake rates were constant along the lupin roots which had the same diameter along the entire length of roots (Hamza and Aylmore, 1992). Increasing salt (NaCl) concentrations decreased the rates at which both radish and lupin roots could extract water from the soil. Salt also accumulated linearly at the root surface (measured by Na^+ microelectrode; Hamza and Aylmore, 1992). These non-destructive techniques appear to have great potential.

III. Induced Flow Through Excised Roots and Root Systems.

Water uptake into roots has also been studied using excised roots and detopped root systems. These types of experiment gained interest once the thermodynamic basis of water transport was understood about 33 years ago (references in Boyer, 1985). It was shown that "water movement is governed by two fundamental factors: the driving force and the conductance of the flow path" p.474, Boyer (1985). Rather than measuring the volumetric fluxes into the roots, these studies have been investigating various aspects of the driving forces and conductances (or permeabilities) of the flow path for water.

It was first thought that water flow into roots was osmotically-driven, since root pressure caused xylem sap to be exuded from the cut stump of a detopped root system and since this exudation ceased following addition of solutions of increasing osmotic strength. Flow into these roots was also induced by applying hydrostatic pressures to the roots or root systems in pressure chambers and measuring the rates at which sap exuded from the cut end which extended through a hole in the chamber. Flow has also been induced into roots immersed in water by applying suction to the cut ends of the roots (Steudle and Jeschke, 1983).

The forces causing water movement include differences in solute concentrations across a membrane, gravitational forces (these are important only in water movement up tall trees), and matrix forces caused by the adhesion of water to solid surfaces. The forces which drive water through plants can be stated in solute and pressure terms. These forces are expressed in units of free energy (ergs/cm³) which, when the free energy is divided by the partial molal volume of water, can be expressed as the water potential Ψ_w (in units of bars or megapascals; 1 MPa = 10 bars). The solute forces and pressure forces can therefore be described as osmotic potentials (Ψ_o) and pressure potentials (Ψ_p) respectively, which influence the water potential (Ψ_w). Thus

$$\Psi_w = \Psi_o + \Psi_p \quad (1).$$

As water moves along a path it encounters frictional resistances to these forces causing the movement (Boyer, 1985). This can be expressed as a permeability (L_p), assuming that conditions are at a steady state (even though many authors admit that steady states rarely occur in the real world). Therefore water flow across a membrane has been described by the equation:

$$J = L_p (\Delta\Psi_w) \quad (2)$$

where $\Delta\Psi_w$ is the gradient of water potential causing the movement of water. The permeability coefficient (L_p), rather than the volumetric fluxes, has been used to describe flow into roots. The permeability of the roots of 4 - 6-day old wheat seedlings (*Triticum aestivum*) ranged from 1.0- 15.0 $\mu\text{l h}^{-1} \text{cm}^{-2} \text{MPa}^{-1}$, L_p of individual cells ranged from 28 - 79 $\mu\text{l h}^{-1} \text{cm}^{-2} \text{MPa}^{-1}$ (Dainty, 1985).

This model has also been modified to describe water flow across roots to incorporate pressure gradients (ΔP), solute gradients ($\Delta \pi$), and a reflection coefficient (σ) for weighting any osmotically-induced flow:

$$J = L_p (\Delta P - \sigma \Delta \pi) \quad (3)$$

(As Nobel (1991) discussed, applying such models to describe fluxes into plants is somewhat illegitimate since the driving forces are borrowed from irreversible thermodynamics and used to describe open systems which are not at equilibrium.)

Even though a great deal of research was needed for the development of this model, it does not accurately represent observed non-linear relationships between J and ΔP or $\Delta \pi$ (Newman, 1976; Passioura, 1984; Dainty, 1985). A great deal more research has been done in response to this discrepancy. Methods have been developed to accurately measure osmotic and pressure potentials while also measuring the flux into the root very precisely (Boyer, 1985). It remains unclear which is the principal driving force of water into roots under such conditions. Several researchers have also expressed concerns that artifacts may exist in excised roots. Excised roots may suffer damage when handled and are starved of metabolites (Passioura, 1988). Salim and Pitman (1984) and Passioura (1988) have also speculated that excised roots which are immersed in nutrient solution and then pressurized may exhibit behaviour which is irrelevant to intact plants at normal pressures, and that pressure-induced flow may be through spaces which are not normally conductive (e.g. intercellular spaces).

It has recently been shown using a pressure probe, that the xylem tensions of tissue measured by the Scholander bomb may have been too high (Balling and Zimmermann, 1990), and this suggests that water potentials measured in the bomb may not be accurate. Zimmermann *et al.* (1992) found that there was a gradient of increasing osmotic pressure and turgor pressure in the outermost to innermost cortical cells of *Aster tripolium* roots.

This gradient of turgor and osmotic pressure collapsed 15 - 30 min after the root was excised from the plant; the turgor pressure throughout the cortex was then intermediate (0.3 MPa). It was speculated by Zimmermann *et al.* (1992) that excised roots may be artifactual systems. This research has caused a great deal of turmoil in the field of plant water relations.

Methods and models derived from this research have provided much information about many aspects of water uptake and provided a useful framework in which to study water uptake (Passioura, 1984, 1988). However these methods are time consuming and technically difficult and are therefore not well suited to studying water uptake into a root system. These studies have unfortunately been performed without any knowledge of the pathway of water flow into roots; this represents one of the major drawbacks of these methods.

IV. Water Fluxes Measured by the Rate of Accumulation of a Marker Solute

The size of the root system of a maize plant at maturity places several constraints on the method which must be used to study water uptake by such roots. Large plants with mature root systems are required to conduct studies of water fluxes into both immature regions and mature regions of nodal roots (sheathed versus bare regions respectively) and into the many fine branches on the mature regions. These first order branches are very heterogeneous under field conditions (Varney *et al.* 1991) and therefore the method must provide easy access to this mature root system so that large enough samples can be collected to adequately study this population of branches. (These two requirements rule out potometers, and the length of time required to collect roots from the field prevents physiological studies since most roots are damaged during the lengthy collection procedure.) The ideal method should also study water uptake into roots on intact plants which are transpiring normally (which rules out using excised roots or root systems). The method must have a spatial resolution of about 1 cm to measure fluxes of water into the

main axes and branches. It should also have a temporal resolution of about an hour so that water fluxes can be measured over the course of a day's transpiration.

The method used in this thesis has two main procedures. The first part of this method involves growing plants of maize by nutrient mist culture (aeroponic culture) for 4 - 8 weeks in large 'mist boxes'. The root systems of the aeroponically-grown plants are structurally and developmentally similar to the roots of field-grown maize. Although the roots of plants from the mist boxes are more uniform, the diameter and lengths of branches and axes are similar to those of field-grown plants. The frequency and distribution of first order branches on the main axes of plants grown in the mist boxes are also similar to the branches on main roots from the field. (see Ch. 3). The late metaxylem of the main axes and branches also mature at about the same distance from the root apex (Ch. 4). In addition to the similarity of the roots of aeroponically-grown plants to those of field-grown plants, the major advantage of this method is that it provides ready access to a mature root system (through a door in the side of the box). Large samples of roots can therefore be collected with minimal disruption of the normally-transpiring plants.

The second part of the method which has been developed during the present study represents a new idea for measuring water uptake by the roots of aeroponically-grown plants of maize. The method is based on observations of the movement of a marker solute (Sulphorhodamine G, abbreviated SR) through the apoplast of dicotyledon leaves (Canny 1990a, b). When an aqueous solution of SR is fed to a transpiring leaf through the cut end of the petiole, it is carried up the xylem and the SR accumulates in the apoplast at the ends of the finest veins. The SR accumulates there because it is osmotically filtered by the plasma membrane as the water crosses the plasma membrane and enters the symplast of the surrounding mesophyll cells. These accumulations of dye have been called *sumps*, and the sites where the water enters the symplast were called *flumes* (Canny, 1990a). Canny reasoned that root tissue may behave similarly to the leaf tissue, and the hypotheses were made 1) that water entering roots would cross the cell membranes at the root surface, and

consequently, 2) that solutes which did not pass through the flumes would be filtered osmotically and remain on the surface forming sumps there. The locations of the flumes would be revealed by the location of the sump, and the rate at which water enters the flume (the rate of water flux) could be measured by the rate of solute accumulation in the sump. Therefore, if SR were added to the nutrient solution in the mist culture system, it might accumulate faster on some parts of the root system to reveal the sites and rates of water uptake on that part of root. This method could not be used in water culture since any SR accumulations on the root surface would be dispersed by diffusion and convection. Preliminary studies were directed at determining if excess mist droplets which drain down the root would wash away the accumulated SR.

Some solutes in the soil solution which are carried to the root surface by mass flow can be accumulated at the root surface, provided these solutes are carried to the root surface faster than they are taken up by the root (Wild, 1981). It has been calculated that calcium and magnesium, which are only slowly absorbed by roots, can accumulate at the surface of roots in soil (Wild, 1981). Hamza and Aylmore (1992) have recently used an Na^+ specific microelectrode to show that Na^+ can build up on the surface of radish and lupin roots grown in soil which was watered with different concentrations of NaCl . Therefore, the hypotheses that a marker solute may accumulate at the root surface is similar to the accumulation of some solutes in the soil. Ehwald (1974) proposed that water uptake be measured by the rate of accumulation of dextran in the solution surrounding a root in a potometer chamber, however this method was not followed up.

The use of tracers to study water movement has been discounted (and rightly so) in the reviews mentioned at the outset of this introduction (Passioura, 1988; Boyer, 1985; Newman, 1976). They pointed out that in these experiments, the tracer had arrived somewhere in the root because it had diffused there. This method is based on a very old idea which had been used by Kny (1898, from Popesco, 1926), Popesco (1926) and Höhn (1934). The early researchers hoped that the dye solutions fed to roots might reveal the

sites where water enters the roots by entering with the water and staining the tissue. Höhn (1934) demonstrated that dyes diffused into roots whether they were absorbing water or not. This was also confirmed by Hülsbruck in 1944 and Bauer in 1953 (from Canny, 1990a). The use of dye movement into tissue to follow water movement must be sharply distinguished from the use of SR in this project which requires that the apoplastic tracer not follow the water, but accumulate on the root surface as the water enters the root leaving the dye behind. The diffusion of a dye through a cell wall is unrelated to the movement of water into the wall. Canny (1990c) has shown that SR movement away from sumps through cell walls in leaves was by diffusion of SR through the walls (the diffusion of SR was 100 to 10,000 times slower than in water). This indicated that there was no flow of water through the wall apoplast (Canny, 1990c). It has also been shown that SR diffuses into the wall apoplast of roots at the same rate as it does in the walls of leaves (Canny and Huang, 1993).

In the present thesis, the accumulation of SR has been used to measure water volume flux J . I have measured volume flux per unit surface (J_S) and per unit length (J_L) of root. The former is more laborious to measure because length and diameter must be measured, however, it is more useful for measurement and comparison of roots which have a wide range of diameters. Note that

$$J_L = \pi d J_S \quad (4)$$

where d is the root diameter, and that if J_L is measured routinely, J_S can be found for a particular root for which d is known. This formula is explained in more detail in the Materials and Methods section. Water flux has been expressed in a variety of units, many of which are expressed as the exponent to many negative powers of ten. Such numbers are easily confused but not easily visualised at the scale of a small branch. The SI units for J_L and J_S

$$J_L : \text{m}^3 \text{ s}^{-1} \text{ m}^{-1} (= \text{m}^2 \text{ s}^{-1})$$

$$J_S : \text{m}^3 \text{ s}^{-1} \text{ m}^{-2} (= \text{m s}^{-1})$$

are no exception, and the units of Sierp and Brewig (1935) have been adopted instead (however modified so that $\text{mm}^3\text{H}_2\text{O} = \mu\text{l}$):

$$J_L : \mu\text{l h}^{-1} \text{ cm}^{-1}_{\text{root}}$$

$$J_S : \mu\text{l h}^{-1} \text{ cm}^{-2}_{\text{root}}$$

These units are easily visualized and the measured fluxes are numbers between 1 and 20. Fluxes measured in other studies will be converted to our units for comparison. Sometimes for this it was necessary to guess at the root diameters to convert measured J_L into estimates of J_S .

In the present study, the rates of water flux into the mature root system of maize plants were measured by the rate at which the roots accumulated the marker solute (SR) from a mist of 0.5 mmole l^{-1} solution of SR in distilled water. Preliminary methods were developed to: 1) measure the concentration of SR on roots; 2) determine the length of time needed to elute SR from the root tissue and 3) measure the 'water free space' of the roots to SR. Early experiments investigated the rate of accumulation of SR on branches to (1) determine the time course and duration of dye accumulation and (2) investigate the variability in measured water fluxes into branches. In subsequent SR accumulation experiments, water fluxes into branches and main roots were measured over the whole root system. The procedures developed during these studies to feed SR-via-mist were used for preparation of plant material for subsequent structural studies to determine the sites where the water entered the root symplast (Ch. 6).

MATERIALS AND METHODS

The idea of using the accumulation of a marker solute (SR) to measure water uptake is a new one. Therefore, before water uptake rates could be measured, it was necessary to work out several details of the methods required to conduct these studies. A procedure to measure SR concentration was developed, followed by a determination of the time required to elute SR from root tissue. Pilot experiments of SR accumulation revealed that there were low levels of SR accumulation by the roots of non-transpiring control plants, and it was hypothesized that this was due to SR uptake into the 'water free space' of the root tissue. Therefore this led to determination of the volume of root which made up the 'water free space'. The procedures used during the SR accumulation experiments designed to measure water uptake are then outlined, followed by the calculations used to convert SR accumulation rates into water fluxes.

I. Preliminary Studies

1. Measurement of SR concentration

SR concentration was measured spectrophotometrically. These measurements were done in quartz cuvettes with an LKB Biochrom Ultraspec model 4050 spectrophotometer (Serial # 00747). All experiments were performed using solutions of sulphorhodamine-G dye in distilled water (figure 1; M.W. 552.61, Aldrich Chemical Co., Acid Red 50, C.I. 45220, Lot #. JL 6301 HK, KW 00103 KW). The maximum solubility of SR at room temperature is about 0.4% w/v (Canny and McCully, 1986). All stock solutions of SR were prepared with glass-distilled water. SR was also eluted from root tissue into distilled water unless stated otherwise.

The absorption spectrum and wavelength of maximum absorption were determined using a 0.1 mmole l⁻¹ SR solution (see figure 2). The wavelength of maximum absorption of SR was 520 nm, therefore all spectrophotometric measurements of SR concentration

were made at this wavelength. Stock solutions of SR in distilled water were prepared in volumetric flasks which were washed in 6M H₂SO₄. Their concentrations of SR ranged from 1×10^{-7} M to 1×10^{-4} M. Three replicate measurements of the absorbance at 520 nm of each stock solution of SR were made. These values were then plotted against concentration to construct a standard curve (figure 3). The absorbance (x) of the samples collected was converted into concentration of SR in mmole l⁻¹ from the equation of this line ($y = 0.00217 + 0.0038x + 0.01506x^2$, where x is absorbance at 520 nm).

Figure 1: Molecular structure of sulphorhodamine G.

Figure 2: Absorption spectrum of sulphorhodamine-G in distilled water (0.1 mmole l^{-1})

Figure 3: Standard curve of sulphorhodamine-G in distilled water.

2. Measurement of SR elution time

The length of time required to elute SR from the roots was measured using tissue which had been prepared in two ways. In the first experiment segments were collected from roots of three plants (58-65 days) which had 0.5 mmole l⁻¹ SR fed via the mist for 24 h. Segments of roots from two soil-grown plants (50 days) were used in the second experiment. Roots from the pots were washed thoroughly and soaked in water for 1 hour and then soaked in 1 mmole l⁻¹ SR for 2 hours.

The elution of SR was measured by soaking the SR-treated root segments in 1 ml distilled water for the appropriate elution time (0, 5, 20, 80, 400 or 1320 min). Segments were then transferred into second tubes of distilled water to elute any remaining dye from the tissue. In the first experiment, the tissue was ground up before the second wash to enhance elution of the dye. The amount of SR eluted during both washes was measured as outlined above, and the amount of dye extracted over time expressed as follows:

$$\frac{\text{Amount of SR eluted during 1st wash}}{\text{Total amount SR eluted during 1st and 2nd wash}} \times 100 = \text{Percent SR extracted}$$

3. Measurement of 'WATER FREE SPACE' (WFS)

The amount of WFS of roots grown in the mist boxes was measured with SR. Main roots were removed from plants, then washed and soaked in water for 1-2 h. Since these roots did not have the soil sheaths found on roots from the field, root segments were collected from regions of the axes which would have been bare or sheathed if they were in the soil. Segments of "sheathed" roots were collected from the unbranched, distal 20 cm of the main roots; and segments of "bare" roots were collected from the proximal end of the main roots.

Root segments were excised, sealed at their ends with cocoa butter or molten dental wax, then soaked for 2 hours in 1 mmole l⁻¹ SR. Each segment was rinsed quickly (5-10

sec) in running water to remove the surface film of SR, then transferred to separate 1 ml aliquots of distilled water for 2 hours to elute the SR from the tissue. The segments of root were then removed and stored in 30% ethanol. The volume of each root segment and the volume of SR eluted from each sample were determined, and then used to calculate the volume of 1 mmole l⁻¹ SR eluted from the piece of root. Twelve samples of each type of tissue were collected in each experiment. The percent of root volume of each segment which was WFS was calculated as follows:

$$\frac{\text{Volume of 1 mmole l}^{-1} \text{ SR taken up by root}}{\text{Volume of root}} \times 100 = \% \text{ WFS of root}$$

The number of plants from which the roots were collected and the age of these plants, as well as any procedural variations are summarized in table 1.

Table 1: Details of the Water Free Space experiments

Experiment number	Number of plants	Age (days)	Procedural changes
1	2	50	- eluate H ₂ O & NaCl (0.5 mmole l ⁻¹). -pot-grown plant used.
2	3	70	-elution time 4h.
3	8	28, 50	-soaked in SR with branches attached

II. Water Uptake Experiments

1. Measurement of SR accumulation

Plants were grown in the mist boxes until their root systems were at an appropriate state of maturity (50 - 100 cm-long main roots). The plants required 25 - 70 days growth in the mist boxes for their roots to achieve this length. All SR accumulation experiments were

performed in the mist boxes with plants grown in these boxes. Details of the ages and number of plants used in each experiment have been summarized in table 2.

(i) Plant preparation

The day before each experiment, the plants were prepared by replacing the nutrient solution with distilled water, so that the roots were in a dilute solution of nutrient solution. The non-transpiring control plants were also prepared at this time, by either cutting off the shoot (detopped controls) or covering the shoot with a large, black-plastic bag and then aluminum-foil (bagged controls). The preparations were made 12 - 24 h before each experiment to allow the water status of the tissues to equilibrate to these changes (except experiment 1). With the exception of experiments 1 - 3, some of the main roots were excised at the base of the plant leaving only 12 - 15 main roots. These roots were removed 1 - 5 days before the experiments in an attempt to increase water uptake rates in the remaining roots. In each SR accumulation experiment there were three to seven transpiring plants, and one or two non-transpiring plants.

(ii) Experimental procedure

On the morning of each experiment (0800-0900 h), enough crystalline SR was added to the reservoir of distilled water to make a final concentration of 0.5 mmole l^{-1} SR (Preliminary experiments 1-3 were started between 1030-1145 h). Four samples of this SR-solution were collected and the SR concentration determined. This 0.5 mmole l^{-1} SR-solution was circulated through the mist box system to produce a mist of SR-distilled water, which was applied to the root systems of the transpiring and non-transpiring plants.

The amount of SR accumulated on the roots from the SR-water mist was measured by excising pieces of root tissue and placing the tissue in aliquots of water to elute the SR from the surface of the root for at least 24 h at 4°C . The surface area of the root was measured as outlined in Ch.3 section 2 and the amount of SR in the eluate was measured

spectrophotometrically. The accumulation of SR was monitored during the experiment (6-12 h), by collecting samples of root tissue at 1 or 2 h intervals. The plant activity was also monitored during these experiments at 1 or 2 h intervals as described in section 3. In order to overcome any uneven misting effects, plant position was changed every 1-2 h by rotating the lids with the plants in them through 180° (except experiments 1-3). Samples of root tissue were collected by two different procedures, depending on the objective of the experiment.

Table 2: Summary of the SR accumulation experiments (1-5, and 1.1-1.4).

Experiment Number	Number of plants Transpiring	Controls	Plant age (days)	Number of roots sampled
Branches				
1	3	1 ¹	64	4 ³ , 4 ⁴
2	3	1 ¹	58-65	12 ³ , 6 ⁴
3	4	1 ¹	34	6 ³ , 6 ⁴
4	4	1 ¹	70	12 ³ , 6 ⁴
5	4	2 ¹	50	12 ³ , 6 ⁴
Whole Root system				
1.1	5	1 ¹	37	3 ⁵ , 1 ⁶
1.2	7	1 ¹	47	3 ⁵ , 1 ⁶
1.3	4	1 ¹ , 1 ²	50	3 ⁵ , 1 ⁶
1.4	6	1 ¹ , 1 ²	38	3 ⁵ , 2 ⁶

¹ detopped plants, ² bagged plants, ³ transpiring plants, ⁴ control plants, ⁵ main roots from transpiring plants, ⁶ main roots from control plants.

(iii) Sample collection

(a) SR accumulation on branches

The preliminary experiments were developed to determine if the accumulation of SR could be used to study water uptake by roots absorbing water from a mist of SR-

distilled water. Therefore, only branches were sampled in these early experiments (numbered 1-5). Branches which were red due to the build-up of SR were excised, shaken free of any excess solution, then put into 1 ml aliquots of water. The SR was eluted from the surface of the root by soaking for at least 24 h at 4 °C. The branches were then removed and their surface area was either measured immediately, or they were stored at 4 °C in 30% ethanol until they were measured. The surface area of the branch and amount of SR in the eluate were determined for each root sampled.

The amount of SR accumulated on each branch was calculated by dividing the amount of SR eluted from the root surface by the surface area of the branch. This accumulation was expressed as pmoles SR mm^{-2} root surface. The mean level of SR accumulation at each sample time was calculated from the amount of SR accumulated by each branch collected. Mean SR accumulation was plotted as a function of time. The mean absorbance of the samples collected at time zero was subtracted from the means of all other sample times to compensate for any changes in absorbance caused by substances released by the tissue during the elution period. The absorbance at time zero was always very close to zero.

(b) SR accumulation on the root system

The second sampling procedure was developed in order to measure water uptake by the branches and main roots from different parts of the root system (mature regions versus immature regions). These experiments were therefore numbered 1.1-1.4. At each sample time (every second hour), entire nodal roots were excised at the base of the plant, then blotted or shaken to remove any drops of excess SR solution. Up to three main roots (one root/plant) were collected at each sample time from the transpiring plants; one main root was collected from the control plant(s). The main roots were all treated similarly. Two-centimetre long segments of the main roots were collected from different positions along the length of root. SR on the 2-cm segments of main roots and branches on the segment were eluted separately to determine the amount of SR accumulated on each. Each 2-cm segment was treated as described below.

The branches (or the basal 5-cm of very long branches) were excised from the segment of main root. All the branches from the segment were pooled in an aliquot of distilled water (3-5 ml) and then stored at 4 °C. Branches on segments from the same position from other roots were also excised and pooled in separate aliquots of water. (The branches collected from each segment were pooled in order to increase sample sizes, once the variability in SR accumulation between branches was determined from the individual root sampling method (Part i).) The segments of main roots were pooled in an aliquot of water with segments of similar position from other roots collected at that time. This procedure was repeated at each position along the main axes. The SR was eluted from the root surface for at least 24 h at 4 °C. The amount of SR in the eluate and the total surface area of root in each vial was measured. These values were then used to calculate the amount of SR accumulated per surface of root in $\text{pmoles SR} \cdot \text{mm}^{-2} \text{ root surface}$.

Segments were collected from the main roots using two different procedures. In the first method, segments of main root were collected at each tenth centimetre starting 5 cm from the base of the stem and extended down to 45 cm from the stem base (Experiments 1.1 - 1.3). This therefore did not always include samples from the apical regions of the main roots. These experiments are pooled and presented together in the results section II.1. A fourth experiment was conducted to include the apical parts of the main axes, using the second method. In this experiment, segments of main roots were collected at various distances from the root apex (Experiment 1.4). Segments of main root and attached branches were collected at each fifth centimetre starting 2.5 cm behind the root tip, and extended up to the stem base in order to include the entire length of the main axes. Experiment 1.4 will be presented separately since this sampling procedure was more complete (Results section II.2.).

2. Calculation of water fluxes

The amount of SR accumulated per surface area of root is calculated and expressed as $\mu\text{moles SRmm}^{-2}\text{root}$. The rate of SR accumulation can be either calculated by dividing the amount of SR by the accumulation time, or estimated from the slope of the line drawn through the plot of SR accumulation against time.

SR accumulates because water is absorbed by the root and the SR is left behind and builds up on the surface of the root. Water flux per unit surface (J_s) is the volume (V) of water which enters a surface area of root (surface area of a cylinder = $\pi d L$, where d = diameter, L = length) in a given time (t), or

$$J_s = \frac{V}{t \pi d L} \quad (5)$$

The water flux of the root can be calculated from the amount of SR on its surface by dividing the rate of SR accumulation by the concentration of SR in the mist.

$$J_s = \frac{\text{rate of SR accumulation}}{\text{concentration of SR in mist}} \quad (6)$$

The concentration of SR in the mist is:

$$\begin{aligned} \frac{0.5 \text{ mmoles}}{\text{l}} &= \frac{0.5 \mu\text{moles}}{\text{ml}} = \frac{0.5 \text{ nanomoles}}{\mu\text{l}} \\ &\frac{0.5 \text{ nanomoles}}{\mu\text{l}} \times \frac{1000 \text{ picomoles}}{\text{nanomole}} \\ &= 500 \frac{\mu\text{moles SR}}{\mu\text{l H}_2\text{O}} \quad (\mu\text{l} = \text{mm}^3 = \text{mg H}_2\text{O}) \end{aligned}$$

For example, a build up of $25 \text{ pmoles SR} \cdot \text{h}^{-1} \cdot \text{mm}^{-2}_{\text{root}}$ is converted into water flux (J_s) per unit surface:

$$J_s = \frac{25 \text{ pmoles SR}}{\text{mm}^2_{\text{root}} \cdot \text{h}} \cdot \frac{500 \text{ pmoles SR}}{\mu\text{l H}_2\text{O}}$$

$$J_s = 0.05 \mu\text{l H}_2\text{O} \cdot \text{h}^{-1} \cdot \text{mm}^2_{\text{root}}$$

$$J_s = 5 \mu\text{l H}_2\text{O} \cdot \text{h}^{-1} \cdot \text{cm}^2_{\text{root}}$$

For comparison with published work in which J_s is not given, calculations were made also of the flux per unit length of root (J_L). Water flux per unit length (J_L) is the volume of water (V) entering a length of root (L) in a given time (t), or

$$J_L = \frac{V}{L \cdot t} \quad (7)$$

and by substitution

$$J_s = \frac{J_L}{\pi d} \quad \text{or} \quad J_L = \pi d J_s \quad (8) \text{ \& } (9)$$

This equation must be used in comparing J_s or J_L for roots of different diameter. The branches conveniently have a diameter of 0.34 mm or approximately $1/\pi$ ($= 0.318$). So for branches, $J_L = J_s$ and these may be used interchangeably since a 1 mm long segment of branch has 1 mm^2 of surface area. For main roots which are 1 mm in diameter, if $J_s = 0.5 \mu\text{l} \cdot \text{h}^{-1} \cdot \text{mm}^2_{\text{root}}$, then J_L may be calculated as follows:

$$J_L = \pi d J_s$$

$$= \pi \times 1 \text{ mm} \times 0.5 \mu\text{l} \cdot \text{h}^{-1} \cdot \text{mm}^2_{\text{root}}$$

$$= 0.5 \pi \mu\text{l} \cdot \text{h}^{-1} \cdot \text{mm}^{-1}_{\text{root}} \times (10 \text{ mm/cm})$$

$$= 5\pi \mu\text{l}\cdot\text{h}^{-1}\cdot\text{cm}^{-1}\text{root}$$

$$= 15.3 \mu\text{l}\cdot\text{h}^{-1}\cdot\text{cm}^{-1}\text{root}$$

The units for J_S and J_L used by Sierp and Brewig (1935), used subsequently by Brewig (1936a,b, 1937) and Brouwer (1933a,b, 1954) were chosen because they are easily visualized at the scale of a small branch and yield values between 1 and 20 for measured fluxes.

$$J_S : \text{mm}^3\cdot\text{h}^{-1}\cdot\text{cm}^{-2}\text{root}$$

$$J_L : \text{mm}^3\cdot\text{h}^{-1}\cdot\text{cm}\text{root}$$

These units also have the advantage that they are easily converted into volumes because $1 \mu\text{l} = 1 \text{ mm}^3$, therefore to avoid any confusion between units of volume ($\text{mm}^3 \text{ H}_2\text{O}$) and surface area (mm^2root), volume will be expressed in microlitres throughout this project.

$$J_S : \text{mm}^3\cdot\text{h}^{-1}\cdot\text{cm}^{-2}\text{root} = \mu\text{l}\cdot\text{h}^{-1}\cdot\text{cm}^{-2}\text{root}$$

$$J_L : \text{mm}^3\cdot\text{h}^{-1}\cdot\text{cm}^{-1}\text{root} = \mu\text{l}\cdot\text{h}^{-1}\cdot\text{cm}^{-1}\text{root}$$

These units may be converted to SI units by multiplying by 2.778×10^{-11} . The SI units were not chosen because the fluxes in SI units are very small numbers expressed as negative exponents to many powers of 10 making them difficult to work with. Also, these units (below) are not easily grasped by the mind at the scale of a small branch and will therefore be avoided.

$$J_S = \text{m}^3\cdot\text{s}^{-1}\cdot\text{m}^{-2} (= \text{m}\cdot\text{s}^{-1})$$

$$J_L = \text{m}^3\cdot\text{s}^{-1}\cdot\text{m}^{-1} (= \text{m}^2\cdot\text{s}^{-1})$$

3. Measurement of Physiological Conditions.

Conditions were monitored with a Li-Cor Inc. Steady State Porometer (model LI-1600, serial # SSP 1070) equipped with a Quantum Light Meter (model LI-190S-1). Leaf temperature ($^{\circ}\text{C}$), light intensity ($\mu\text{mole}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), transpiration rate ($\mu\text{g}\cdot\text{cm}^{-2}\cdot\text{s}^{-1}$) and diffusion resistance ($\text{s}\cdot\text{cm}^{-1}$) were measured during each experiment (except experiment 1). A circular leaf-chamber (leaf area enclosed = 1 cm^2) was used to take measurements on the abaxial surface, 10-20 cm from the tip of the leaves. One reading was taken on two leaves from each of three or four plants at each sample time. Mean values were calculated by pooling the measurements from all plants at each time. Measurements were made at various time intervals for the duration of each experiment which measured water uptake. Concurrent measurements of the transpiration rates of pot-grown plants in the same glasshouse were also collected for comparison with plants in the mist-boxes. The values of the pot-grown plants were more uniform, therefore fewer measurements were taken.

RESULTS

I. Preliminary Studies

1. SR elution experiments

These experiments were undertaken to determine the length of time required for the elution of SR from main roots and branches. The amount of SR eluted from the root tissue did not change after 80 min. For branches up to 80-90% of the SR was eluted after 80 min, while for the main roots approximately 50% of SR was eluted after 400 min (figure 4).

Figure 4: Elution rates of SR from branches and main roots into distilled water. Bars = standard deviation.

2. Water free space experiments

The volume of root which is 'water free space' (WFS) was measured by the volume of 1 mmole l^{-1} SR taken into the WFS of the root tissue. The results from these WFS experiments are summarized in table 3. The branches had the highest percentage of their volume as WFS to SR (Mean $27.8 \pm 7.3\%$ WFS). The basal regions of the main

roots (basal 40-60cm) had $22.1 \pm 6.4\%$ root volume as WFS. The apical regions only had $14.4 \pm 5.1\%$ WFS as measured by SR (table 3).

Table 3: Water Free Space of branches and main roots measured with 1 mmole l^{-1} SR.

Experiment	Tissue	Number of Samples	Mean WFS $\pm$ S.D.
1 ¹	branches	12	$27 \pm 9 \%$
1 ¹	bare main	6	$16 \pm 5 \%$
1 ¹	sheathed main	6	$10 \pm 2 \%$
1 ²	branches	12	$21 \pm 5 \%$
1 ²	bare main	6	$17 \pm 4 \%$
1 ²	sheathed main	6	$12 \pm 7 \%$
2	branches	12	$26 \pm 8 \%$
2	bare main	12	$30 \pm 6 \%$
2	sheathed main	12	$14 \pm 4 \%$
3	branches	12	$38 \pm 17 \%$
3	bare main	12	$25 \pm 8 \%$
3	sheathed main	12	$21 \pm 15 \%$
Mean	branches	48	$28 \pm 7 \%$
Mean	bare main	36	$22 \pm 6 \%$
Mean	sheathed main	36	$14 \pm 5 \%$

¹ roots from pot-grown plants, ² SR eluted into 0.5 mM NaCl solution.

II. Water Uptake Experiments

1. SR accumulation on branches

Rates of accumulation of SR from a SR-distilled water mist by branches

(Experiments 1 - 5) on transpiring and non-transpiring plants (controls) are presented in figures 5, 6, 8, 10 and 12. Net SR accumulation rates were determined from the figures by subtracting the 'background' SR accumulation levels of the controls from the SR accumulation levels of the roots on the transpiring plants. The net SR accumulation rates were then converted into net water fluxes for branches. Mean light levels and transpiration rates of the plants during each experiment are also presented (figures 7, 9, 11, and 13).

In each experiment the branches on the transpiring plants accumulated more SR than branches on the control plants. The accumulation of SR was attributed to water uptake by the branches on the transpiring plants, and taken as evidence that the accumulation of SR could be used to measure the rate of water uptake.

Accumulation of SR by the branches of transpiring plants was higher than the branches on non-transpiring controls in the first three experiments (figures 5, 6, and 8), however the rate of accumulation varied between these experiments, ranging from 10 $\text{pmoles}\cdot\text{h}^{-1}\cdot\text{mm}^{-2}_{\text{root}}$ (expt 2, fig. 6) to 50 $\text{pmoles}\cdot\text{h}^{-1}\cdot\text{mm}^{-2}_{\text{root}}$ (expt. 1, fig. 5). The amount of SR accumulated from the SR-distilled water mist decreased after 3-4 hours during experiments 1 and 3. Branches on control plants also accumulated 30-50 $\text{pmoles}\cdot\text{mm}^{-2}_{\text{root}}$ in 1 to 2 h, but remained at this level for the rest of the experiment. Since these experiments were done during the winter months, daylight faded early in the afternoon. Light levels and transpiration rates both decreased during experiment 2 (figure 7), and varied during experiment 3 (figure 9). Note that these experiments (1-3) began late in the morning, at 1030 h (expt. 1 and 3) or 1145 h (expt. 2).

In the light of this experience, procedural modifications were made during experiments 4 and 5 (see figures 10 and 12). This produced higher levels of SR accumulation on the branches of the transpiring plants, while the controls stayed at about 40-50 $\text{pmoles}\cdot\text{mm}^{-2}_{\text{root}}$. These modifications were: 1) the experiments were started earlier in the morning (0800-0900h) to take advantage of the daylight of the short winter days; 2) the controls were prepared the day before each experiment; and 3) some main roots were removed to increase water uptake rates of the remaining roots. The transpiration rates paralleled light intensity in experiment 5 (figure 13).

The amounts of SR accumulated on all branches from all five experiments were pooled in figure 14. SR accumulation varied considerably on branches from transpiring plants. The highest rate of SR accumulation, achieved by a half dozen branches, was 250 $\text{pmoles}\cdot\text{h}^{-1}\cdot\text{mm}^{-2}_{\text{root}}$ (line figure 14A). There were also many branches on transpiring

plants which had the same amount of SR on their surface as the controls (compare figure 14 A and B). In contrast, SR levels on branches from the control plants showed little variation compared to branches on transpiring plants (figure 14B). Branches on the controls accumulated SR only slowly for 3 - 4 hours, then levelled off at about 50 - 100 $\text{pmoles}\cdot\text{mm}^{-2}\cdot\text{root}$.

The rate of SR accumulation of each branch was calculated by dividing the amount of SR accumulated by the period of accumulation from SR-water mist. SR accumulation rates of all branches in experiments 1 -5 are presented as a histogram (figure 15). The SR accumulation rates of branches on transpiring and control plants are both log-normally distributed (tested using the Probit transformation method, Wardlaw, 1985). Because of the large variances of the rates of accumulation and because the values are not normally distributed, simple comparisons of means and errors are useless. Comparisons must be made between the distributions, taking account of their variability.

The branches on the transpiring plants (figure 15 A) had a broad range of SR accumulation rates ($0.69\text{-}230.2 \text{ pmoles h}^{-1} \text{ mm}^{-2}\cdot\text{root}$). The mean was $40 \pm 40 \text{ pmoles h}^{-1}\cdot\text{mm}^{-2}\cdot\text{root}$, mode $20\text{-}30 \text{ pmoles h}^{-1}\cdot\text{mm}^{-2}\cdot\text{root}$. The rate of SR accumulation of branches on the non-transpiring plant showed little variation (range $0\text{-}35.5 \text{ pmoles SR}\cdot\text{h}^{-1}\cdot\text{mm}^{-2}\cdot\text{root}$, figure 15B). The mean rate for the controls was $10.0 \pm 7.1 \text{ pmoles SR}\cdot\text{h}^{-1}\cdot\text{mm}^{-2}\cdot\text{root}$. Fifty percent of the branches on transpiring plants had SR accumulation rates over 35 $\text{pmoles}\cdot\text{h}^{-1}\cdot\text{mm}^{-2}\cdot\text{root}$ (the upper limit of branches on the non-transpiring plants).

SR accumulation rates of the branches on transpiring and non-transpiring plants were normalized by taking the $\log_{10}$ of each and compared by a t-test. The means were different at the $P>0.0001$ ($t=15.599$). The means were also different at the $P>0.0001$ when compared by an analysis of variance.

Branches were selected from the transpiring plants which had SR accumulation rates above the 95% confidence limit of the distribution of the controls (95% C.L. = mean $\pm t$ (SEM); $P>0.05$, $t = 1.96$, SEM = S.D./N). Because of the continuous misting, the

background SR accumulation on the controls ($10 \pm 0.96 \text{ pmoles} \cdot \text{h}^{-1} \cdot \text{mm}^{-2} \text{root}$) was subtracted from the SR accumulation rates of the branches on transpiring plants to produce values of net SR accumulation rates. These rates were then converted to net water fluxes ($J_s, \mu\text{l H}_2\text{O} \cdot \text{h}^{-1} \cdot \text{cm}^{-2} \text{root}$) by dividing by 5 (figure 16).

The distribution of net water fluxes of the branches is presented in figure 16. Mean water flux was $8 \pm 8 \mu\text{l H}_2\text{O} \cdot \text{h}^{-1} \cdot \text{cm}^{-2} \text{root}$. The geometric mean is a better measure of the central tendency of log-normally distributed data (Wardlaw, 1985) and in these terms most branches have a water flux of $4.7 \mu\text{l H}_2\text{O} \cdot \text{h}^{-1} \cdot \text{cm}^{-2} \text{root}$. Water fluxes of these branches ranged from 0 - $44 \mu\text{l H}_2\text{O} \cdot \text{h}^{-1} \cdot \text{cm}^{-2} \text{root}$, and therefore any values up to this maximum seem possible. Eighty percent of branches had fluxes between 0 - $10 \mu\text{l H}_2\text{O} \cdot \text{h}^{-1} \cdot \text{cm}^{-2} \text{root}$; 10% of branches had fluxes greater than $14 \mu\text{l H}_2\text{O} \cdot \text{h}^{-1} \cdot \text{cm}^{-2} \text{root}$, 5% had fluxes above $18 \mu\text{l H}_2\text{O} \cdot \text{h}^{-1} \cdot \text{cm}^{-2}$, and 1% had fluxes over $40 \mu\text{l H}_2\text{O} \cdot \text{h}^{-1} \cdot \text{cm}^{-2} \text{root}$.

Figure 5: Accumulation of SR by branches on transpiring plants (n=3) and control plants from a SR-distilled water mist. Each point is the mean of 4 roots (Experiment 1).

Figure 6: Accumulation of SR by the branches of transpiring plants (n=3) and a control plant from a mist of SR-distilled water. Each point is the mean of 9 roots. Data from experiment 2.

Figure 7: Transpiration rates of the plants and light intensity during experiment 2 (fig.6). Each point is the mean of 9 readings, from 3 plants.

Figure 8: Accumulation of SR by branches on transpiring plants (n=4) and a control plant from a SR-distilled water mist. Each point is the mean of 6 roots. From experiment 3.

Figure 9: Transpiration rates of the plants and light intensity during the SR accumulation experiment 3 (fig.8). Each point is the mean of 8 readings, n=4 plants.

Figure 10: Accumulation of SR by branches on transpiring (n=4) and control plants from a SR-distilled water mist. Each point is the mean of 12 roots (Experiment 4).

Figure 11: Transpiration rates of the plants and light intensity during SR accumulation experiment 4 (fig.10). Each point is the mean of 16 readings, n=4 plants.

Figure 12: Accumulation of SR by branches on transpiring plants (5 plants) and a control plant from a SR-distilled water mist. Each point is the mean of 12 roots (Experiment 5).

Figure 13: Transpiration rates of the plants and light intensity during SR accumulation experiment 5 (fig.12). Each point is the mean of 15 readings, n=5 plants.

Figure 14: SR accumulation on all branches from experiments 1 to 5.
(A) Branches on transpiring plants (n=310), line= max.accum.
(B) Branches on non-transpiring plants (control,n=216).

Figure 15: SR accumulation rates of all branches. (A) Branches on transpiring plants. (B) Branches on non-transpiring control plants.

2. SR accumulation on the root system

SR accumulation sampled widely throughout the entire root system was used to measure water uptake by the branches and main roots over the entire root system to determine where the highest fluxes of water occurred. As mentioned in the Methods and Materials two sampling procedures were used. Branches and main roots were sampled from the 'basal' regions of the root system (near the stem base) in the upper regions of the box. This sample collection did not always include the apical regions depending on the length of the main roots (expts 1.1 - 1.3, part i). A final experiment in which samples were collected basipetally from the root apex up to the stem base was conducted to measure water flux over the whole length of the main roots (Expt. 1.4, Part ii). This therefore provided a complete survey of the root system and this will be presented separately.

(i) SR accumulation on the basal regions of the root system

In experiments 1.1, 1.2, and 1.3, SR accumulation rates by branches and main roots were measured separately at different distances from the base of the plant. Water uptake was measured at 5, 15, 25, 35, and 45 cm from the base of the plants. Two-centimetre long segments of main axes and the attached branches were collected at each position. The branches were removed from each segment and the SR eluted from the main axes and branches in separate vials. The amounts of SR accumulated over time on the main roots and branches at each position are illustrated in figures 17, 19, and 21.

In experiment 1.1 (figure 17), the main roots at 45 cm from the stem base (near the root apex) had the greatest SR accumulation (see 4 and 8 h). The branches on basal regions of the main axes accumulated the most SR of any branches in this experiment (200 pmoles·mm⁻², at 8 h). The light levels and transpiration rates during this experiment increased during the day, and dropped off at the end of the day (figure 18).

During experiment 1.2, the branches on the axes 5-25 cm from the stem base accumulated more SR than the main roots (4 and 6 h, figure 19). After 8 h of transpiration in SR-water mist, the branches 15 - 45 cm from the base of the plant also accumulated much SR. SR accumulation on the main roots varied, more widely than in experiment 1.1. SR levels on the roots of the non-transpiring plants (controls) also varied considerably and was sometimes higher than on the roots of the transpiring plants. Light intensity and transpiration rates both increased during the day (figure 20).

In experiment 1.3 (8.5 h, figure 21), the branches again accumulated more SR than the main axes. The main axes also had high SR accumulation levels near the apex (55cm). SR accumulation on non-transpiring control plants was around 50 pmoles·mm⁻² after 8.5 and 22.5 h. Light intensity and transpiration rates were highest at 6 h, then decreased at 9 h (figure 22). There was much heterogeneity in the amount of SR accumulated between sample times and between experiments. During these 3 experiments, branches near the

stem base (0 -25 cm) accumulated more SR than the main roots. High accumulations of SR on the main roots were found on apical regions 45 cm from the plant.

The SR accumulation levels at each position were pooled and mean SR accumulation was calculated at times 4, 6 and 8 h for branches and axes (figure 23 A - D). The amount of SR accumulated on the main roots did not change from 4 - 8 h (figure 23, A-C); at 8 h the amount of SR was the same on the main roots of transpiring as on those of non-transpiring plants (figure 23 C). Branches on transpiring plants accumulated more SR than branches on non-transpiring plants (8 h, figure 23, D). When the amounts of SR accumulated at 4, 6 and 8 h are pooled, the branches have the highest levels of SR at each point, while the main roots and controls (all control axes and branches) were similar (figure 23 E).

Each measurement of SR accumulation rate from experiments 1.1-1.3 (at 4-8 h) was converted into water fluxes. These results were pooled and the water fluxes of the main roots and branches were graphed as a function of distance from the base of the stem (Main roots, figure 24; Branches, figure 26). The values of water flux were similar on the main roots of transpiring plants (2 or 3 root segments per sample) and control plants (1 or 2 roots per sample) (figure 24). However the main roots on the transpiring plants often had higher fluxes of water than the controls. There was a slight dependence of water fluxes with distance from stem base, caused by a few samples which had high fluxes at 25 and 45 cm from the stem base (figure 24). When the water fluxes of the main roots from transpiring and non-transpiring plants were plotted as histograms, they were not very different (figure 25). Mean flux into main roots on transpiring plants (3.13 ± 3.12 , range 0 - $16.2 \mu\text{l H}_2\text{O}\cdot\text{h}^{-1}\cdot\text{cm}^{-2}_{\text{root}}$) was close to the fluxes of the controls (2.03 ± 1.59 , range 0 - $7.31 \mu\text{l H}_2\text{O}\cdot\text{h}^{-1}\cdot\text{cm}^{-2}_{\text{root}}$). The water fluxes of the main roots from transpiring plants were not statistically different from the non-transpiring plants (normalized data tested by a one-factor ANOVA, and Fisher tests).

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Also, branches sampled at these positions from the main roots of transpiring and non-transpiring plants had similar fluxes (figure 26). (Each point from the transpiring plants is the average of 30 - 60 branches from 2 or 3 plants, each point from the control plants is the average of 18 - 30 pooled branches.) Although, the branches on transpiring plants often achieved very high water fluxes, many samples from transpiring plants had fluxes similar to the controls between 3 - 5 $\mu\text{l H}_2\text{O}\cdot\text{h}^{-1}\cdot\text{cm}^{-2}_{\text{root}}$. The means of these two groups of branches were also very close to each other (mean flux of branches on transpiring plants $2.18 \pm 2.14 \mu\text{l H}_2\text{O}\cdot\text{h}^{-1}\cdot\text{cm}^{-2}_{\text{root}}$; mean flux of controls $1.90 \pm 2.15 \mu\text{l H}_2\text{O}\cdot\text{h}^{-1}\cdot\text{cm}^{-2}_{\text{root}}$), and the frequency distribution of the fluxes was also similar (figure 27). These two populations were not statistically different (same statistical tests as main roots above). There were several samples of branches from the transpiring plants which had much higher water fluxes than any of the branches collected from the control plants. Note that the mean water fluxes of the main roots and branches of the control plants (2.034 and $1.897 \mu\text{l H}_2\text{O}\cdot\text{h}^{-1}\cdot\text{cm}^{-2}_{\text{root}}$), convert back to SR accumulation rates of 10 pmoles $\text{SR}\cdot\text{h}^{-1}\cdot\text{cm}^{-2}_{\text{root}}$, which is the same SR accumulation rate as found on branches collected from the control plants in part 1. The fluxes calculated for roots of the controls were therefore caused by the background misting of SR, rather than by water uptake.

The variability of fluxes measured on the roots of both transpiring and non-transpiring plants was large (SD 80 - 100% of means). With such large variabilities in the samples, another approach was necessary to sort out useful comparisons. This was to select measured values that were significantly outside the range of variation of the controls, and consider only these selected values. To distinguish clearly net fluxes above the mean 'background' level of the controls, only samples which had fluxes above the upper limit (95% C.L.) of the normal distribution of the control values were selected. The 95% confidence limit (C.L.) of the control mean was calculated using the formula $95\% \text{ C.L.} = \text{mean} \pm t (\text{SEM})$ where the t value is from a student t -table for N samples, and the standard error of the mean (SEM) is the standard deviation divided by square root of the sample size

(N). Samples from the transpiring plants which had fluxes above this limit were selected as having fluxes which were clearly different from the controls.

The mean flux of water into main roots from the control plants was $2 \pm 1.6 \mu\text{l H}_2\text{O}\cdot\text{h}^{-1}\cdot\text{cm}^{-2}_{\text{root}}$. Thirteen of the fifty samples of main roots from transpiring plants were significantly different from the controls (above the 95% C.L. = $2.74 \mu\text{l H}_2\text{O}\cdot\text{h}^{-1}\cdot\text{cm}^{-2}_{\text{root}}$, $P>0.05$, $t=2.074$). The mean flux of these specially-selected samples of main root was $6.45 \pm 3.6 \mu\text{l H}_2\text{O}\cdot\text{h}^{-1}\cdot\text{cm}^{-2}_{\text{root}}$. The net flux of the main roots was $4.42 \pm 3.6 \mu\text{l H}_2\text{O}\cdot\text{h}^{-1}\cdot\text{cm}^{-2}_{\text{root}}$ when the background mist was subtracted ($10 \text{ pmoles SR}\cdot\text{h}^{-1}\cdot\text{mm}^{-2}_{\text{root}} = 2.034 \mu\text{l H}_2\text{O}\cdot\text{h}^{-1}\cdot\text{cm}^{-2}_{\text{root}}$).

Twenty-two branches from the transpiring plants had fluxes above the 95% confidence limit of the controls (Mean controls $1.9 \pm 2.2 \mu\text{l H}_2\text{O}\cdot\text{h}^{-1}\cdot\text{cm}^{-2}_{\text{root}}$; upper limit $2.8 \mu\text{l H}_2\text{O}\cdot\text{h}^{-1}\cdot\text{cm}^{-2}_{\text{root}}$; $P>0.05$, $t=2.064$). The mean flux of the branches which were above the distribution of the controls was ($7.1 \pm 5.4 \mu\text{l H}_2\text{O}\cdot\text{h}^{-1}\cdot\text{cm}^{-2}_{\text{root}}$), which yielded a net flux of $5.2 \pm 5.4 \mu\text{l H}_2\text{O}\cdot\text{h}^{-1}\cdot\text{cm}^{-2}_{\text{root}}$ when the background flux was subtracted.

Samples of axes and branches were collected at various distances from the stem base to determine where water fluxes were highest along the main axes. As illustrated in figure 3 in chapter 2 there was a vertical gradient in mist distribution inside the mist boxes so that the volume of mist solution captured in collection vials decreased with distance from the bottom of the box. To compensate for this uneven misting in the boxes, the mean accumulation levels (expressed as fluxes) of the controls at each position were subtracted from the corresponding mean fluxes of the roots of transpiring plants. The fluxes at each position were means calculated from the samples selected on transpiring plants in the previous section which were significantly above the distribution of the controls. The net fluxes of branches and axes at each position are summarized in figure 28. Branches close to the stem base had the highest net fluxes of water; the water fluxes of branches decreased towards the apex of the main root. The main axes had highest fluxes 45 cm from the stem base near the root apex. It is unclear if the apex of the root was at 45 cm or beyond. The

tip of the main roots was probably within 10 to 20 cm from the furthest sample at 45 cm. In the next experiment, 1.4, segments of main root were collected at the apex of the main roots and then at intervals along the main roots up to the base of the plants, to overcome this limitation of the sampling method used during experiments 1.1 - 1.3.

(ii) SR accumulation throughout the entire root system. (Expt 1.4)

The amount of SR accumulated after 4 and 8 h on the branches and main roots along the entire length of these axes are presented in figure 29 (and the transpiration rates are presented in figure 30). After 4 h there were higher levels of SR accumulated on the apical 40 cm of the axes, however the controls (1 bagged plant, 1 detopped plant) had even greater accumulations of SR (figure 29). SR accumulation was low in the basal 60 cm (40 - 112 cm from the apex) on the roots of both transpiring and control plants. A similar pattern occurred at $t = 8$ h, where both branches and axes in the apical regions had high amounts of SR. SR accumulation levels of the controls was lower than the transpiring plants. The levels of SR accumulated during this experiment were higher than most other experiments.

The net values of SR accumulation were calculated by subtracting the amount of SR accumulated on the root of the control plants from the SR accumulation levels on the roots of the transpiring plants at each distance. These net SR accumulations of branches and main roots at each position were converted to water fluxes ($J_s \mu\text{l H}_2\text{O} \cdot \text{h}^{-1} \cdot \text{cm}^{-2}_{\text{root}}$) (figure 31). The apical regions (20 - 40 cm) of the main roots had the highest water fluxes. The branches became active between 25 - 40 cm from the tip. Branches and axes in the basal regions (50 cm from the root apex) both had low water fluxes from 50 cm ($0.25 - 0.5 \mu\text{l H}_2\text{O} \cdot \text{h}^{-1} \cdot \text{cm}^{-2}_{\text{root}}$; figure 31).

The amount of water collected by the branches and axes at each distance from the root apex was calculated by multiplying the water flux (J_s) by the surface area of branches and axes respectively. The amount of water collected by 1 cm segments of main roots were

collected by all the branches on 1 cm of main root. The length of branches on each fifth centimetre of axis was measured in chapter 3 (figure 6) and used to determine the surface area of branches at each position of flux measurement (figure 32). The branches on a centimetre of main root clearly collected more water than the centimetre of main root. In the apical regions of the main roots where there were no branches or only very few short ones, the axes collect more water than the axes at 25 - 70 cm from the tip of the main roots (figure 31). The branches on the basal regions of the main roots (50 - 110 cm from the apex) continued to collect more water than the main axes. At 75 cm the branches were very long (Ch.3 figure 6) and produced a high flux (circle above line, figure 32). If the length of branches at positions close to 75 cm was used, a lower water collection value was calculated, which was consistent with the amount of water collected at other positions (line figure 32).

3. Summary of physiological conditions during each experiment

Environmental conditions and plant activity during the water uptake experiments are summarized in table 2. Leaf temperatures of the plants ranged from 18.5 to 29.4 °C with a mean leaf temperature of 24.8 ± 3 °C. Mean light intensity during each experiment varied from 94 to 706 $\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$, however the overall mean light intensity was 261 ± 205 $\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$. Diffusion resistance was about four times higher on plants in the mist boxes (39.4 ± 33.3 $\text{s}\cdot\text{cm}^{-1}$) than pot-grown plants in the same greenhouse (9.51 ± 4.17 $\text{s}\cdot\text{cm}^{-1}$). Transpiration rates of pot-grown plants (range 2-4; mean 2.0 ± 0.91 $\mu\text{g H}_2\text{O}\cdot\text{cm}^{-2}\cdot\text{s}^{-1}$) were twice those of the mist box-grown plants (range 0.4-1.2, mean 0.83 ± 0.29 $\mu\text{g H}_2\text{O}\cdot\text{cm}^{-2}\cdot\text{s}^{-1}$).

Table 4: Environmental conditions and physiological activity of plants in the mist boxes during the water uptake experiments.

Experiment		Leaf Temperature (°C)	Light Intensity ($\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$)	Diffusion Resistance ($\text{s}\cdot\text{cm}^{-1}$)	Transpiration Rate ($\mu\text{gH}_2\text{O}\cdot\text{cm}^{-2}\cdot\text{s}^{-1}$)
2	PG	21.9 ± 0.6	100 ± 20	6.5 ± 0.2	2.04 ± 0.02
	EXPTL	24.0 ± 1.0	170 ± 70	37 ± 14	0.73 ± 0.34
3	PG	21.5 ± 0.3	94 ± 30	10 ± 1.2	1.35 ± 0.15
	EXPTL	21.2 ± 0.6	180 ± 30	30 ± 7.0	0.96 ± 0.27
4	PG	25.9 ± 1.9	340 ± 210	6.6 ± 2.0	3.71 ± 1.10
	EXPTL	28.5 ± 2.4	690 ± 380	120 ± 130	0.47 ± 0.24
	BX	29.4 ± 1.4	706 ± 221	28 ± 9.6	1.19 ± 0.13
5	EXPTL	25.7 ± 1.4	150 ± 50	27 ± 9.4	0.87 ± 0.29
1.1	PG	24.6 ± 3.7	105 ± 90	8.7 ± 2.7	1.81 ± 1.32
	EXPTL	24.6 ± 4.2	140 ± 90	13 ± 5.6	1.23 ± 0.76
1.2	PG	23.9 ± 0.2	470 ± 500	7.5 ± 3.6	2.55 ± 1.75
	EXPTL	27.8 ± 3.9	200 ± 190	26 ± 6.7	1.17 ± 0.67
1.3	PG	26.6 ± 1.2	320 ± 335	18 ± 10	1.97 ± 1.16
	EXPTL	26.9 ± 1.5	110 ± 135	30 ± 7.6	0.72 ± 0.19
1.4	EXPTL	18.5 ± 0.9	150 ± 110	33 ± 7.2	0.48 ± 0.14
X _{PG}		24 ± 2	240 ± 160	9.5 ± 4	2.0 ± 0.9
X _{EXPTL}		25 ± 3	225 ± 190	39 ± 33	1.0 ± 0.3
X _{all}		25 ± 3	260 ± 200	27 ± 28	1.4 ± 0.9

EXPTL = experimental plants, PG = pot-grown plants, BX = plants in other box. Each value is the mean $\pm$ standard deviation of all means at each sample time for that experiment.

The transpiration rates may be converted into $\mu\text{mol H}_2\text{O}\cdot\text{cm}^{-2}\cdot\text{s}^{-1}$ or $\text{mmol H}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ by multiplying

$$\mu\text{g H}_2\text{O}\cdot\text{cm}^{-2}\cdot\text{s}^{-1} \quad \text{by } 0.555 \mu\text{mol H}_2\text{O}\cdot\text{cm}^{-2}\cdot\text{s}^{-1}$$

$$\text{or } 0.555 \text{ mmol H}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$$

given 1 mole H_2O = 18.05 cm^3 at 20°C (from Nobel, 1991).

Figure 17: Accumulation of SR by the roots of maize plants transpiring with their roots in a SR-distilled water mist (sample time in upper left corner of each panel, Expt. 1.1, n=5 plants, 50 days, 1 control).

Figure 18: Light intensity and transpiration rates of plants during SR Accumulation experiment 1.1.

Figure 19: Accumulation of SR by the roots of maize plants transpiring with their roots in a SR-distilled water mist (sample time in upper left corner of each panel, Expt. 1.2, n=7 plants, 47 days, 1 control).

Figure 20: Light intensity and transpiration rate of plants during SR Accumulation Experiment 1.2.

Figure 21: Accumulation of SR by the roots of maize plants transpiring with their roots in a SR-distilled water mist (sample time in upper left corner of each panel, Expt. 1.3, n=4 plants, 50 days, 1 control).

Figure 22: Light intensity and transpiration rate of the plants during SR Accumulation Experiment 1.3.

Figure 23: SR accumulation on main roots and branches as a function of distance from the stem base (cm). Pooled data from experiments 1.1 - 1.3. Controls are roots from non-transpiring plants (detopped and bagged). **Fig. A.** Accumulation on branches and main roots after 4 h transpiration in SR mist. **Fig. B.** Accumulation of branches and main roots after 6 h transpiration in SR mist. **Fig. C.** Accumulation on main roots after 8 h in mist, transpiring and controls. **Fig. D.** Accumulation on branches after 8 h in SR mist, transpiring and controls. **Fig. E.** Mean accumulation on branches, main roots and controls (4, 6 and 8 h).

Figure 24: Water fluxes (J_s) of main roots on transpiring and non-transpiring plants from experiment 1.1, 1.2, and 1.3.

Figure 25: Water fluxes (J_s) of main roots on transpiring (A) and non-transpiring plants (B) from Expts 1.1, 1.2 & 1.3.

Figure 26: Water fluxes (J_s) of branches on transpiring and non-transpiring plants from experiments 1.1, 1.2, and 1.3.

Figure 27: Water fluxes of branch es on transpiring (A) and non-transpiring plants (B) measured during Expts 1.1, 1.2, & 1.3.

Figure 28: Net water fluxes of branches and main roots on plants transpiring with their roots in a SR-distilled water mist (4 - 8 h) from experiments 1.1 - 1.3.

Figure 29: Accumulation of SR by the roots of maize plants transpiring with their roots in a SR-distilled water mist (sample time in upper left corner of each panel, Expt. 1.4, n=4 plants, 50 days, 2 controls).

Figure 30: Light intensity and transpiration rate of the plants during SR accumulation experiment 1.4.

Figure 31: Water fluxes (J_s) into branches (O) and main roots ($\diamond$) as a function of distance from the apex of the main root (from experiment 1.4).

Figure 32: Water collection by branches (O) and main roots (◊) as a function of distance from the apex of the main root (from experiment 1.4).

III. Summary

Most of the SR was eluted from branches in 80 min.(80 - 90 %), while only 50 % of SR was eluted from the main roots even after 400 min. The volume of root which was 'water free space' to SR was highest in branches (27.8 %), lowest in the apical regions of main roots (14.4 % WFS) and intermediate in the basal portions of main roots (22.1 % WFS; S.D. 30 % of means).

Table 5: Water fluxes into branches and main roots measured by the accumulation of SR from a SR-distilled water mist.

	J_L	J_S	
Expts 1 - 5 (Branches)	($\mu\text{l H}_2\text{O h}^{-1} \text{ cm}^{-1}_{\text{root}}$)	($\mu\text{l H}_2\text{O h}^{-1} \text{ cm}^{-2}_{\text{root}}$)	
Mean flux	0.76	7.6 ± 7.8	
Geometric mean flux	0.47	4.7	
Maximum flux	4.4	44	
80 % branches range	0 - 1	0 - 10	
10 % branches range	> 1.4	> 14	
5 % branches range	> 1.8	> 18	
1 % branches range	> 4.0	> 40	
<u>Expt 1.1 - 1.3</u>			
Branches	0.52	5.2 ± 5.4	max. 22
Main roots	1.39	4.4 ± 3.6	max. 16
<u>Expt 1.4</u>			
Branches	0.15	max. 0.4	1.5 ± 1.3 max. 4.4
Main roots	1.0	max. 2.5	3.1 ± 2.3 max. 7.8

The accumulation of SR from a SR-distilled water mist by the mature roots of transpiring plants of maize was used to measure water fluxes. The range of measured fluxes J_S and J_L (table 5) of water into branches was extremely large. Branches sampled

from all over the root system had water fluxes (J_s) ranging from 0 - 44 $\mu\text{l H}_2\text{O h}^{-1} \text{cm}^{-2}_{\text{root}}$, with a mean flux (J_s) of 4.7 $\mu\text{l H}_2\text{O h}^{-1} \text{cm}^{-2}_{\text{root}}$. Most branches (80 %) had water fluxes of less than 10 ($\mu\text{l H}_2\text{O h}^{-1} \text{cm}^{-2}_{\text{root}}$), 10 % had fluxes over 14 $\mu\text{l H}_2\text{O h}^{-1} \text{cm}^{-2}_{\text{root}}$, 5 % had fluxes over 18 $\mu\text{l H}_2\text{O h}^{-1} \text{cm}^{-2}_{\text{root}}$ and one percent of the branches had fluxes over 40 $\mu\text{l H}_2\text{O h}^{-1} \text{cm}^{-2}_{\text{root}}$. Branches collected from the basal 45 cm of main axes (Expts. 1.1 - 1.3) had fluxes of $5.2 \pm 5.4 \mu\text{l H}_2\text{O h}^{-1} \text{cm}^{-2}_{\text{root}}$, while branches collected from along the entire length of main axes had a mean flux of $1.5 \pm 1.3 \mu\text{l H}_2\text{O h}^{-1} \text{cm}^{-2}_{\text{root}}$ (from expt. 1.4). The basal regions of main roots had a mean water flux of $4.4 \pm 3.6 \mu\text{l H}_2\text{O h}^{-1} \text{cm}^{-2}_{\text{root}}$ (Expts. 1.1 - 1.3), however the samples of main roots collected from the entire lengths of axes had a mean flux of $3.1 \pm 2.3 \mu\text{l H}_2\text{O h}^{-1} \text{cm}^{-2}_{\text{root}}$ (expt. 1.4, table 5).

The flux of water was highest into branches at 20 - 40 cm from the apex of the main roots. The apical 40 - 60 cm of the main roots had the highest water fluxes. However, when the volume of water collected per hour by branches and main roots was calculated (by multiplying J_s by the total surface area of 1 cm of axes or the total surface area of all branches on 1 cm axes), most (8 times more) water was collected by the branches which have 8 - 14 times more absorptive surface than the main roots (table 5).

DISCUSSION

I. Preliminary Studies

1. SR elution time

The elution of SR was determined by soaking tissue in SR for 1 - 2 h, then eluting the dye from the tissue by soaking the tissue in two washes of distilled water and determining the amount of SR eluted during each wash. Elution of SR from the root tissue into distilled water occurred in 80 min. Most SR (80 - 90 %) was eluted from branches during the first elution, while only 50 % of the SR was eluted from the main roots during this period. The

build up of SR on the branches was caused by more binding of SR to branches than on main roots. Since more SR was eluted from the branch roots than the main roots, it is unlikely that more SR bound to the branches, causing more SR to accumulate on the branches.

When tissue is soaked in a solution of SR, dye will diffuse into the tissue until the concentrations of dye inside and outside are in equilibrium. Binding of dye to tissue, which removes the dye from solution, may cause more SR to be taken up by the tissue than if binding did not occur. Staining of various tissues by SR has been studied by Canny (1988). The fluorescent intensity of tissue was enhanced 1.7:1 over the fluorescent intensity of the staining solution. When the tissue was soaked for several hours in a SR solution then washed with water, fluorescent intensity was 3 - 7 % of the initial fluorescence which was the same as autofluorescence. Canny (1988) found that SR which is negatively-charged at normal pH (pH 7), washed out of even lignified walls of wheat leaves.

Although Canny (1988) found that SR mostly washes out of leaves, some SR must remain bound in the tissue. This SR may be either (i) bound to wall material; (ii) in the WFS of the root at equilibrium with the eluate or (iii) sequestered in the vacuoles of cells which may be permeable to SR or have substances in their vacuoles which may bind SR (this SR would not be measured since it would not be eluted). Since the amount of SR accumulated on the roots from a SR-water mist was used to measure the amount of water uptake, any dye which remains bound to the tissue will reduce the water fluxes measured by the method. This binding of SR should not nullify the method, but may lead to underestimates of the fluxes. Comparison of fluxes by the different roots (branches vs main roots) could be complicated by any differences in binding of SR to branches and main roots.

The amount of SR which washed out of the main roots during the second elution period may be SR in the water free space of the root (see next section). Even though the

branches have a higher WFS (30 % of their volume) than the main roots (19 % WFS), the absolute volume of WFS is greater in the main roots. The volume of WFS can be calculated for 1 cm long segments of main roots and branches. A 10-cm long segment of main root (diameter 1 mm) has 150 μ l of WFS while a branch of similar length (diameter 0.3 mm) has 0.2 μ l of WFS. This may explain why only 50 % of the SR was eluted during the first elution period (figure 4). The amount of SR eluted from main roots can be increased by eluting into a large volume of water so that most of the SR will elute from the root before an equilibrium will be achieved with the eluate. The SR accumulated on main roots during the water uptake studies was therefore eluted into 3 - 5 ml aliquots of water.

2. Water free space

If a plant cell or piece of plant tissue is soaked in a salt solution, the salt solution will be passively absorbed in a two phase process. Salt solution is absorbed quickly for about 60 minutes, and then absorbed slowly over a longer period of time. Salt solution can be rapidly washed out of the tissue in about 60 min. upon transfer to distilled water. The space into which the solute and solvent can readily diffuse has been defined as the 'water free space' or apparent free space. This space was determined using slices of *Beta* disks, and is considered an extension of the environment (Briggs and Robertson, 1957). A water free space was first demonstrated in roots by Hope and Stevens in 1952 (as quoted by Epstein, 1956). Water free space has been reviewed by Brouwer (1965).

The free space of roots may be estimated by the passive uptake of anions. The volume of the roots of aeroponically-grown plants of maize which was WFS was determined by the uptake of the anion SR (pKa 5.2). These values are compared to the values for WFS of the roots of other plants in table 6. The published values for water free space of roots varies widely from 11 - 34 % WFS and the results from the roots of aeroponically-grown maize fall within this range (14 - 28 %). Much of the variation in the published values of WFS of roots has been attributed to procedural differences between

these studies. It has also been suggested that WFS increases with thinness of tissue slices or roots (This was supported by the WFS experiments; recall the diameters of branches, basal and apical regions of main roots which are 0.349 mm, 1.0 mm and 1.3 mm respectively.). It has been suggested that WFS has been overestimated because of the layer of solution adhering to the root surface. Levitt (1957) calculated a WFS of 8 % after correcting for this surface layer. Ingelsten and Hylmö (1961) found that the WFS of wheat (*Triticum aestivum*) roots was 11 - 14 % using a centrifugation method to remove the surface layer (table 6). The surface film could also be removed by a brief (5 sec) rinse in water (Ingelsten and Hylmö, 1961). Presumably the 5 - 10 sec rinse removed the surface film of SR from the maize roots, and the corresponding WFS values are accurate.

Table 6: Water Free Space in Roots.

Species	Root type	Percent WFS	Source, comments
<i>Zea mays</i>	branches	28 %	this study, measured with SR, surface film washed off with H ₂ O
	Axes (basal 40 cm)	22 %	
	Axes (apical 25 cm)	14 %	
<i>Hordeum vulgare</i> Barley	Axes	18 - 20 %	Jacobson <i>et al.</i> , 1958, measured with various salts, film blotted off
<i>Hordeum vulgare</i>	Axes	23 %	Epstein, 1956, measured with various salts, film blotted off
<i>Triticum aestivum</i> Wheat	Axes	18 - 25 %	Kylin & Hylmö, 1957, blotted
Wheat	Axes	24 - 34 %	Butler, 1953, measured with various salts, film blotted off
Wheat	Axes	11 - 14 %	Ingelsten & Hylmö, 1961, centrifugation used to remove surface film

Regardless of the absolute magnitude of the volume of root tissue which is water free space, the main objective was to determine if low levels of SR uptake in the roots of the non-transpiring control plants were due to the WFS for SR. These results suggest that

Table 7: Water fluxes into roots. Values of fluxes calculated (either J_S or J_L) from published data.

Species	J_L ($\mu\text{l h}^{-1} \text{cm}^{-1} \text{root}$)		J_S ($\mu\text{l h}^{-1} \text{cm}^{-2} \text{root}$)		Comments
	Range	Max	Range	Max	
<i>Hordeum vulgare</i> ¹					Sanderson, 1983. 15 - 18 day old plants
main, basal 20 cm	0.4 - 0.6	0.6	1.3 - 1.9	1.9	
main, apical 4 - 10 cm	0.4 - 1.2	2.7	1.3 - 3.7	8.6	
branches	0.08 - 0.1		0.8 - 1.0		
<i>Hordeum vulgare</i> ¹					Graham <i>et al.</i> 1974.
main		3.4		1.1	
<i>Hordeum vulgare</i> ¹					Clarkson <i>et al.</i> , 1973.
main, basal 30+ cm	mean 0.3		1.0		
main, apical 0 - 7 cm	mean 1.4		4.5		
Branches, basal regions	mean 0.04		0.4		
apical 1-5 cm	mean 0.09		0.9		
<i>Vicia faba</i> ²					Brouwer, 1953a, b 28 - 35 days old
Basal 7 - 12.5 cm	8 - 14	155	25 - 44	493	
Apical 7 cm	4 - 6	21.4	13 - 19	68	
<i>Citrus</i> ³					Hayward <i>et al.</i> , 1942. 2 years old
Basal regions		2.9		4.7	
<i>Zea mays</i> ¹ (cv Mexican June)					adventitious roots, 10 - 15 cm long
Basal 8 - 15 cm	6.3 - 9.4	10	20 - 30	32	
Apical 10 cm	0.94 - 5.0	39	3 - 16	125	

¹. J_S calculated assuming 1 mm diameter axes, 0.3 mm diameter branches. ². J_S calculated assuming 1 mm diameter roots. ³. J_S calculated assuming 2 mm diameter roots. there is a volume of root which is free space for SR.

The exact location of the water free space of the maize roots for SR will be studied with microscopic examination of roots which were fed SR then freeze-substituted and

anhydrously processed. The volume of WFS will be the same for both roots of transpiring plants and non-transpiring plants. Therefore, to obtain values of water uptake the level of SR accumulated on the controls will be subtracted from the SR accumulation of the roots of transpiring plants to yield net SR accumulation rates.

3. Water uptake rates

Table 8: Water fluxes (J_S) into epidermal cells.

Species	J_S ($\mu\text{l h}^{-1} \text{cm}^{-2}$)	Source, comments
<i>Allium cepa</i>		Rosene (1954)
trichoblasts (hairs)	4.4 - 20	on adventitious roots
atrachoblasts (hairless)	5.4 - 14	
<i>Raphanus sativus</i>		Rosene (1943)
root hairs	2 - 31	
young hairs	13.8	Rosene (1950)
old hairs	5.5	
<i>Secale Cereale</i>		Rosene (1955)
root hairs	1.3 - 4.1 (mean 2.6)	

(i) Comparison with published values.

The rates of water flux into roots and root hairs are assembled in tables 7 and 8 respectively. These rates have been measured during potometric studies of water uptake into the roots of several species. All fluxes have been converted to the units used during the present study (J_L in $\mu\text{l h}^{-1} \text{cm}^{-1}_{\text{root}}$ and J_S in $\mu\text{l h}^{-1} \text{cm}^{-2}_{\text{root}}$). Several assumptions of root diameter were made in order to convert the water flux per unit length (J_L) into flux per root surface (J_S) (summarized in table 7). Water flux per length of root is influenced by root diameter, since larger diameter roots have greater absorptive surface per root length and consequently higher values than smaller diameter roots. To overcome this limitation, rates of water uptake into different plants will be compared using values of J_S .

The range of water fluxes into branches and axes of maize (table 5) is very similar to that listed in tables 7 and 8. The roots of barley (*Hordeum vulgare*) have the lowest

water fluxes ranging from 2.3 - 3.7, maximum $8.6 \mu\text{l h}^{-1} \text{cm}^{-2}_{\text{root}}$ into axes; branches have even lower fluxes ($0.8 - 1.0 \mu\text{l h}^{-1} \text{cm}^{-2}_{\text{root}}$). Second order lateral roots of broad bean (*Vicia faba*) have water fluxes of $13 - 44 \mu\text{l h}^{-1} \text{cm}^{-2}_{\text{root}}$, at the high end of the range of fluxes into maize ($0 - 44 \mu\text{l h}^{-1} \text{cm}^{-2}_{\text{root}}$). The maximum fluxes into *Vicia* roots ($493 \mu\text{l h}^{-1} \text{cm}^{-2}_{\text{root}}$) far exceeds the rates of water uptake into maize roots measured during this study and by Hayward *et al.* (1942). The extreme fluxes into the *Vicia* roots were measured under high suction tensions (high transpiration rates and osmotic gradients, Brouwer 1953a, b). It is also possible that the extreme values were produced by damaged roots or leaky potometers. The range of water fluxes into maize roots measured with potometers ($3 - 30 \mu\text{l h}^{-1} \text{cm}^{-2}_{\text{root}}$) by Hayward *et al.* (1942) was also similar to the fluxes into aeroponically-grown roots measured in the present study. The maximum flux measured by Hayward *et al.* (1942) ($125 \mu\text{l h}^{-1} \text{cm}^{-2}_{\text{root}}$) was three times the maximum value presented in table 5. Water flux into root hairs (table 8, $1.3 - 31 \mu\text{l h}^{-1} \text{cm}^{-2}_{\text{root}}$) was also similar to that into roots.

(ii) Comparison of the water fluxes into branches and main roots.

Mean fluxes into branches and main roots measured during experiments 1.1 - 1.3 were similar (branches $J_S = 5.2 \mu\text{l h}^{-1} \text{cm}^{-2}_{\text{root}}$; main roots $J_S = 4.4 \mu\text{l h}^{-1} \text{cm}^{-2}_{\text{root}}$). In experiment 1.4, water flux into branches ($J_S = 1.5 \pm 1.3 \mu\text{l h}^{-1} \text{cm}^{-2}_{\text{root}}$) and main roots ($J_S = 3.1 \pm 2.3 \mu\text{l h}^{-1} \text{cm}^{-2}_{\text{root}}$) was lower than that in experiments 1.1 to 1.3. This difference may be the result of pooling large samples of branches and main roots; this possibility will be discussed in section 4. Sanderson (1983) and Clarkson *et al.* (1973) measured water flux into branches and main roots of barley. These authors concluded that water uptake into branches was low because they used water flux per unit length (J_L) of branches and main roots were used for comparison (J_L of main roots = $0.3 - 2.7 \mu\text{l h}^{-1} \text{cm}^{-1}_{\text{root}}$; branches = $0.08 - 0.1 \mu\text{l h}^{-1} \text{cm}^{-1}_{\text{root}}$). The values of J_S of the basal parts of the barley main roots and branches were not very different (J_S main roots = $1.3 - 1.9 \mu\text{l h}^{-1} \text{cm}^{-2}_{\text{root}}$; branches =

0.8 - 1.0 $\mu\text{l h}^{-1} \text{cm}^{-2}_{\text{root}}$). This may explain why the role of branch roots in the functioning of root systems has been so overlooked.

Nuclear magnetic resonance imaging (NMR imaging) was recently used to show that small branches of loblolly pine (*Pinus taeda*) were more efficient (per unit surface) at absorbing water than larger diameter roots (MacFall *et al.*, 1991). This technique can show only relative differences in water uptake rates rather than produce quantitative (absolute) data. MacFall *et al.* (1991) did not determine if there is any difference in water absorption between the basal or apical zones of the roots. Water fluxes (J_S) into basal and apical zones of the roots of maize did vary (table 7), and this will now be discussed in detail.

(iii) Water uptake along the length of main roots.

The main objective of the present study was to determine the rate of the major fluxes of water into the different components of a root system of maize. This reexamination was in response to the observations of St.Aubin *et al.* (1986) who found that the late metaxylem remained immature, living cells in the apical 20+ cm of main roots of field-grown maize elements. This observation cast doubt on the traditional theory that the major fluxes of water enter the apical regions (root hair zone) of the main axes of a root system, since the axial conductance of these young regions is insufficient to carry the fluxes required by a transpiring maize plant (St.Aubin *et al.*, 1986; McCully and Canny, 1988). The fluxes (J_L) of water into the apical and basal regions of barley roots measured by Sanderson (1983) and Clarkson *et al.* (1973) support the traditional theory. Water fluxes were higher in the apical regions of the barley axes in both studies (J_L in apical regions 0.4 - 1.2 $\mu\text{l h}^{-1} \text{cm}^{-1}_{\text{root}}$, max 3.4 $\mu\text{l h}^{-1} \text{cm}^{-1}_{\text{root}}$; J_L in basal regions 0.4 - 0.6 $\mu\text{l h}^{-1} \text{cm}^{-1}_{\text{root}}$). The plants of barley used during these studies were young and immature (15 - 18 days old). These studies overlooked the dependence of J_L on root diameter and the surface area of the basal regions and branches and their role in water collection.

Brewig (1936a, b) and Brouwer (1953a, b) also found the apical regions of *Vicia* roots had higher fluxes (J_S) when the plants had low transpiration rates. However, when the plants were under high transpiration rates, the basal parts of the roots had the highest fluxes ($J_S = 25 - 44 \mu\text{l h}^{-1} \text{cm}^{-2}_{\text{root}}$), at the upper range recorded in maize branches ($44 \mu\text{l h}^{-1} \text{cm}^{-2}_{\text{root}}$). Water flux into the basal 8 - 15 cm of main roots of maize was higher (table 7; $J_S 20 - 30 \mu\text{l h}^{-1} \text{cm}^{-2}_{\text{root}}$) than water flux into the apical 10 cm ($J_S = 3 - 16 \mu\text{l h}^{-1} \text{cm}^{-2}_{\text{root}}$; Hayward *et al.*, 1942). The extremely high flux into the apical zones of maize recorded by Hayward *et al.* (1942) is rather suspect since all other values were in the range $10 - 20 \mu\text{l h}^{-1} \text{cm}^{-2}_{\text{root}}$. The basal regions of 2 year old *Citrus* roots, which were presumably suberized, had mean water flux $J_S = 4.7 \mu\text{l h}^{-1} \text{cm}^{-2}_{\text{root}}$, similar to the mean flux into maize roots ($J_S = 5 \mu\text{l h}^{-1} \text{cm}^{-2}_{\text{root}}$). Others have also found that considerable amounts of water are absorbed by woody, suberized roots (see references cited by MacFall *et al.*, 1991).

SR accumulation on branches and main roots at various positions along the main roots was measured to determine the rates of water flux into main roots and branches throughout the root system of maize. The accumulation of SR on the roots of the control plants was subtracted from the SR accumulation on roots of transpiring plants at each sample position to compensate for any mist gradients in the boxes. Net SR accumulation was converted into water flux in the branches and main roots at each position.

Water uptake in the root system was studied using two sample collection methods. In the first method, segments of main roots with their branches were collected at 10 cm intervals up to 45 cm from the stem base (Expts. 1.1 - 1.3). This method did not provide a complete survey if the roots extended far beyond 45 cm from the stem base. These results are summarized in figure 28. At the base of the plant, branches had higher water fluxes than the main roots. In the apical regions (45 cm from the plant) the main roots had higher fluxes than the branches (figure 28).

The second sample collection (expt. 1.4) was more complete. Segments of main root and branches (on the segment) were collected from the apex of the main roots and at 5

- 10 cm intervals along the main roots up to the base of the plants. There were two advantages to this procedure. Collecting samples from the root apex and at intervals along the axes provided samples of similar age and development at each position (assuming the roots grew at the same rate). Extending the sample collection from the tip to the stem base provided a complete survey of the entire root system. The water flux (J_s) of branches and main roots at each position are summarized in figure 31. Water fluxes are highest into the apical regions of the main roots (apical 25 cm). Branches near the apex have lower fluxes than the axes, however water flux into branches increased at 40 cm to exceed water flux into the main roots. Water flux into main roots and branches was the same in the basal 60 cm of the main axes. The volume of water collected by the roots was calculated by multiplying the flux (J_s) by the surface area (figure 32). The main roots collected more water than the branches in the apical zones (where there were few immature branches). Proximal to 25 cm from the apex, branches collected 8 times more water than the main roots each hour. As shown in chapter 4, the LMX of the main roots become open and conducting vessel elements 25 - 40 cm from the apex. Therefore the high fluxes into the branches occur where the LMX vessels of the main roots are open to collect and conduct these flows. The observations of delayed maturation of LMX in the apical 20+ cm of the main roots (St.Aubin *et al.*, 1986) now finds a clear and satisfying answer. The destination of the fluxes of water into the apical regions the main roots is uncertain. Since the low axial flows found in these parts of the main roots are through mature protoxylem and early metaxylem, one may speculate that the absorbed water be used by the rapidly-growing apical meristem.

The zone of high water fluxes into branches 25 - 70 cm from the tip will continually move through the soil as the main root grows down through the soil. Tiers of main roots of maize develop successively from stem nodes in the crown and lower stem nodes above the soil (Hoppe *et al.*, 1986). Soil under a maize plant will therefore be repeatedly reexploited by each successive tier of main roots as they develop and penetrate

the soil. By measuring the amount of water absorbed by individual roots in potometers, Navara (1987) also found that main roots played a major role in water uptake. Water uptake increased significantly with the formation and development of main roots from each successive tier. Formation of branches on the main roots was correlated with marked increases in water uptake (Navara, 1987).

(iv) Comparison with soil conductance and water uptake rates of roots in the soil

Soil water moves in response to a gradient in water potential energy, from points of high to low water potential. The water potential is the sum of the matric potential (Ψ_m), the gravity potential (Ψ_g) and the osmotic potential (Ψ_p). Water movement through soil has been described by D'Arcy's Law:

$$V = -k \frac{dH}{dx}$$

where V is the water flux in $\text{cm}^3 \text{ cm}^{-2} \text{ s}^{-1}$, k is the hydraulic conductivity of the soil in $\text{cm}^3 \text{ cm}^{-2} \text{ s}^{-1}$, and $\frac{dH}{dx}$ is the water potential gradient over distance x (Nye and Tinker, 1977;

Wild, 1981). In saturated soil k is constant, however, in unsaturated soil k decreases with reduced soil water content (θ). Since water potential is also influenced by changes in water content, the situation and mathematical models become exceedingly complicated. To summarize and simplify, water moves through soil towards roots by mass flow in response to a difference in water potential (created by the absorbing root).

Agronomists have shown that there is a defined range of soil water content at which water is available to plant roots. Saturated soil represents the upper limit of the range, however soil is saturated only during irrigation or heavy rain. Following 2 - 3 days drainage, the soil will be at field capacity (-0.01 MPa ; $\theta = 17 - 35 \%$ volume depending on soil type) which is optimum for plant growth. As the soil dries and soil water is less available, plants begin to wilt to reduce transpiration when they cannot absorb enough water to meet their transpirational demand; they regain turgor overnight. The point at

which soil water content is reduced so that the plants do not regain turgor is the wilting coefficient of the soil (-1.5 MPa; $\theta = 0.08 - 0.10$ % volume). Soil water which is within this range of potential ($-0.01 - -1.5$ MPa; from field capacity down to the wilting coefficient) is available to plant roots (more water is also available in saturated soil). The hydraulic conductivity of soil (average of sandy loam or clayey soil) within this range is also similar to the range of water fluxes into maize roots ($0 - 44 \mu\text{l h}^{-1} \text{cm}^{-2}_{\text{root}}$). At field capacity, $k = 42 \mu\text{l h}^{-1} \text{cm}^{-2}_{\text{soil}}$, but at the wilting coefficient, $k = 0.004 \mu\text{l h}^{-1} \text{cm}^{-2}_{\text{soil}}$. The low conductivity of soil at the wilting coefficient may explain why wilting occurs. This comparison is simplistic since most descriptions of flow into roots are similar to flow into cylinders (Nye and Tinker, 1977).

There is also much debate in the literature regarding whether water uptake by roots is limited by the resistance of the root or of the soil. The work of Dunham and Nye in 1973 (in Nye and Tinker, 1977) suggests that the answer is both. When onion (*Allium cepa*) roots which are absorbing water at $J_s = 6.1 \mu\text{l h}^{-1} \text{cm}^{-2}_{\text{root}}$, are placed against a block of soil at $q = 0.35$ % (which would have a $k = 42 \mu\text{l h}^{-1} \text{cm}^{-2}_{\text{soil}}$), no changes in q near the root surface were observed. Water uptake in soil at high θ is therefore limited by the rate of water flux into the root, since the k exceeds the rate of J_s . Onion roots absorbing water at this rate from soil at $\theta = 20 - 25$ % cause the θ of adjacent soil to decrease to 15 %. Under these conditions water uptake is limited by the k of the soil ($k = 0.004 \mu\text{l h}^{-1} \text{cm}^{-2}_{\text{soil}}$).

Models of water fluxes through soil into roots have been developed. These models have incorporated the soil water content and hydraulic conductivity. The maximum flux of water through soil into roots predicted by the models was $42 \mu\text{l h}^{-1} \text{cm}^{-2}_{\text{root}}$ or $4.1 \mu\text{l h}^{-1} \text{cm}^{-1}_{\text{root}}$ (Lang and Gardner, 1970 and Whistler, Klute and Millington, 1970; in Nye and Tinker, 1977). This is surprisingly similar to the maximum flux measured during this study ($44 \mu\text{l h}^{-1} \text{cm}^{-2}_{\text{root}}$ or $4.1 \mu\text{l h}^{-1} \text{cm}^{-1}_{\text{root}}$).

The fluxes of water into roots from the soil were estimated by dividing the rate of change in soil water content by estimates of root density (fluxes listed below from Willatt and Taylor, 1978). Lawlor in 1972 found that rye-grass (*Lolium perenne*) in large pots had $J_L = 0.25 \mu\text{l h}^{-1} \text{cm}^{-1} \text{root}$. Fluxes into maize roots from soil also varies ($J_L = 0.04 - 1 \mu\text{l h}^{-1} \text{cm}^{-1} \text{root}$, Taylor and Klepper, 1973; $J_L = 0.12 - 10 \mu\text{l h}^{-1} \text{cm}^{-1} \text{root}$, Allmaras *et al.*, 1975). Roots of cotton (*Gossypium hirsutum*) absorbed water from soil at lower rates ($J_L = 0.013 - 1.75 \mu\text{l h}^{-1} \text{cm}^{-1} \text{root}$, Taylor and Klepper, 1971; $J_L = 0 - 0.29 \mu\text{l h}^{-1} \text{cm}^{-1} \text{root}$, Taylor and Klepper, 1975). Soya bean (*Glycine max*) roots had water fluxes ($J_L = 0 - 8.3 \mu\text{l h}^{-1} \text{cm}^{-1} \text{root}$; Willatt and Taylor, 1978) which were also similar to those of maize. The water fluxes into roots from the soil are similar to the fluxes measured aeroponically and potometrically. Gradients in water fluxes along the roots were noted in roots in the soil (Willatt and Taylor, 1978). These gradients were similar to the gradient of water fluxes into the roots of aeroponically-grown maize.

Davis (1940) grew maize plants at one end of a rectangular wooden box so that the roots grew horizontally through the soil in this box. Root penetration was easily monitored through glass on one side of the box. Water content of the soil was monitored by tensiometers placed in the soil every 10 cm along the box. As the roots grew through the soil, there was always a reduction in the water content of the soil 15 - 20 cm behind the root apices. He also noted that the plants wilted when the soil surrounding the basal regions of the root system (near the stem base) dried to the wilting coefficient, even though the apical parts of the roots were in soil at much higher water contents (well above the wilting coefficient). Upon rewetting, soil water was always absorbed from the basal regions first (Davis, 1940). These absorption of water from the soil by the basal regions of the main roots rather than the apical regions supports the observations of St.Aubin *et al.* (1986) who found low axial conductance in the apical regions of the main roots of field-grown maize. This suggested to St.Aubin *et al.* (1986) that water is absorbed in the basal regions of the main roots (possibly by the branches). Willatt and Taylor (1978) also found

a gradient in the water fluxes into soya bean roots at different soil depths. They noted that water fluxes were highest in the deepest horizons, calculated by measuring changes in soil water content then dividing this by the rooting density at that depth. (Slight changes in water content divided by the extremely low rooting density in the deep horizons must have produced the higher water fluxes.) Soil water content was always markedly reduced over time in the regions of highest root density (basal regions), 50 - 100 cm behind the apices of the roots. The water content of deeper soil regions also decreased as the zone of high root density moved down the soil profile (Willatt and Taylor, 1978). Shallow soil remained at higher water content than soil further down the soil profile, suggesting that the highest water fluxes occur within a defined zone of the root system. In maize this actively-absorbing zone was limited to the branches on the main root, 25 - 75 cm from the tip (figure 32).

(v) Assessment of the method

Culturing plants aeroponically initially appears to be very unnatural. However, roots growing through soil often penetrate cracks and voids in the soil, and under dry conditions roots may shrink away from the soil so that they are surrounded by a gap. Cole and Aston in 1974 (as quoted in Nye and Tinker, 1977) measured a 60 % reduction in the diameter of wheat roots. There is, however, much water vapour in the voids in soil, and water may indeed be absorbed in the vapour phase. Under a gradient of -5 bars, up to $0.29 \mu\text{l h}^{-1} \text{cm}^{-1}_{\text{root}}$ may cross a 0.04 mm gap between root and soil as water vapour; however under normal conditions little water is transported to roots in the vapour phase (Nye and Tinker, 1977). More commonly, water vapour is lost from roots to soil under drought conditions (Nobel and Cui, 1992).

The water fluxes into maize roots measured aeroponically are self-consistent and agree with the published fluxes measured in potometers and in the soil. The variation in water fluxes along the roots is also consistent with roots in soil. The heterogeneity of the

measured fluxes is probably real. Since roots often do not make full contact with soil, short lengths of roots may have higher fluxes (Nye and Tinker, 1977). The results of the present study support this suggestion.

The method has several advantages and disadvantages. Any small plants which can be cultured aeroponically can be grown to maturity. Running a mist box continually for 1 - 2 months proved to be one of the major challenges of this method (root system of maize will die after a 15 - 30 min interruption in misting), but this can be overcome with patient and skillful technical assistance. Ultrasonic mist generators which are now available will greatly improve the reliability and expand the uses of the method. Experiments on the roots can be performed easily and non-destructively, under normal (or at least only slightly reduced) transpiration rates. Sample sizes can be large enough to study a heterogeneous phase space like a root system.

Hubick *et al.* (1982) found that *Helianthus annuus* (sunflower) plants grown aeroponically had higher fresh and dry weights than plants grown by continuous flow techniques or traditional pot-grown plants. Therefore aeroponics is a good method for growing plant material for research on root function. Since some roots were removed prior to the experiments to increase water uptake rates, this may have had an influence on the plants. Brouwer (1981) found that removal of main roots of maize plants, so that only the seminal root or 2 - 4 main roots remained, had little effect on the final weight of roots or shoots. Thus, the removal of roots should not have seriously affected the present experiments.

Using an apoplastic tracer like SR to locate and measure zones of high water flux has the disadvantage that any bound or sequestered dye may reduce measured water fluxes but this was not a serious problem in the present study. That rapidly-absorbing roots or regions of roots can be visually identified by their intense red colour is an obvious advantage since these can then be collected for structural studies. This has been done in order to determine the answer to the question which had been left unresolved by this

chapter: "Where does the water enter the root symplast and leave the dye behind?" These answers are presented in Chapter 6.

CHAPTER 6

SITES OF WATER UPTAKE INTO THE ROOT SYMPLAST OF AEROPONICALLY-GROWN PLANTS OF MAIZE

INTRODUCTION

Physiologists have used a variety of methods to measure water uptake into roots.

Although these studies have shown that the regions of maximum water uptake into roots vary considerably, the most widely accepted work came from the potometric studies of Robards *et al.* (1973), Graham *et al.* (1974) and Sanderson (1983). These studies indicated that water fluxes into barley and marrow root were highest in the apical 10 cm of the main axes. Low fluxes of water into the roots behind the apical 10 cm were attributed to the presence of a mature endodermis, which had developed Casparian strips and suberin lamellae in their walls in these regions (Robards *et al.*, 1973; Clarkson and Robards, 1975).

Casparian bands and suberin lamellae are composed of large organic molecules (lignin and suberin-like compounds) which are hydrophobic. These compounds were originally isolated and chemically characterized from structures which prevent water loss (periderm from trees (bark) and potato storage tissue). The Casparian strip and suberin lamellae were therefore considered to make the walls impermeable to the movement of water and solutes (Preistley, 1921; Van Fleet, 1961). Robards *et al.* (1973) and Clarkson and Robards (1975) hypothesised that the flow through the apoplast was blocked by the Casparian strips and the suberin lamellae at the endodermis so that the water and solutes must enter the symplast at that point; "the endodermis has long been considered to act as an effective barrier to mass flow and diffusion of solutes into the vascular tissue, and it is frequently identified as the major resistance to water flow through the root" (Robards *et al.*, 1973). This must have led many people to conclude that regions of roots which possess a mature endodermis were not important in the absorption of water; and that the unsuberized regions (apical 10 cm) were the sites of water uptake (as supported by the potometric studies).

Although this theory was once widely accepted, other evidence has led to movement away from it (Dainty, 1985). There is a great deal of confusion in our understanding of (1) the movement of water and solutes through the apoplast and symplast of plant tissue; (2) the movement of water *versus* the movement of solutes through plant tissue and; (3) the function of the Casparian strips and suberin lamellae. Dainty *et al.* (1981) and Boyer (1985) point out one of the more serious problems (or limitations) faced by those studying water uptake and movement into roots: we do not know the pathway taken by water or solutes entering a root.

Tracers have been used to follow the paths of water through plants. Many types of tracers have been used including various inorganic chemicals, both radioactive and nonradioactive and heavy metals, and a variety of dyes, both fluorescent and non-fluorescent. Tracers which remain in the apoplast (large, negatively-charged molecules) and tracers which move via the symplast have also been used to study apoplastic and symplastic pathways respectively (see review of tracer dyes by Canny, 1990a).

The idea of using tracers to study the flow of water through plant tissue is an old one. It is based on the assumptions that the dye moves with the water into and through the tissue, staining the tissue where the water flows through the plant, and that water flows through the walls (Sachs' Imbibition Theory, from Canny, 1990a). The movement of tracers into roots was studied by Kny in 1898, Popesco (1926), Höhn (1934) and others (see Popesco, 1926 and Canny 1990a). Dyes were fed to leaves in the transpiration stream *via* the xylem vessels through the cut end of the petiole to follow water movement by Strugger in 1939 to test Sachs' theory (as quoted in Canny, 1990a). The movement of dye away from the veins through the walls supported Sachs' theory and led Strugger to conclude that the walls were a pathway for movement of water and solutes away from the veins (Extended Cohesion Theory, Canny, 1990a).

Two important aspects of the movement of the dye through leaves led Canny to question these interpretations: (1) the movement of dye away from the veins in the walls

was very slow and (2) the dye did not accumulate in the walls of the mesophyll cells lining the stomatal cavity as the water evaporated from their surfaces. Canny fed the apoplastic dye SR to leaves in the transpiration stream, then used freeze-substitution and anhydrous embedding methods to immobilize the water-soluble dye *in situ* for observation. He found that the movement of SR through the cell walls away from the veins was by diffusion, unassisted by water flow (Canny, 1990a, c). It had been shown many years ago that dyes moved by diffusion through the cell walls of roots (Höhn, 1934), whether the roots were absorbing water or losing water (Hülsbruch, 1944, as quoted in Canny, 1990c). SR also moves by diffusion through the wall apoplast in the roots of field-grown maize (Canny and Huang, 1993). Even though it has been clearly shown that there is no flow of water in the wall apoplast, many researchers continue to use tracers in search of pathways through the apoplast.

Canny also found dye accumulations in the freeze-substituted leaves, but these accumulations were not found on the surface of the mesophyll cells lining the stomatal cavities as he had expected. Instead the SR accumulated in the apoplast at the ends of the finest veins, where the water leaves the xylem vessels. It was hypothesized that the water crosses the plasma membrane and enters the symplast of the cells surrounding the xylem vessels, then moves symplastically to the evaporative surfaces. The low-solubility SR accumulates in the apoplast because it is osmotically-filtered as the water crosses the plasma membrane. The places where dye accumulates were called *sumps*; the positions of the sumps show where the water enters the symplast through *flumes* (Canny, 1990a, b). The location of sumps and flumes varies in the leaves of different species of plants, in response to structural differences (Canny, 1990a).

This accumulation of SR in leaves led Canny to hypothesize that roots might also accumulate SR at the places where the water crosses the plasma membrane and enters the root symplast, leaving the dye behind. Although he speculated that the SR would accumulate on the root surface at the epidermis, the theory of Robards *et al.* (1973) would

predict an accumulation of SR on the outer walls of the endodermis, where they believe the water enters the symplast. It is also possible that the SR accumulates somewhere else. To test these ideas in the present series of experiments, an aqueous solution of SR was fed via the mist to the roots of transpiring plants of maize. This had the advantage that accumulations of SR on the root surface would not be swept away by convection or diffusion which would occur if the roots were immersed in a solution of SR.

To find where the dye was left behind when the water entered the symplast, the accumulation of SR from a SR-water mist on the roots of transpiring plants of maize was investigated by light microscopy. The water-soluble dye was immobilized *in situ* by freeze-substitution and anhydrous processing into plastic resin for structural observation (Canny and McCully, 1986). These observations revealed the location of SR accumulation and sites of entry of water into the root symplast.

MATERIALS AND METHODS

I. Plant Material

Plant material was grown in a mist of nutrient solution according to the methods outlined in Chapter 2. The nutrient solution was replaced with distilled water and the plants were left transpiring with their roots in the mist of water (now a very dilute nutrient solution) for 12 - 16 h. Crystalline SR was added to the reservoir of water to make a final concentration of 0.5 mmole SR l⁻¹. SR-water solution was fed via the mist to the root systems of transpiring and non-transpiring control plants (detopped or covered with a black bag) for 2 to 8 hours; roots were then harvested and prepared for observation. (The methods for feeding dye in the mist are described in detail in Chapter 5.)

II. Sample Collection

Root tissue was collected during two SR accumulation experiments (4 -6 plants in each experiment). During the first experiment roots were collected randomly throughout the root systems during a day's transpiration. In the second experiment root tissue was collected from 3 main roots (1 root/plant) at specific distances (5, 15, 30, and 45 cm) from the apex. Branches were collected at 15, 30 and 45 cm from the apex of each main root. Also, five branches, which were bright red due to large accumulations of SR were collected and processed separately.

For non-transpiring control samples, roots were collected from plants which had their shoots covered with black bags and aluminum foil. For comparison of autofluorescence of unstained roots with that of roots which were fed SR via the mist, segments of main roots (3 roots) and branches were collected from plants which were transpiring with their roots in a mist of very dilute nutrient solution without the SR (the nutrient solution had been replaced by distilled water the day before sample collection). Segments were collected at similar positions to those listed above.

III. Tissue Preparation

Pieces of root were freeze-substituted and processed anhydrously following the techniques of Canny and McCully (1986) to preserve the water-soluble SR dye in place. All solvents and materials used in freezing, freeze-substitution and embedding were dried over molecular sieve (BDH type 4A and 13X). Manipulations subsequent to the freeze-substitution were performed in a dry box apparatus ventilated with air which was dried over activated silica gel.

1. Freeze-substitution

Roots were excised from the base of the stem, and placed in covered plastic trays which were lined with damp paper towels. Segments of main root and branches (1 - 2 cm long)

were excised from various distances behind the apex of the main root. Pieces (1 - 2 mm long) were excised from the root segments by gentle cutting with sharp, surgical-steel blades on a piece of dental wax, then picked up with teflon-coated forceps and quickly plunged into methyl cyclohexane:isopentane (1:1) at its melting point (-160 °C). Tissue pieces were collected in a droplet of the freezing mixture with a bacteriological loop, and transferred to vials of ether:acrolein (9:1 w/v) which were frozen solid with liquid nitrogen, then warmed enough to melt on the surface (-127 °C). Vials of tissue in ether:acrolein were held at -96 °C in acetone frozen with liquid nitrogen during sample collection and transport to the laboratory, then stored for at least 2 weeks at -84 °C in an Ultra-Deep freezer.

2. Anhydrous processing

Anhydrous processing of root tissue subsequent to freeze-substitution was in a dry box apparatus. Air which had been passed through three columns (3.5 x 45 cm) of activated silica gel was circulated through the dry box during processing. All dilutions, measurements, mixing, and solvent and Spurr's resin changes were done in the dry box. (Spurr's resin from J. B. EM Supplies, Pointe Claire, Dorval, P. Q., H9R 4S8).

Vials of tissue in ether:acrolein were first warmed to room temperature. The root tissue was infiltrated with resin by replacing the ether:acrolein solution with solutions of a graded series of ether:Spurr's epoxy resin mixtures. Tissue was transferred every 30 min to solutions of increasing concentrations of resin by the following series (2.5, 5, 10, 15, 30, 50, 70, 90, and 3 x 100 % resin). The tissue was then transferred to vials of fresh resin daily for 4 - 6 days, embedded in fresh resin and then polymerized overnight at 70 °C.

3. Sectioning

Thin sections (1 - 5 μm) were cut dry on glass knives on a Porter-Blum Ultra-microtome (model MT-1, Ivan Sorvall Inc.). Sections were mounted and flattened onto clean glass slides by covering the sections with wax paper and pressing them onto the slide with a rubber eraser. Sections were mounted under a coverslip in non-fluorescent immersion oil (Olympus) and the slides stored flat to prevent movement of the coverslips. Sections were cut from at least three blocks in both longitudinal and transverse planes from samples at each collection position in the root system.

IV. Microscopy and Photography

Observations were made on a variety of microscopes including three models by Olympus (Vanox, BH-2 and CH-2), and a Zeiss Axiophot photomicroscope. Whole mounts of root tissue in blocks of resin (method of Wang and Canny, 1985) and thin sections were observed with Nomarski optics, brightfield illumination, polarized light, and epifluorescence optics (Ultraviolet-induced fluorescence: UG-1 exciter filter, DM 400 dichroic mirror and L-420 barrier filter, and; Green-induced fluorescence: IF-545 + B36 exciter filters, DM 480 dichroic mirror and O-590 barrier filter). Images were recorded on Fujichrome 35 mm slide film (ASA 50) and Kodak TMAX 100 Black and White film (35 mm, processed for ASA 160). Fluorescence images induced by ultraviolet light were recorded on the colour slide film; fluorescence images induced by green light were recorded on black and white film. When viewed with brightfield illumination, the contrast of images recorded on black and white film was enhanced with colour filters. Prints were made on Ilford Ilfobrom paper using both condensor and diffusion enlargers.

RESULTS

The results of chapter 5 indicated that roots on transpiring plants accumulate high levels of SR from a SR-water mist, while the roots of non-transpiring plants (detopped or bagged controls) do not. The whole-mounted branches in blocks of resin in figs. 1A and 1B were collected from transpiring and non-transpiring plants respectively. These figures illustrate and corroborate the results of chapter 5, since there are many SR crystals on the branch from the transpiring plants (fig. 1A) and few crystals on the control plant (fig. 1B). The branch in fig. 1A was one of the very red roots which have the highest fluxes (Ch. 5, fig. 16). Some of the crystals may have been present on the living root. The dry processing has crystallized all the SR present on freezing. As shown in Chapter 5 (fig. 14), there was a great deal of variability in the amount of SR accumulated by branches. This was reflected by the amount of crystals found on the surface of branches.

Another striking feature of fig. 1A is the concentration of SR crystals in the grooves between the epidermal cells. This pattern was observed on about 30 % of the dark red branches but never on main roots. In TS, the crystals on the branch in fig. 1C (which was also a dark red root) are lying in the grooves above the radial walls of the epidermal cells. There are also crystals on the root hairs grazed in this section (lower right and arrows, fig. 1C). These figures show that there is a build up of SR on all epidermal cells of the roots, supporting the first hypothesis made in Chapter 5 (that the SR would accumulate on the root surface).

When the field in fig. 1C was viewed with epifluorescence optics, the SR crystals on the epidermis are orange, and the walls of the epidermis and the outer tangential and radial walls of the hypodermis fluoresce bright greenish-yellow (fig. 1D). Maize roots which have been frozen, freeze-substituted and embedded by this method, autofluoresce blue under UV excitation light (similar to the walls in the lower left corner, fig. 1D). Therefore the bright green fluorescence in the walls is produced by the low levels of SR

which has diffused into these walls and intercellular spaces. When the concentration of SR in the intercellular spaces is high the walls appear orange, similar to the crystals.

This spread of SR through the walls away from the sumps at the epidermis is by diffusion. The rate of diffusion of SR into field-grown maize roots has been determined by Canny and Huang (1993). The theory and methods for determining the rate of diffusion of SR in cell walls are presented in that paper. The diffusivity (D) of SR into maize roots ranges from 10^{-8} to $10^{-10} \text{ cm}^2 \text{ s}^{-1}$ (*cf* D of SR in water $5 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$).

Two processes occur at the root surface: (1) there is an accumulation of SR on the root surface at the *sumps* (surface of the epidermal cells), and (2) SR diffuses away from the sumps through the walls of the apoplast in all directions. The concentrations of SR in the crystals will be compared with the concentration of SR which has diffused into the walls in the discussion section. Other patterns of accumulation and penetration were found, and these are illustrated in fig. 2.

Autofluorescence of branches is very weak when viewed with epifluorescence optics and green excitation light (figure 2A). The negative of this micrograph was recorded with a very long exposure time, then printed on a higher contrast paper with a very short exposure time to increase contrast, otherwise the image would be black. SR fluoresces brightly under green excitation and is easily distinguished from autofluorescence by black and white film (*cf.* figs. 2A and 2B).

The permeability of the branch root in fig. 2B varied around its circumference. SR diffused only slowly into the anticlinal walls of the hypodermis on the left side of the root (7-9 o'clock), however the SR diffused through the anticlinal walls of the hypodermal cells around the rest of the root's circumference. The SR did not penetrate beyond the walls of the hypodermis into the cortex of the root (fig. 2B). In another branch however, SR did permeate the walls of the hypodermis and enter the cortex (fig. 2C). SR also entered walls of the cortical cells and intercellular spaces; low levels of SR were also found inside some of the cortical cells. SR did not diffuse inwards beyond the

outer tangential walls of the endodermis (fig. 2C). Two types of branch were distinguished: (1) branches into which SR diffused only very slowly, and (2) branches which were very permeable to the diffusion of SR into their cortices.

When the branch in fig. 2C was viewed with brightfield illumination, many crystals of SR were found on the surface of the root (fig. 2D). The red crystals appear black because a green filter (the complementary colour to red) was used during photography to enhance image contrast. The outer tangential walls of some of the hypodermal cells and some of the intercellular spaces between the hypodermis and epidermis contained such accumulations of SR (see arrows fig. 2D). This is the second location of the *sumps*; at the outer tangential walls of the hypodermal cells. These sumps are more pronounced and more common in the main roots (see fig. 3).

Main roots on transpiring plants also accumulate SR on their surface. The crystals of SR are randomly-distributed on the surface of the main roots (not shown). In TS, SR is limited to the walls of the epidermal cells and hypodermis (*cf.* figs. 3A and B with the control which was not fed SR, fig. 3F). Normally SR penetrates the anticlinal walls (radial and tangential walls) only very slowly and infrequently, but in some cases SR will diffuse across the hypodermis (root in figs. 3A and 3B). In most of the sections of main roots observed during the study, SR diffuses up to the radial walls of the hypodermis, crossing it only rarely even after 6 - 8 h (fig. 3C).

The sumps, where SR accumulated and made the walls appear dark red, were located on or around the epidermal cells (fig. 3D). Sumps were also common on the outer tangential walls of the hypodermis of main roots (fig. 3E). When observed with brightfield illumination, these walls normally have very low contrast (fig. 3G). The epidermal cells above sumps on the hypodermis often had spaces between them. In some of these places there were low concentrations of SR in some side of the epidermal cells, but the printing suppressed this fluorescence (see figs. 3A - C).

SR enters roots which have been damaged (fig. 4A and B). In damaged roots SR permeates the tissue of the cortex and enters the stele, often staining the protoxylem and EMX. There is variation in the penetration of SR through branch junctions. SR enters some junctions but not others. SR may also enter main roots through branch junctions (figs. 4C-E). SR moves into some branch junctions either through the cortical tissue of the branch or through the gaps between the branch and main root. SR moves away from the branch junctions, diffusing through the walls of the hypodermal cells which are enlarged near branch junctions (fig. 4D). Accumulations of SR also occur on the surface of cortical cells adjacent to branch junctions, which suggests that sumps may also form at the surface of these cortical cells (fig. 4E).

Figure 1. Branch roots fed a mist of 0.5 mmole l^{-1} SR for 8 h, then frozen, freeze-substituted and anhydrously-embedded in Spurr's resin. Figures A and B. Whole mounts of branches in blocks of resin viewed with polarised light; transpiring plant (fig. A), non-transpiring plant (fig. B). Bars = $100 \mu\text{m}$. Figures C and D. Transverse sections through a branch from a transpiring plant which was fed SR-water mist for 6 h, then processed into resin and sectioned anhydrously. Bars = $20 \mu\text{m}$. **Figure A.** Branches from transpiring plants had many crystals of SR on their surface. Some branches have rows of crystals in the grooves between epidermal cells. The crystalline nature of the SR is verified by its birefringence in polarised light. **Figure B.** Branches from a non-transpiring plant stained slightly pink by the SR mist. Very few crystals of SR are found on the surface of the root or in the grooves between the epidermal cells (arrows). **Figure C.** SR crystals are found on the surface of root hairs grazed in this TS (arrows and lower right) and on the surface of the epidermal cells. There are also many crystals in the grooves above the anticlinal walls of the epidermal cells. Nomarski optics. **Figure D.** Same frame as shown in fig. C viewed with epifluorescence optics. Green/yellow fluorescence in the wall apoplast of the epidermal cells and outer tangential and radial walls of the hypodermis caused by the diffusion of low concentrations of SR into these walls. Crystals of SR are orange because their fluorescence is quenched. UV excitation.

Figure 2. Transverse sections of branches which were frozen, freeze-substituted, embedded and sectioned anhydrously. Figures A-C viewed by epifluorescence optics (Green excitation). Figure D, brightfield illumination with a monochromatic green filter. Bars = 50 μm ; e = epidermal cell; h = hypodermal cell. **Figure A.** Autofluorescence of a branch from a root system of a plant which was not fed SR in the mist. Printed on a higher contrast paper than figures B-D. **Figure B.** Branch from root system of a plant which was fed a mist of 0.5 mmole l^{-1} SR for 6 h. SR in the walls of the epidermal cells is fluorescing strongly. SR penetrates the anticlinal walls of the hypodermis in most regions of this root, except on the left side where it does not cross the hypodermis. **Figure C.** SR penetrates the walls of the epidermis and hypodermis into the cortex of this branch. There are also low levels of SR in some cortical cells. Diffusion of SR in the wall apoplast and intercellular spaces is limited to the cortex at the endodermis. **Figure D.** When the section in C is viewed with brightfield illumination, many crystals of SR are visible on the root surface. SR also accumulates on the outer tangential walls of some hypodermal cells (arrows). The low levels of SR in the walls of the cortical cells visible with epifluorescence optics (fig. C) can not be distinguished with brightfield illumination.

Figure 3. Transverse sections of main roots which were fed a mist of 0.5 mmole F^{-1} SR for 6 h, then frozen, freeze substituted, embedded and sectioned anhydrously (controls in figures F and G were not fed SR). Figures A, B, C and F viewed by epifluorescence optics (Green excitation). Figures D, E, and G viewed by brightfield illumination with a monochromatic green filter. e = epidermal cell; h = hypodermal cell. **Figure A.** The strong fluorescence of the SR in the walls of the epidermis and the radial and outer tangential walls of the hypodermis is easily distinguished from the weak autofluorescence of the root (cf fig. F). Bar = 50 μm . **Figure B.** During the 6 h exposure time, SR had diffused through the anticlinal walls of the hypodermis into the intercellular space between the cortical cells and the hypodermis. Bar = 25 μm . **Figure C.** Diffusion of SR is very slow through the anticlinal walls of the hypodermis of this root (and most others). Small amounts of SR have diffused further into the anticlinal walls of the lowest hypodermal cell. Bar = 10 μm . **Figure D.** The walls of the epidermis and outer tangential walls of the hypodermis are dark because of the accumulation of SR (cf with control fig. G). SR is also found in some intercellular spaces between the epidermis and hypodermis. Bar = 20 μm . **Figure E.** SR accumulation on the outer tangential wall of two hypodermal cells. There are also small crystals of SR on the epidermis of this main root. The concentration of SR in the anticlinal walls of the hypodermis is too low to be visible by brightfield illumination. Bar = 10 μm . **Figure F.** Autofluorescence of the epidermis, hypodermis and cortex of a main root which was not fed SR in the mist. (Image contrast of the weak autofluorescence enhanced by printing on a higher contrast paper.) Bar = 20 μm . **Figure G.** Same frame as fig. F, viewed with brightfield illumination. Bar = 20 μm .

Figure 4. Sites of SR penetration into roots. Figures A-D. Transverse sections viewed by epifluorescence optics (Green excitation). Figure E viewed with brightfield illumination and a monochromatic green filter. Bars = 50 μ m. **Figure A.** SR penetrates this damaged branch entering the walls and lumen of the damaged cells, as well as the intercellular spaces in the cortex. The walls of the endodermis, protoxylem and EMX have also been penetrated by SR. **Figure B.** SR has entered the damage site and permeated the walls of the cortical cells and EMX of this main root. Note that the SR did not diffuse past the anticlinal walls of the hypodermis in the undamaged regions (lower right). **Figure C.** Junction of a first order branch and main root. SR has diffused along the wall of the cortical cells of the branch (arrows). Hypodermal cells on either side of the junction are enlarged. The walls of these cells are stained with SR which entered through the gap in the junction. **Figure D.** TS of a main root (above or below a branch junction). The walls of the enlarged hypodermal cells shown in fig. C (asterisks) are stained by SR, which entered through the junction. **Figure E.** LS of a main root at the edge of a branch junction. SR accumulates in the intercellular spaces and on the surface of cortical cells (arrows).

DISCUSSION

I. Location of the Sumps and Flumes.

The answers to the question asked at the end of Chapter 5 : "Where does the water enter the root symplast and leave the dye behind?", are found in the results of these studies on the accumulations of SR on the freeze substituted roots. These accumulations of SR (or sumps) reveal the sites where water enters the roots (at the flumes). Sumps were formed in two places on the maize roots. First, crystals of SR were found on the surface of the epidermal cells (figs. 1 and 2). Water must cross the plasma membrane to enter the symplast of the epidermal cells, leaving behind the SR which is osmotically-filtered by the plasma membrane (dyes can only be removed from solution through osmotic filtration by a membrane or by evaporation of the water, Canny 1990a, b).

Second, in some roots sumps also formed on the outer tangential walls of the hypodermis and intercellular spaces between the epidermis and hypodermis. Water must therefore cross flumes on the surface of the hypodermal cells, forming sumps of SR on their surfaces as the dye is left behind (figs. 2 and 3). The SR on the outer walls of the hypodermis was not crystalline (since it was not birefringent when viewed with polarized light). The build up of SR on the hypodermis usually occurred near places where the epidermal cells had been pulled apart or disrupted. SR occasionally entered the epidermal cells near such sites which suggests that these epidermal cells were damaged or dead. It is unclear whether the SR entered through the gaps between the epidermal cells or through dead epidermal cells. The death of the epidermis in older regions is a common feature of roots of grasses grown in the field (McCully and Canny, 1986; Wenzel and McCully, 1991).

Prior to freezing and processing of the roots which were fed SR, there may have been some crystals on their surface. Many crystals must have also been formed during the freeze-substitution process. It is not clear why there were dense concentrations of SR

Several explanations seem likely: (1) the grooves are sites of high fluxes of water and therefore high concentrations of SR ; (2) substances in the grooves cause the crystals to form there first (seeding) or to be immobilized there (mucilage) or (3) the size and geometry of the branches causes the crystals to move into or stay in the grooves.

II. Diffusion of SR into the Root Tissue

The accumulation of SR on the root surface was accompanied by the diffusion of low concentrations of SR through the cell walls. The radial diffusion of SR into main roots was usually very slow, crossing the radial walls of the hypodermis only rarely even after 6 - 8 h. Movement of SR into branches varied, not crossing the hypodermis of some branches, penetrating the walls of cortical cells up to the endodermis of others. The movement of SR was very rapid into damaged roots.

These results agree with those of Canny and Huang (1993), who measured the rates of diffusion of SR into the roots of field-grown maize. Diffusion of SR into immature regions of main roots varied widely ($D = 0 - 1.3 \times 10^{-7} \text{ cm}^2 \text{ s}^{-1}$), but was uniformly low into mature regions of main roots ($D = 4.9 \times 10^{-9} \text{ cm}^2 \text{ s}^{-1}$). Canny and Huang (1993) also found two groups of branches, those into which SR has a high diffusivity ($D = 1.3 \times 10^{-7} \text{ cm}^2 \text{ s}^{-1}$) and those into which it has a low diffusivity ($D = 2.6 \pm 10^{-9} \text{ cm}^2 \text{ s}^{-1}$).

III. SR Crystals and SR in the Walls.

Concentrated SR solutions are red (M.W. 552; solubility 0.4% = *ca* 7 mmol l^{-1}), and dilute SR solutions are pink. Concentrated solutions of SR are only weakly fluorescent at about 560 nm (Canny, 1988) because of quenching. Dilute solutions of SR are faint pink but strongly fluorescent at $10^{-4} \text{ mole l}^{-1}$, and a fluorometer is required for comparisons below this concentration (Canny, 1988). The SR which diffuses into the cell walls fluoresces strongly when viewed with epifluorescence optics, but is too faint to be

viewed with brightfield illumination. Therefore, the concentration of SR which has diffused into the walls must be about 10^{-4} moles l^{-1} . A 10^{-4} mole l^{-1} solution contains 55 g SR per m^3 volume. Assuming the density of a crystal is 1, 1 g of crystalline SR will make 18 000 ml of 10^{-4} mole l^{-1} SR solution. Therefore SR crystals are 18 000 times more concentrated than SR in the walls.

IV. Comparison with the Literature.

As mentioned in the discussion in chapter 5, the accumulation of a marker solute on roots has not been used to measure water uptake which makes comparisons with the literature difficult. Hamza and Aylmore (1992) measured changes in Na^+ concentrations at the surface of radish and lupin roots in soil maintained at different NaCl concentrations. Na^+ levels increased linearly as the roots absorbed water; the rates of increase in the Na^+ concentrations being dependent on transpiration rates. This study did not use Na^+ accumulation to measure rates of water uptake, nor did they determine where the salt accumulated (Hamza and Aylmore, 1992). The roots of mangroves can apparently filter the salt from the sea water which bathes their roots, which maintains a very low salt concentration in the xylem sap. Scholander (1968) found that the ability to exclude salt from the xylem sap ceased only when the plasma membrane of the cells in the roots were disrupted with organic solvents, which led to the conclusion that the membrane removed the salt from the seawater by simple ultrafiltration.

Peterson and coworkers have conducted exhaustive studies on the movement of a wide variety of tracer dyes in the hope of determining the pathway of water and solutes into roots. They showed that there are Casparian strips and suberin lamellae in the walls of the hypodermis (Peterson *et al.*, 1978; Peterson *et al.*, 1982). The diffusion of apoplastic tracer and sulphate ions into roots through the wall apoplast is limited or reduced at the walls of the hypodermis (Peterson, 1988). The slow diffusion of SR

across the hypodermis of the main roots of maize in the present study agrees with their observations

Peterson and others have also searched for places where the wall apoplast is not blocked by suberin lamellae and Casparian strips (short passage cells in the hypodermis, Peterson *et al.* (1978), branch junctions (Peterson *et al.*, 1981; Moon *et al.*, 1986) and most recently the root tip (Enstone and Peterson, 1992). It is not surprising that the diffusion of tracers into the wall apoplast of these sites was not much different than the diffusion of tracers into the suberized regions of the root since it has been shown that the movement of tracers through the walls is by diffusion, and that there is no flow in the wall apoplast of roots or leaves (Canny and Huang, 1993; Canny 1990a, c).

It was shown in chapter 4 that the walls of the hypodermis contain Casparian bands and suberin lamellae in the branches and axes throughout the entire root system except the apices. These suberized regions correspond to the locations of very high fluxes of water into these roots, which suggests that the suberization has little effect on water uptake. Clarkson *et al.* (1987) found that roots grown at 13 °C had higher rates of water uptake than roots grown at 20 °C, even though the hypodermis was more suberized in the roots grown at 13 °C. Both these results and the results of the present study conflict with the correlation of low water fluxes and the appearance of suberin lamellae in the walls of the hypodermis and endodermis (Clarkson *et al.*, 1987).

The build up of SR on the surface of the epidermis which is not suberized occurs as the water crosses the plasma membrane and is separated from the dye. When epidermal cells are pulled apart or are disrupted (and dead?), the plasma membrane of the hypodermal cells must then begin to filter out the SR as the water enters the symplast there. The Casparian strips and suberin lamellae would only reduce the rate at which the SR and other solutes diffuse through the wall apoplast. Therefore, in maize roots, the major resistance (if any) to water uptake occurs at the root surface (epidermis and hypodermis) where the water enters the root symplast.

CHAPTER 7

GENERAL CONCLUSIONS

The hypothesis proposed in the Introduction viz. that water uptake can be studied by the accumulation of a dye at sumps has been verified. The question about the role of the branch roots has been answered: they collect *ca* 88% of the water. The paradox that the region of high axial conductance in main roots is far from their apices has been resolved; this is the zone where the branch roots start to feed in their contribution. A further question has been answered: the water path into roots is a symplastic path from the living cells at the root surface. In addition, a major misconception has been disproved, namely that suberization of cell walls in roots affects water uptake.

Values of water flux measured by the accumulation of SR from the SR mist were consistent with published values of water flux. Fluxes into branches and main roots were similar (*ca* $J_s = 5 \mu\text{l h}^{-1} \text{cm}^{-2}_{\text{root}}$); however, the branches had 8 times more surface and therefore absorbed 8 times more water. The pattern of water fluxes measured throughout the root system was also consistent with published data. Although the apical regions of the main roots had high water fluxes, the branches 25 -75 cm from the apex of the main roots collected most of the water absorbed each hour. The LMX of the main roots mature 15 - 30 cm from the root apex, becoming open conduits to carry the high fluxes of water collected by the branches in that region. The LMX vessels were open only in the basal regions of the branches found 30 - 45 from the apex of the main roots.

SR accumulation on the root surface was investigated by preparing tissue for observation with freeze-substitution and anhydrous embedding in resin. Observations by light microscope revealed accumulations of SR on the epidermis and hypodermis. This indicates that water enters the root symplast at the epidermis and hypodermis. Dense accumulations of SR crystals in the grooves between the epidermal cells may be the result of high water fluxes at these sites. Roots or regions of roots which are rapidly absorbing water are revealed with this method by their bright red appearance due to large accumulations of SR. This is the most important advantage of this method because these same roots can be collected for structural observations.

The hypodermis and endodermis were suberized in branches and main roots throughout the root system. Many of the regions of the root system which had high water fluxes were suberized. This suggests that the development of suberin lamellae (and Casparian bands) may be unrelated to water uptake.

It was clear that high water fluxes into branches occurred at the point where the LMX vessels of the main root were open, but it was not clear if the LMX vessels of these branches were also open. From the survey of branch LMX maturity, only the LMX in the basal regions of the branches were open. As discussed by Wenzel *et al.* (1989), the tension (negative pressure) in the xylem vessels created by the transpirational pull of the shoot is scarcely attenuated over long distances in regions of roots with open LMX vessels, but quickly attenuated in regions of root with closed LMX. Since the water need move only a short distances along the branch to the place where the LMX are open, open LMX along the entire length of the branch may be unnecessary. This question warrants further investigation with careful observation of the state of maturity of EMX/LMX in actively absorbing branches. Structural observations should also be made to explain the variation in rates of water uptake along the main roots and branches. Examination of the walls and plasma membranes of the epidermal and hypodermal cells might reveal why the water enters the symplast of these cells.

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