ABSORBING SURFACE AREA OF SOUTHERN PINE ROOT SYSTEMS:
PHOSPHORUS AND POTASSIUM UPTAKE BY ROOT SYSTEMS OF TWELVE-
YEAR-OLD SLASH PINE TREES

By

JOSE A. ESCAMILLA-BENCOMO

A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1997

ACKNOWLEDGMENTS

*-Hey you, what are you going to do when hope dies?
-Hope does not die. Hope has relatives and patrons
to come to its aid; there are plenty of very devout,
optimistic people in the world, who will protect hope.*

*José Joaquín Fernández de Lizardi
El PERIQUILLO SARMIENTO*

Through the course of the last four years of my life, many people have provided assistance and support which, to a large degree, have made a reality of my Ph.D. program and this dissertation.

First, I thank my main adviser, Dr. Nicholas Comerford ("Nick"), for providing constructive criticism, intellectual support and friendship. I also appreciate Dr. Konda R. Reddy, Dr. Suresh Rao, Dr. David Sylvia and Dr. Kimberlyn Williams for serving on my committee.

Second, I am grateful for the financial support provided for this project by USDA Grant No. 722122712 and by the industrial members of the Program of Pine Productivity Research (P³R) at the University of Florida. I also acknowledge Jefferson-Smurfit Corporation for providing the land for field studies. Also for providing funds during my graduate education I acknowledge the following organizations: National Council of Science and Technology in Mexico (CONACYT), Centro de Investigación Científica de Yucatán

(CICY), and the Florida Mexico Institute of the Latin American and Caribbean Center.

My special thanks to Mary McLeod for logistic support, help in all phases of lab work, and her friendship. Thanks also to Randall McCrady for his essential help and camaraderie during greenhouse and field work. I also recognize the vital and tedious root separation work done by Karen Wenner-Pedersen and Sharon Wright.

. Friends are as important as academic advice. Pauline Grierson and John Tredinnick provided a home away from home, during their time in Gainesville. Special thanks to Pauline for eliciting key scientific discussions and lengthy manuscript editing. Thanks to Andres Nazario for his counseling and support, and to Arlete Freitas, Leslie Cooperband, Christine Bliss, Karen Wenner-Pedersen, Dave Farmer, Steve Trabue, Ian Bjørsvik and Scott Brinton for their friendship. They all were players in those fun events that kept me going throughout these years of graduate school.

Lastly, but not less important, I thank my friends and family in Mexico, too numerous to list. However, none of this has been possible without my parents, Armando y Beatriz, and their unconditional love, encouragement and understanding during this very long process.

TABLE OF CONTENTS

ACKNOWLEDGMENTS	ii
LIST OF TABLES	vii
LIST OF FIGURES	ix
ABSTRACT	xi
CHAPTERS	
1 GENERAL INTRODUCTION.....	1
2 NUTRIENT ABSORBING-SURFACES OF TREES AND THE NUTRIENT UPTAKE PROCESS.....	6
Introduction.....	6
Definition of Absorbing-Surfaces of Trees.....	7
Nutrient Uptake for the Different Root Surfaces of Trees	11
Pathways of Nutrient Uptake	11
Nutrient Uptake by Root Trees	14
Evidence of Nutrient Uptake in Brown Roots.....	18
Michaelis-Menten Kinetics as a Description of Active Nutrient Uptake	20
Appropriateness of the Equation for Root Uptake	20
Active Root Uptake Mechanism and Root Anatomy	23
Importance of Root Uptake Kinetics under Field Conditions	26
I_{max} , K_m and C_{min} under field conditions.....	26
Temporal and spatial changes in root uptake kinetics under field conditions	30
Concluding Remarks.....	34
3 A METHOD FOR MEASURING NUTRIENT DEPLETION BY ROOTS OF MATURE TREES IN THE FIELD.	35
Introduction.....	35
Materials and Methods.....	40
Site Description	40
Root Regeneration System	41
Nutrient Uptake Root Chamber Design and Construction	43
In-situ Nutrient Uptake System	46

Testing of the Nutrient Uptake System.....	51
Laboratory studies	51
In-situ field studies	53
Chemical Analyses	55
Data Analyses	56
Laboratory studies	56
Field studies	56
Results.....	57
Laboratory Studies (No roots Present in Chambers)	57
Field Studies	58
Root regeneration system	58
Nutrient depletion with air	58
Nutrient depletion with N ₂ gas.	58
Oxygen saturation with air and N ₂ gas..	61
Discussion.....	63
 4 PHOSPHORUS AND POTASSIUM DEPLETION BY ROOTS OF FIELD- GROWN SLASH PINE: AEROBIC AND HYPOXIC CONDITIONS.....	 68
Introduction.....	68
Materials and Methods.....	75
Site Description	75
Root Regeneration System	75
Field Nutrient Uptake System Installation	76
Depletion Studies	77
Pre-depletion treatment.	77
Experiment 1: Nutrient depletion study with air	78
Experiment 2: Nutrient depletion study with N ₂ gas.....	78
Experiment 3: Nutrient depletion study with air after N ₂ gas.....	79
Experiments 4, 5 and 6: Nutrient depletion study with air/ N ₂ gas/ air.....	79
Chemical Analyses	80
Data Analyses	80
Results.....	81
Oxygen Concentration and Temperature in the Chambers	81
Potassium Depletion with Alternating Aerobic and Hypoxic Conditions	83
Potassium Depletion Under Hypoxic Conditions	84
Phosphorus Depletion with Alternating Aerobic and Hypoxic conditions.	84
Phosphorus Depletion Under Hypoxic Conditions..	84
Discussion.....	88
Implications of P and K uptake by roots of pines in reduced soil conditions in the field... ..	93
Conclusions	95

5	PHOSPHORUS AND POTASSIUM DEPLETION BY WOODY-ROOTS OF TWELVE-YEAR-OLD SLASH PINE TREES.....	97
	Introduction.....	97
	Materials and Methods.....	101
	Root System Description.....	101
	Nutrient Uptake system installation.....	102
	Depletion Studies.....	103
	Pre-depletion treatment.	103
	Study #1.P and K depletion by brown roots: Isolation of white roots by waxing.....	104
	Study #2 (1995) and study #3 (1996). P and K depletion by brown roots: Isolation of white roots by cutting.....	106
	Study #4. P and K depletion by whole root systems subjected to NaOCl solution.....	106
	Laboratory Incubation Studies.....	107
	Inoculation with root scrapings.	107
	Inoculation with nutrient solution.	107
	Ion Mass in the Root Free Space.....	109
	Root Measurements.....	109
	Anatomical Observations of Root Sections.....	110
	Chemical Analyses.....	111
	Data Analyses.....	111
	Oxygen saturation, and nutrient solution temperature.....	111
	Phosphorus and K uptake and mass flow influx.	112
	Phosphorus and K influx.	112
	Phosphorus and K uptake, NaOCl solution study.	114
	Laboratory incubation studies.	116
	Results.....	116
	Anatomical Observations and Root System Descriptions.....	116
	O ₂ Concentration and Temperature in the Chambers.....	121
	Microbial Interferences.....	121
	Potassium and Phosphorus Influx.....	124
	Discussion.....	127
6	OVERALL RESULTS.....	136
	LIST OF REFERENCES.....	140
	BIOGRAPHICAL SKETCH.....	155

LIST OF TABLES

<u>Table</u>	<u>page</u>
2-1. Categories of fine-roots in a perennial, dicot, woody- root system.....	8
3-1. List of parts and materials for constructing a nutrient uptake chamber.....	48
3-2. List of parts and materials for constructing a Mariotte flask system.....	52
4-1. Temperature and Dissolved Oxygen concentration in nutrient solution, during consecutive experiments of P and K depletion by pine roots.	82
5-1. Experimental design of Covariance analysis used for P and K Influx data obtained from depletion experiments of studies 1,2 and 3.....	115
5-2. Description of root systems used in study one, where roots were subjected to waxing (1995 depletion experiments.)..	117
5-3. Description of root systems used in study two, where roots were subjected to cutting (1995 depletion experiments.).....	118
5-4. Description of root systems used in study three, where roots were subjected to cutting. 1996 depletion experiments.....	119
5-5. Temperature and Dissolved Oxygen concentrations in the nutrient solution during consecutive experiments of P and K depletion by pine roots. 1995 studies..	122
5-6. Temperature and Dissolved Oxygen concentrations in the nutrient solution during consecutive experiments of P and K depletion by pine roots. 1996 studies..	123
5-7. Comparison of constant rate of depletion (r) and C for P and K depleted pine roots treated with 1% NaOCl. Study four.....	125

5-8. Covariance analysis for P and K influx ($\text{Ln}(\text{Influx})$) by pine root systems of studies 1,2 and 3.....	128
5-9. Mean P and K influx for woody roots and whole roots of 12-year-old slash pine. These untransformed values ($\mu\text{mol cm}^{-1} \text{ h}^{-1}$) are a summary of the three separate studies performed in 1995 and 1996. Cut refers to the studies when white roots were removed by cutting while waxed refers to the study where white roots were covered with wax.....	129

LIST OF FIGURES

<u>Figure</u>	<u>page</u>
2-1. A series of Michaelis-Menten curves	28
3-1. The root regeneration system	42
3-2. Nutrient uptake root chamber	44
3-3. <i>In-situ</i> nutrient uptake system... ..	47
3-4. Mariotte Flask system	50
3-5. Phosphorus and Potassium depletion by seventeen root systems of <i>Pinus elliottii</i> var. <i>elliottii</i> . The roots were gassed with air during the experiment.....	59
3-6. Phosphorus and Potassium depletion by seventeen root systems of <i>Pinus elliottii</i> var. <i>elliottii</i> . The roots were gassed with N ₂ gas during the experiment.....	60
3-7. Nutrient solution temperature and Oxygen saturation in root chambers with lateral root systems of <i>Pinus elliottii</i> var. <i>elliottii</i>	62
4-1. Potassium depletion by seventeen root systems of <i>Pinus elliottii</i> var. <i>elliottii</i> . under aerobic and hypoxic conditions.....	85
4-2. Potassium depletion by root systems of <i>Pinus elliottii</i> var. <i>elliottii</i> . under hypoxic conditions.....	86
4-3. Potassium depletion by seventeen root systems of <i>Pinus elliottii</i> var. <i>elliottii</i> . under series of aerobic conditions.....	87
4-4. Phosphorus depletion by root systems of <i>Pinus elliottii</i> var. <i>elliottii</i> . under aerobic and hypoxic conditions.....	89

4-5. Phosphorus depletion by seventeen root systems of <i>Pinus elliottii</i> var. <i>elliottii</i> . under series of aerobic conditions.....	90
4-6. Phosphorus depletion by root systems of <i>Pinus elliottii</i> var. <i>elliottii</i> . under hypoxic conditions.....	91
4-7. Potassium uptake potential of slash pine roots when subjected to cycles of aerobic and hypoxic conditions....	96
5-1. Calculation of potassium depletion due to mass flow by eight root systems of <i>Pinus elliottii</i> var. <i>elliottii</i>	126
5-2. Calculation of phosphorus depletion due to mass flow by eleven root systems of <i>Pinus elliottii</i> var. <i>elliottii</i>	130
5-3. Calculation of phosphorus depletion due to mass flow by eleven root systems of <i>Pinus elliottii</i> var. <i>elliottii</i> . The root chambers were gassed with N ₂ gas....	131

Abstract of Dissertation Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Doctor of Philosophy

ABSORBING SURFACE AREA OF SOUTHERN PINE ROOT SYSTEMS:
PHOSPHORUS AND POTASSIUM UPTAKE BY ROOT SYSTEMS OF TWELVE-
YEAR-OLD SLASH PINE TREES

By

Jose A. Escamilla-Bencomo

December, 1997

Chairman: Nicholas B. Comerford
Major Department: Soil and Water Science Department

Our understanding of ion uptake by pine trees is principally based on studies of white roots of seedlings. However, root systems of trees consist of white, brown (woody and nonwoody) and mycorrhizal roots including external hyphae. It is unclear whether brown roots are functional in nutrient absorption. However, brown and woody roots may contribute over 80% of the fine-root system of slash pine (*Pinus elliottii* Engelm. var. *elliottii*). The aim of this research was to study P and K uptake by root systems of twelve-year-old slash pine growing under field conditions.

The first objective of my research was to develop a field method for measuring nutrient uptake by roots of trees. The design included (i) a root chamber, (ii) a system for

controlling the O_2 level of the root chamber, and (iii) a Mariotte flask system.

The second objective was to (i) compare P and K depletion by lateral root systems of slash pine when the roots are subjected to a short-term, hypoxic treatment, and (ii) to document the ability of roots to absorb P and K following the removal of the hypoxic treatment. N_2 gas was used to achieve hypoxic conditions during the experiments ($<50 \mu M O_2$). Slash pine is grown extensively on soils where surface horizons are subject to short-term hypoxic conditions due to seasonal, fluctuating watertables. I demonstrated that that pine roots grown in aerobic soil conditions were capable of P uptake during short-duration hypoxic conditions. In contrast, K depletion was totally inhibited. Once the hypoxic condition was corrected, uptake of both P and K resumed.

The final objective of my research was to compare P and K influx rates between whole and woody roots of slash pine, while evaluating the mechanism of nutrient uptake by woody roots through the use of N_2 gas as a metabolic inhibitor of ion absorption. I concluded that woody roots absorb P and K and that rates of ion uptake were similar to whole roots. I also documented an active K uptake mechanism by woody roots.

The results indicated that estimates of the surface area of woody roots and sensitivity of K uptake under aerobic and hypoxic conditions, should be included in nutrient uptake models of slash pine ecosystems.

CHAPTER 1 GENERAL INTRODUCTION

Water and mineral nutrient limitations occur frequently in southern pine forest (Neary et al., 1990; Peters, 1990). These limitations are compounded by the fact that intensive forest harvesting and site preparation can change the nutrient capital and availability of nutrients for the next rotation of trees (Morris and Miller, 1994). Therefore, nutrient management in forest plantations increasingly is focusing on matching nutrient supply, from soil or fertilizers with nutrient demand by crop (Smethurst and Nambiar, 1990). Since nutrient availability is a limitation to productivity, then an understanding of nutrient uptake, allocation and use by pine trees is fundamental to predicting the consequences of harvesting, as well as to ameliorating negative harvesting effects.

Predictions of nutrient uptake and demand by the tree at the root surface can be contrasted with the ability of the soil to supply nutrients. Tree nutrient uptake can be described as a set of processes occurring simultaneously in three phases (Comerford et al., 1994a). The first phase is the effect of processes that release nutrients from their solid form to the soil solution. The second phase is the nutrient movement through the soil solution to the absorbing surface of the tree (via mass flow and/or diffusion). The

last phase is the uptake process at the absorbing surface of the tree. If the rate of absorption is less than supply by mass flow nutrients will accumulate at the root surface. Whereas a zone of depletion will occur if mass flow is less than uptake (Barber, 1984; Nye and Tinker, 1977). In the case of depletion, uptake is an active process because the plant must transport these nutrients into cells against a concentration gradient (Clarkson and Grignon, 1991). The absorption of nutrients by roots depends on aerobic metabolism to produce high energy potentials to drive active membrane transport processes (Mengel, 1974). Sudden oxygen deprivation reduces or eliminates active nutrient uptake. Hence uptake is governed by root kinetics, and is often described mathematically by Michaelis-Menten kinetics (Claassen and Barber, 1976).

Total nutrient accumulation of plants can be predicted reasonably well from nutrient uptake model calculations. These calculations are based on parameters such as root kinetics of the plant species, and nutrient concentrations in the soil solution (Barber and Cushman, 1981; Nye and Tinker, 1977). Such predictions from model calculations are less successful for plants grown at low nutrient supply (Jungk and Claassen, 1986) and in tree species under field conditions (Smethurst and Comerford, 1993). Calculations of average uptake rates over the entire root system of a plant are inappropriate in tree species under field conditions because (i) tree roots are irregularly distributed in the soil

(Comerford et al., 1994b), (ii) tree root systems are comprised of root zones with different morphology and physiology (Clarkson et al., 1978) and (iii) soil supply of nutrients is highly heterogeneous both spatially and temporally (Lechowicz and Bell, 1991; Koch and Matzner, 1993).

Root kinetic parameters have been studied for most ions either with excised roots, or with whole root systems of intact plants (reviewed by Epstein, 1972; Nye and Tinker, 1977). However, these investigations have been largely restricted to annual species or herbaceous perennials. Pine root systems include from white roots to mycorrhizal roots to brown roots with secondary growth (woody roots). Consequently, differences in root morphology could influence nutrient uptake to varying extents. There is no question that white roots and mycorrhizal roots of forest tree species are active in nutrient uptake. However a main question is whether woody roots function in uptake, and if so, is it active uptake (VanRees and Comerford, 1990). The question is important because of the tremendous woody fine root surface area in pine trees (Comerford et al., 1994a). Information of this kind is very limited for tree species (Atkinson and Wilson, 1979; Bhat, 1981), and only few root kinetics parameters have been determined for commercial forest tree species, and in all cases for tree seedlings (Van Rees et al., 1990; Kelly and Barber, 1991).

Pine roots of the southern United States encounter shallow water tables that severely reduce soil- O_2 supply to large parts of the root system (Comerford et al., 1996). Forested Spodosols of the Southeastern lower Coastal Plain typically have perched, fluctuating water tables. Sudden oxygen deprivation reduces or eliminates active nutrient uptake of white roots (Fisher and Stone, 1990b; Topa and McLeod, 1986b). A main question is whether under field conditions if a short-term reduced O_2 supply will affect permanently pine roots ability for ion uptake from the soil solution.

Clarkson et al. (1978), stated that the nutrient uptake effectiveness of a root system depends on (i) the relative amounts of different absorbing surfaces present, (ii) the nutrient absorption rates of each type of surface, (iii) differential response of the absorbing surfaces to environmental variables. A major limitation to test the above hypothesis with roots of forest trees is the lack of methodology for field studies of nutrient uptake.

This dissertation describes a nutrient uptake system that I had to implement to document if (i) woody fine root function in nutrient absorption, and (ii) differential response of fine pine roots to changing aeration in the soil environment.

The overall objective of my research was to investigate absorption of phosphorus (P) and potassium (K) by the root surface area of twelve-year-old slash pine trees under field

conditions. Chapter 2 is a literature review of the definition of the nutrient-absorbing surface of trees with a discussion of the active uptake process in the context of root kinetics. Chapter 3 is the description and testing of a field method that I developed to measure nutrient depletion by roots of trees under field conditions. Chapter 4 addresses the effect of low oxygen on the uptake of P and K by roots of pine trees under field conditions. Chapter 5 is a study of P and K depletion by woody roots of pine trees under field conditions. Each of the following 4 chapters is an independent manuscript for journal publication. I chose to study K and P uptake because (i) P availability limits growth of pine (Pritchett and Comerford, 1982; Waring, 1981); ii) P and K uptake by slash pine seedlings were successfully predicted under field conditions (Van Rees et al., 1990a; Smethurst and Comerford, 1993), (iii) P and K uptake by roots have contrasting responses to low oxygen supply, and (iv) K and P have contrasting nutrient cycles.

CHAPTER 2
NUTRIENT-ABSORBING SURFACES OF TREES AND THE NUTRIENT UPTAKE
PROCESS

Introduction

This review addresses the question of nutrient uptake and focuses on the root-hypha absorbing system of the plant. It is based mainly on a review article published by Comerford et al. (1994a), of which I was a coauthor. This review includes sections from that article, which are relevant to the topics covered in my dissertation.

Clarkson et al. (1978), stated that the nutrient uptake effectiveness of a root system depends on (i) the relative amounts of different absorbing surfaces present, (ii) the nutrient absorption rates of each type of surface, (iii) differential response of the absorbing surfaces to environmental variables.

I address these issues in relation to pine trees by considering first the definition of the nutrient-absorbing surface of trees. Once the root categories are defined, this review will address what are the pathways of nutrient uptake for different root surfaces and whether all categories have a nutrient uptake function. Then I discuss the evidence for nutrient uptake by fine brown and fine woody roots, and the potential importance of this uptake in pine forests based on

the account that they are a large proportion of the total fine root surface area. In turn I discuss the active uptake process in the context of Michaelis-Menten kinetics, the biofeedback between the plant and the uptake process, and the response of the root uptake process to changing soil environments, specifically under low-oxygen or hypoxic condition. Last, I framed this information in the the overall objectives of the research described in the following four chapters.

Definition of Absorbing-Surfaces of Trees

A perennial, dicot, woody root system is interesting because secondary xylem growth occurs, and this "woody" portion of the root system may be the major contributor to the fine root population. Fine roots in pine trees are defined as roots < 2 mm in diameter (Van Rees and Comerford, 1986). As an illustration, mature stands of *Pinus elliotii* Engelm. var. *elliotii* (slash pine) had fine roots accounting for about 90% of the total root length (Van Rees and Comerford, 1986), while mature *Pinus taeda* (loblolly pine), had 67% of its root system surface area in roots with diameters less than 1.3 mm (Kramer and Bullock, 1966).

A perennial dicot, woody, fine root system can be described by considering the types of roots that constitute it. The following fine root classification (Table 2-1) is based on the definitions of Sutton and Tinus (1983), the

Table 2-1. Categories of fine-roots in a perennial, dicot, woody-root system (Comerford et al., 1994a).

<u>ROOT CATEGORY</u>	<u>DESCRIPTION</u>
<u>LATERAL ROOTS</u>	
White Roots	New primary lateral roots white in color
Brown Roots Primary	Lateral roots, still having primary growth but the epidermis/cortex has turned brown from either suberin/lignin deposition or cortical cell decay
Woody	Roots showing evidence of secondary growth by having secondary xylem
<u>SHORT ROOTS</u>	
	A root of diminutive length (often less than 2 mm) arising from a lateral root. It may or may not be bifurcated and is not considered capable of further growth
Mycorrhizal	A short root infected with symbiotic fungi. May have a fungal mantle surrounding it if ectomycorrhizal
Non-mycorrhizal	A short root not infected with symbiotic fungi
<u>ROOT HAIRS</u>	
	A small tubular outgrowth from an epidermal cell.
<u>EXTRAMATRICAL HYPHAE</u>	
	Hyphae and rhizomorphs emanating from the mycorrhiza and distributed in the soil.

descriptions of Chung and Kramer (1975) and Kramer and Bullock (1966). Most studies of fine-root systems have generally not recognized nor included all these categories in their analyses.

However, most root studies in forest species have recognized that brown roots are the dominant fine-root category while white roots represent less than 1% of the surface area (Kramer and Bullock, 1966; Van Rees and Comerford, 1990). The majority, or even all, of the tree fine-root system can be brown depending on seasonal and environmental conditions (Hendrick and Pregitzer, 1992). Loblolly pine, growing in the Piedmont of North Carolina (Kramer and Bullock, 1966), had the following surface area distribution (i) white roots 0.3%; (ii) mycorrhizas (not including extramatrical hyphae) 2.7%; (iii) brown roots <1.3 mm in diameter 64.1%; and brown roots >1.3 in diameter 32.7%. In this study, as in most root biomass studies, it is unclear if the brown root category is woody or just brown in color with no secondary growth (primary).

Fine roots of most species common in forests (e.g., spruce, fir, pine, beech, oak, birch, poplar) are colonised by ectomycorrhizal fungi (Harley and Harley, 1987). Other tree species (e.g., elm, ash, maple, fruit trees) are colonised by endomycorrhizal (arbuscular mycorrhizal) fungi (George and Marschner, 1996). In a number of tree species, both ectomycorrhizal and arbuscular mycorrhizal, can occur simultaneously (Lodge, 1989). Some roots of all trees, in

particular the fast-growing long roots, usually remain non-mycorrhizal. The surface area of extramatrical mycorrhizas found in pine forest might very well dwarf the surface area of the other root categories. Under controlled conditions external mycelium of mycorrhizal pine seedlings accounted for 75% of the total surface area (Rousseau et al., 1994) however, this contribution is likely to be lower under more natural conditions due to presence of competitors and predators. With current technology it is not possible to measure mycorrhizal hyphae length or surface area under field conditions. Therefore there are no reliable estimates of extramatrical mycorrhizal hyphal length or surface area in natural environments.

Root hairs are part of the epidermis of the root. They are involved in water and ionic exchange between the plant and the growth medium. Therefore, when root hairs are present the root surface can be considerably enlarged depending on root hair length and density of hairs on the epidermis. Root hair length varies between 80 and 1500 μm , with diameters of 5-20 μm , depending on species and cultivars (Dittmer, 1949; Caradus, 1979). Although the location of the root hair zone is generally known in some plant species (1 to 4 cm long and behind the zone of active root elongation (Jaunin and Hofer, 1986), root hairs are not always present. Little work has been done to investigate the abundance of root hairs on pine roots. However Kozlowski and Scholtes (1948) reported root hairs on roots of 7-week-old loblolly pine seedling with hair

densities of 217 hair cm^{-2} Roots developing an ectomycorrhizal structure will not have root hairs (Mexal et al., 1979), but root hairs are common on some endomycorrhizal roots (Lyford, 1975).

Most studies on nutrient absorption by root hairs have been done in agronomic crops where they have been shown to be important for increasing root surface area. (Itoh and Barber, 1983). Still, their contribution to the root system surface area of trees is largely unknown.

Nutrient Uptake for the Different Root-Surfaces of Trees

Once the root categories are defined, the next question is whether all categories have a nutrient uptake function. I will address this question by first describing the pathways for nutrient uptake and then reviewing the nutrient uptake by different root surfaces of trees.

Pathways of Nutrient Uptake

In any root there exists two pathways for nutrient uptake. These are the apoplastic and symplastic pathways. As defined by Dumbroff and Pierson (1971) the apoplastic pathway is a route through the apoplast where the ion does not have to pass a selectively permeable membrane but moves through intercellular space, while the symplastic pathway is where the ion must pass a semipermeable membrane to enter the cytoplasm and moves radially in the cytoplasm by passing from cell to cell via plasmodesmata. Transport of water and ions

through the apoplast of the root outside the endodermis is only stopped by Casparian bands on the inner cortical layer in the endodermis.

A white root includes the area from the root tip to some point in the zone of primary tissues. Therefore, a white root can have no endodermis, can have a developing endodermis, and can have a fully developed endodermis; depending on position along the root. In the youngest white roots, the casparian bands are absent but the xylem vessels are immature, so only very limited apoplastic entry to the xylem is possible. As the root matures the casparian band becomes continuous and forms a barrier to apoplastic nutrient flow.

In some cases a Casparian band can develop in the hypodermis, sometimes referred to as the exodermis (Peterson et al., 1981; Perumalla and Peterson, 1986). This has been shown in some plants to be an effective barrier to apoplastic flow (Peterson et al., 1981). Yet, it is not clear if this strip is fully equivalent to that of the endodermis as proposed by Shone and Clarkson (1986). The presence and degree of development of the suberin deposition is related to stress, with high water stress causing it to form closer to the tip of the root.

In brown roots, a fully developed endodermis can be compromised by short and lateral root development leaving a partial pathway for apoplastic water and nutrient flow where the roots exit the pericycle through the endodermis. If secondary xylem growth occurs, it can cause corruption of the

endodermis with the cortex progressively sloughing away (Troughton, 1957). With time, cork tissue with a cork cambium develops, becoming another possible barrier to apoplastic flow.

In short roots with ectomycorrhiza, the fungal sheath becomes the surface area for absorption of both water and nutrients. Ectomycorrhizae may have a well developed sheath which forms a tightly packed layer between the soil and root epidermis extending around the root apex, so that the zone of maximum water and mineral uptake of the root is separated from the soil by fungal tissue. Therefore all substances absorbed from the soil must pass through the fungal sheath or mantle before coming in contact with the outer cells of the root (Harley and Smith, 1983). Like the root tissue, the fungal sheath is divided into symplastic and apoplastic compartments (Ashford et al., 1988; 1989). Cellufluor has been used to test the apoplastic permeability of the fungal sheath in *Pisonia mycorrhizae*. Ashford et al. (1988) documented that the apoplastic tracers penetrated as far as the root epidermal cells in tip regions in the immediate vicinity of the mycorrhizal root cap. Behind this area, where both the root and mycorrhiza are likely to be active, the sheath was impermeable to the tracer. Blockage of the apoplast was believed to be at the interface between fungus and root. Therefore it was concluded that the symplastic pathway within the fungus was very important at this stage with the hartig net in a position to deliver water and

nutrients into the intercellular cortex. Any obstructions to apoplastic flow that exist at that point of entry as described above then apply. In short roots with endomycorrhiza the fungal mantle is not present.

Root hairs generally arise from the epidermis. A root hair may increase the absorbing surface area and hence increase the ion and water absorption per unit length of root, but nutrients must still pass a symplastic route. When root hairs develop, a cuticle has been known to develop over them and the epidermis (Curl and Truelove, 1986). The hydrophobic waxy components of the cuticle may increase resistance to water and nutrient flow, but experimental data are lacking making definitive statements difficult. The relative rapid deterioration of the cytoplasm of root hairs of maize roots suggests that root hairs may not function for very long (Fusseder, 1987).

Nutrient Uptake by Root Trees

Most of the research on root nutrient uptake by trees comes almost exclusively from experiments with tree seedlings under laboratory or greenhouse conditions. There is no question that white roots, short roots and mycorrhizal hyphae are active in nutrient uptake of trees (George and Marschner, 1996). However much less is known about nutrient absorption by intact older fine roots of perennial plants in field conditions. The main question in nutrient uptake by trees is whether brown roots function in uptake, and if so, is it by

active uptake? The importance of this question for trees is obvious when one considers the tremendous root length and surface area in this category. For illustration Comerford and Neary (1991) used nutrient uptake models to show that in a semi-mature slash pine stand, the only way one can predict phosphorus (P) root uptake based on observed P soil solution concentrations, was to either adjust mineralization rates or not allow a very large part of root system to function in P uptake. This would suggest that a significant portion of the fine root (brown-woody) system is not absorbing P. It might be that our idea of an "effective" root length density is incorrect. Yet, one would normally include these potentially non-absorbing roots in estimates of root length density when using nutrient uptake models. While the lack of a mechanistic definition of an "effective" root length density has not been a problem in agriculture crops, its importance is more critical in tree root systems. Not only are tree roots irregularly distributed in the soil (Comerford et al., 1994b), but tree root systems also comprise roots with very different morphology and physiology (Table 2-1). Also, the plant available nutrients are distributed with higher heterogeneity in forest soils (Lechowicz and Bell, 1991).

In forest trees, root biomass represents less than 20% of the total biomass (Keyes and Grier, 1981). This relatively lower root biomass is mainly caused by the high turnover rates of tree fine roots. Fine roots of forest trees have a short-life span (George and Marschner, 1996), and a large

amount of nutrients is used each year for the growth of new roots (van Praag et al., 1988; Eissenstat and Van Rees, 1994). The yearly nutrient uptake in adequately growing forest depends on the tree species, but to take up those nutrients, trees need efficient root systems adapted to the usually nutrient-poor forest soils (Koch and Matzner 1993).

If absorption of nutrients by roots of trees is confined to new root growth, is it the browning of roots (accompanied by suberization) that curtails the nutrient uptake activity of roots of trees? Long lived root elements would seem to be advantageous for a plant given energy and nutrient availability considerations. Still the question of root longevity may be viewed in a different perspective. Maintenance of long lived, fine root elements, may in fact be a poor evolutionary strategy. It may be less energetically expensive to form a new root element which systematically explores new microsites for relative immobile elements such as phosphorus, than to maintain a long lived absorbing root element in sufficient density to meet plant nutrient demand. Studies have demonstrated that either alternative, can be favorable to the plant.

With annual plants it is well known that uptake rates of roots change with age (distance along the root axis) (Bowen, 1969; Barber, 1984; Marschner, 1991) and that the important factor in zonality of ion uptake is the structural differences in walls of epidermal cells and in peripheral cells of the cortex, endodermis and stele. For both annual

and perennial plants the term "root browning" has been loosely applied to several developmental processes including those of root dormancy, cortical breakdown and periderm formation (Sutton and Tinus, 1983). Field observations of growing root systems generally corroborate the common assumption that only the new growing root elements are free of the browning (darkening) in color, considered to be a development of suberin layers in older roots (Head, 1967). Even though suberin is impermeable to water, the deposition of suberin layers is not always continuous, particularly if they are deposited in the walls of the exodermis. Even in the case of periderm formation, water could still enter through lenticels, wounds or other breaks or discontinuities in the suberin layer.

The most detailed investigation related to the histochemical nature of root browning was done in jack pine roots and eucalyptus by McKenzie and Peterson (1995a and 1995b). Root browning was not linked to suberization but was caused by deposition of condensed tannins in the walls of all cells external to the stele. The term suberization should not be associated with browning, since the white zones of jack pine and eucalyptus roots were not colored internally, despite the presence of suberin in the endodermis of jack pine and in both the endodermis and exodermis of eucalyptus. Therefore, a white root is not necessarily an unsuberized root, nor is a suberized tissue layer necessarily brown. This

study documented that an apoplastic tracer was unable to penetrate the periderm in secondary roots.

Evidence of Nutrient Uptake in Brown Roots

An important question in nutrient uptake is whether brown roots function in uptake, and if so, is it by active uptake? Uptake by brown (woody?) roots has been reported, but the data base is not extensive. *Citrus* and *Pinus taeda* have both been shown to have brown roots that absorb PO_4 (Crider 1933; Chung and Kramer, 1975). The work by Chung and Kramer even suggested that an effective ion barrier existed allowing selective ion absorption. Kramer (1946) suggested that the selective barrier was either the cork cambium, the vascular cambium or both. Similar uptake rates for *Prunus* were subsequently reported (Atkinson and Wilson, 1979). The same study by Crider (1933) also indicated active uptake of NO_3 by woody (brown?) roots in *Citrus*, again with an effective ion selective barrier implicating active uptake.

The rate of K uptake by brown (woody?) roots of *Prunus* and *Malus* (Atkinson and Wilson, 1980) and slash pine (Van Rees and Comerford, 1990) was shown to be lower or similar to the uptake rate of white roots. In the work on *Pinus elliotii* (Van Rees and Comerford, 1990), K accumulated at a rate faster than that attributable to mass flow, suggesting an active uptake mechanism

So there appears to be some evidence that, as with white roots and short roots, brown roots are capable of nutrient uptake. However, it is not clear if uptake is dominated by mass flow or active uptake mechanisms. If uptake in brown roots is via apoplastic pathways, these pathways could include lenticels, wounds at the base of branch roots (Addoms, 1946) and breaks in the endodermis (Dumbroff and Pierson, 1971; Peterson et al., 1981). However, where active uptake is indicated it is not clear what the barrier to apoplastic flow is.

All of the research on root nutrient uptake by brown roots comes almost exclusively from experiments with either sections of roots (Chung and Kramer 1975) or intact roots from tree seedlings under laboratory or greenhouse conditions. Also in most studies, is it unclear what category of brown roots was being investigated.

Model experiments by Bhat (1981, 1983) showed that the rate of ^{32}P uptake by whole root systems of 3.5 years-old apples trees was 2.5 lower than root systems of apple tree seedlings. In both cases the trees were grown under greenhouse conditions. Unfortunately such detailed uptake studies with root trees in field conditions are rare. Up to now, only very few attempts have been made to develop techniques to study the nutrient uptake of root systems in forest sites (Glavac and Ebben 1986; Marschner et al., 1991; Niederholzer et al., 1994).

Michaelis-Menten Kinetics as a Description of Active Nutrient Uptake

Appropriateness of the Equation for Root Uptake

Nutrient concentrations in the cytoplasm of soil grown roots are usually much higher than in the soil solution at the rhizoplane. Metabolic energy is required for nutrient uptake to occur against this chemical gradient. The transport of nutrients across cell membranes is enzymatically mediated and nutrient specific (Nye and Tinker, 1977), but chemical details about transport to the enzyme carrier, the nature of the enzyme, movement via carrier, and release inside the cell remain largely matters of speculation.

Because active nutrient uptake at low concentrations (e.g. < 1 mM) is enzymatic, the principles of enzyme kinetics have been applied to the mathematical description of nutrient uptake within the appropriate ranges of concentrations. L. Michaelis and M.L. Menten in 1913 developed a general theory of enzyme kinetics, which was later extended by G.E. Briggs and J.B.S. Haldane (Lehninger, 1970). They considered the situation where an enzyme combined with a substrate, then dissociated to release the product. Both steps were associated with forward and reverse rate constants. Once simplifying assumptions were made, this situation resolved mathematically to an asymptotic function between the velocity of the reaction and the concentration of the substrate, i.e.

$$V = V_{\max} * S / (K_m + S) \quad (1)$$

Where V is the velocity of the reaction, S is the concentration of the substrate, V_{max} is the maximum velocity attainable, and K_m is the S at half V_{max} and is also related to the rate constants of the reactions and thereby the affinity of the enzyme for the substrate. The constants V_{max} and K_m are normally empirically fit but can be calculated if forward and reverse rate constants are known. The function is known as the Michaelis-Menten equation and describes the saturation phenomenon observed in enzyme studies at low concentrations.

Claassen and Barber (1976) and Nielsen and Barber (1978), applied this function to nutrient uptake by roots, modified it by including a minimum concentration below which there was no further uptake (C_{min}), and substituted the terms I (inflow to a root) for V , and C (concentration) for S , i.e.

$$I = I_{max} * (C - C_{min}) / (K_m + (C - C_{min})) \quad (2)$$

Hence, this function is a description of nutrient uptake using Michaelis-Menten kinetics (Figure 2-1).

Although this function has fit observations of inflow versus concentration, i.e. $I(C)$, for many nutrients and root types, there are several reasons why observations might deviate from this theoretical form. Nutrient uptake may be more complex than the simple enzyme system on which Michaelis-Menten kinetics are based. For instance, more than one enzyme or type of enzymes may be involved, diffusion to and from the carrier enzyme might be a limitation, energy supply to the enzyme may be a limitation, the rate constants

may be nonlinear with substrate concentration (Lehninger, 1970), and more than one uptake mechanism might be at work (Nye and Tinker, 1977).

This latter remark refers to the phenomenon of multiphasic uptake (Epstein, 1972; Hodges, 1973; Nissen, 1980) whereby Michaelis-Menten kinetics seem to be an adequate description of uptake at normal soil solution concentrations, e.g. < 1 mM, but at higher concentrations the process is not active and hence is unaffected if induction of the carrier enzyme is blocked (Omata et al., 1989; Oaks, 1992). However, the high concentration at which this second mechanism dominates is much higher than the concentration of most nutrients under natural and most managed conditions. With the exception of NO_3 under limited soil management conditions, multiphase uptake is likely to be of no concern for understanding growth under low fertility.

A further complication is that efflux of nutrients from roots may not be related to the activity of the uptake system because it is thought to be largely a diffusive process dependent upon internal and external nutrient concentrations and the integrity of the membrane.

Although Michaelis-Menten kinetics remain a useful description of nutrient uptake by roots at most soil solution concentrations, it is best thought of as an empirical description of nutrient uptake relative to solution concentration, $I(C)$, than a theoretical approach. While based in theory, its application will only approach a theoretical

level when the process is better understood, when active and passive uptake mechanisms are defined for each root category, and when feedback mechanisms regulating uptake are mechanistically incorporated.

Active Root Uptake Mechanism and Root Anatomy

Most evidence suggests that an active mechanism is responsible for the bulk of ion uptake by roots, but many physiologists believe that passive mechanisms also provide at least a small portion of the ions that are absorbed by roots (Brouwer, 1965). In this review I am focusing on P and K uptake by roots of trees; therefore I will only discuss the uptake mechanism of the above mentioned nutrients.

Phosphorus uptake by roots is active as measured by electrochemical gradients (Ulrich-Eberius et al., 1981) and appears to be operated by the pH gradient across the plasmalemma as indicated by Dunlop (1989) and Bowling (1981). Phosphate flux in the roots is affected by transpiration which ensures the flow of solutes in plant tissues. Active P uptake by roots of trees has been demonstrated for loblolly pine (Topa and McLeod, 1986b).

Potassium uptake by root tissue is active (Higinbotham et al., 1967). This was proved by the use of inhibitors of energetic metabolism. In sunflower and wheat roots, K uptake was inhibited by 2,4-DNP (Pettersson, 1981). Antibiotics of the "ionophore" type showed a stimulatory effect on K uptake. Gramicidine-D and nigericine stimulated K influx into the

roots up to from 4- to 8-fold (Hodges et al., 1971). The ability of roots to regulate influx decreases with increasing external ion K concentrations suggesting a feedback mechanism (Pettersen and Jensén, 1979). Active K uptake by roots of trees has been demonstrated for plum (Rosen and Carlson, 1984) and for slash pine species (Fisher and Stone, 1990b; Van Rees and Comerford, 1990).

Ion transfer across the plasmalemma and ion influx or efflux from the apoplast into the symplast is directed by regulation of active processes of metabolic reactions. There is much experimental evidence for the prevalence of symplastic over the apoplastic transport of ions. Anderson (1975) assumed that over 90% of the ions passed into the stele moved through the symplast-apoplast mechanism (not plasmodesmata). For instance P-phosphate ions are transported mainly through the symplast (Clarkson and Sanderson, 1974). However starving roots first take up phosphate by diffusion into the apoplast. After the saturation of the apoplast and when the steady-state of the external solution-tissue system is exceeded, the transport proceeds actively (symplastic) (Ulrich-Eberius et al., 1981). In comparison, K ions are transported mainly through the symplast (Lauchli et al., 1973).

Only few studies have examined the potential role of brown (woody?) and white roots in water and nutrient uptake. Moon et al., (1986) found that the uptake of ions and water in roots of mangrove occurred primarily through the

symplast of the younger (nonwoody?), intact roots (apoplastic flow was estimated at 1%). Furthermore, Moon et al., (1986) found that disturbing roots by removing them from the soil increased estimates of apoplastic water flux by 57X. However time was not allowed for roots to heal from the injury sustained nor were injuries sealed with wax, thus allowing an abnormally high number of direct entry points for apoplastic water flow. Van Rees and Comerford (1990) reported that fine woody roots of slash pine seedlings were nearly as effective in water uptake as were entire root systems (nonwoody and woody). However, Van Rees and Comerford (1990) found that water absorption by fine woody roots with cut ends (the cut ends had several day to callous) was similar to that of woody-roots coated with paraffin wax. The dependence of flow upon development of suberized layers in the endodermal cell is not crucial for apoplastic movement. There are known to be regions of discontinuity in the Casparium band in roots through which unhindered transport in apoplast might occur. Such regions occur at the root where the endodermal cells are not yet mature (Robards and Jackson, 1976) and sites of secondary root initiation. During their development, laterals root cause a gap in the structure of the endodermis. They arise from meristematic initials in the pericycle; as they divide, a dome of cells is produced and the endodermis divide to form the epidermal layer of the emerging lateral (McCully, 1975). At the junction of the lateral and the endodermis there is a ring of cells which lack Casparian bands (Dumbroff

and Peirson, 1971)) which seems to permit apoplast continuity between the cortex and the stele. Apoplastic tracers were seen to enter the stele at this point (Peterson et al., 1981) and water uptake in the zone of lateral emergency of barley roots was higher than elsewhere and very responsive to changes in transpiration rate (Sanderson, 1983). Therefore, probable mass flow pathways are provided at one stage during branch development. Yet, the physiological significance of this apoplastic pathway and a potential passive uptake of nutrients has not been studied in field conditions. All of the research on the mechanisms of nutrient uptake by roots comes from experiments with tree seedlings under laboratory or greenhouse conditions.

Importance of Root Uptake Kinetics Under Field Conditions

I_{max} , K_s and C_{min} Under Field Conditions

The importance of the Michaelis-Menten parameters under field conditions will depend mainly on the concentration of the nutrient in solution at the absorbing surface (C_{la}). The profile of nutrient concentration in solution relative to radial distance from the root can take three basic profiles. When plant uptake is equal to the supply by mass flow, C_{la} is equal to the bulk soil solution concentration. If demand at the root surface is greater than supply by mass flow in the soil, a depletion zone will develop and C_{la} will be less than the bulk soil solution. The higher the plant demand and the

slower the rates of mass flow and diffusion supply, the lower will be C_{1a} . However, if mass flow supply is more than the demand at the root surface, a surplus will build at the root surface and C_{1a} will be greater than the bulk solution concentration.

The sensitivity of nutrient uptake to the value of the Michaelis-Menten parameters depends on C_{1a} . When mass flow is a dominant source of supply and is approaching or exceeding plant demand, then C_{1a} is high, approaching the solution concentration at which I_{max} occurs. Under these conditions, uptake will be dependent on the value of I_{max} . However, if mass flow does not contribute much to soil supply and the supply by diffusion is significantly less than demand by the plant, C_{1a} becomes very low. If C_{1a} gets low enough, it gets to the point on the curves where they begin to converge and now plant uptake is not sensitive to I_{max} . There is a similar but reverse explanation to a plant's uptake sensitivity to C_{min} . As C_{1a} approaches the value of C_{min} , plant nutrient uptake will be very sensitive to the value of C_{min} (Figure 2-1, case A). However, when C_{1a} is much higher than C_{min} (C_{min} is very low relative to C_{1a}), changes in C_{min} will have little effect on plant uptake. (Figure 2-1, case B). For illustration *Fescue* and *Canary grass* roots with low I_{max} values relative to corn (Figure 2-1), can deplete P from the soil solution under lower soil P concentrations when compared with corn roots.

Figure 2-1. A series of Michaelis-Menten curves. Case A represents the condition where the solution concentration is so low that sensitivity to $I_{\max}$ is unimportant. Case B represents the condition where the influx would be sensitive to the value of $I_{\max}$.

Conversely at high soil P concentration corn roots will deplete P faster from the soil solution because higher $I_{\max}$ values when compared with *Fescue* and Canary grass roots. This is extremely important since soil nutrient concentrations will be closer to $C_{\min}$ values in forest soils. Although it is possible that these parameters could affect a plant's competitive ability, little is known about their genetic or environmental controls. Nevertheless, there is evidence that K_m is under genetic and environmental control, and that plants continually presented with nutrients at concentrations approximating K_m will grow at high relative growth rates in the absence of other limitations (Bloom et al., 1993).

Mycorrhizal hyphae may have lower $C_{\min}$ values than the root with which they are associated, thereby enhancing P uptake. However, work by Li et al. (1991) indicated that this was not the case for *Glomus mosseae* colonizing *Trifolium repens* because both surfaces depleted P to similar concentrations. However knowledge of nutrient uptake by ectomycorrhizal fungi comes almost from experiments in the laboratory where small tree seedlings are inoculated with isolates of some species of mycorrhizal fungi. Some of the fungi used in these experiments are not common in forests and often substrates other than forest soils are used (George and Marschner, 1996). Unfortunately, an excavation of mycorrhizal fine roots from soils disrupts the hyphae growing into the soil dissecting the mycorrhizal roots from its major uptake organs. Therefore, uptake studies with intact mycorrhizal

fine roots are only possible in greenhouse conditions (Anderson and Riegwickz, 1991).

The $I_{\max}$ parameter has received more attention than K_m or $C_{\min}$, probably because $I_{\max}$ is more easily measured and it is quite responsive to imbalances between nutrient uptake rates and demand by the plant. For instance, Jungk et al. (1990) found that the $I_{\max}$ of soybean roots increased 376% due to a decrease in pretreatment P concentrations from 30 to 0.03 mM, while K_m values decreased by only 60%. Jackson and Caldwell (1992) have shown for field grown *Agropyron desertorum* roots that the P uptake capacity (i.e., $I_{\max}$) can be increased by at least 73% in response to increases in P supply.

Temporal and Spatial Changes in Root Uptake Kinetics Under Field Conditions

Not only do the uptake kinetics of a root change as a root ages (i.e. with distance behind the root tip), but the uptake kinetics of root systems may vary temporally over short periods, e.g., 2 minutes (Ayling, 1993), or spatially over small distances, e.g., 2 centimeters (Bowen, 1969).

The potential significance of spatial and temporal variability of root uptake kinetics for nutrient competition between plants has been illustrated experimentally and theoretically by Jackson and Caldwell (1992) and Caldwell et al., (1992). They measured P uptake by excised roots of *A. desertorum* grown in microsites to which water or water with nutrients had been added. Plants were 0.5 m apart and control and enriched microsites were located on opposite sides of

individual plants. Four days after treatment, uptake rates of P by excised roots from enriched microsites were up to 73% higher than those by roots from control areas when immersed in 1, 10 or 20 mM P, but there was no comparable effect on N uptake in the range 50-1000 mM N. Model simulations indicated that this mechanism for enhanced P uptake from fertile microsites was more important than root proliferation.

Dighton and Harrison (1990) reported that, despite year to year variation in the P uptake capacity of fine roots of seven *Picea sitchensis* stands aged 14-26 years, increased growth rates after the addition of P fertilizer were accompanied by a decrease in the P uptake capacity by fine roots in fertilized plots. The change in P uptake capacity after fertilization was considered a better indication of P deficiency in these stands than concentrations of P in foliage.

Short-term feedback mechanisms may operate by regulating the energy supply to ion carriers in cell membranes or by altering the configuration of the membrane so that transport of the ion to, with and from the carrier is retarded or enhanced. Long-term feedback on uptake kinetics probably involve regulation of the number of carriers. For example, enzymes like nitrate transporters require a lag phase of exposure to nitrate before uptake can proceed.

Under field conditions absorbing surface areas of trees can also influence nutrient soil-solution concentration, modulating the supply of nutrients to the surface, and

affecting uptake rate. Processes important for forest soils of the southeastern United States include organic exudates and redox potentials. For example several types of organic acids (such as citrate and oxalate) are excreted by roots and mycorrhizal hyphae in response to P deficiency, improving P availability in the soil-solution. Examples of this process in forest soils are given for *Pinus* by Fox et al. (1990) and Fox and Comerford (1990).

Also, roots of some forest species growing in anaerobic conditions have been shown to oxidize their rhizosphere. Taproots of pine trees occur in soil zones which are perennially or temporarily water saturated producing anoxic conditions. Roots of slash pine (*Pinus elliotii*) (Fisher and Stone, 1990a), lodgepole pine (*Pinus contorta* Douglas ex Loudon) (Coutts, 1982) and pond pine (*Pinus serotina* Michx.) growing in oxygen depleted soils induced localized hypertrophy of lenticels on taproots and lateral roots and produced continuous gas filled lacunae in the roots. Internal O_2 transport of these roots provides the ability to absorb nutrient on poorly drained soil. Such a mechanism allows active K uptake under what would appear to be anaerobic conditions (Fisher and Stone, 1990b). This process is important for forest plantations of the southeastern United States where seasonal water tables severely reduce O_2 supply to large parts of the root system. Forested Spodosols of the southeastern lower coastal plain typically have perched, fluctuating water tables. At any single location the

saturated zone can range from near the soil surface to depths > 150 cm during a single year, and often during a single growing season (Phillips et al., 1989). Pine roots growing in those soils encounter shallow water tables which promote reduced soil conditions (Comerford et al., 1996). Reduced soil conditions affect root respiration and, as soil oxygen tension decreases, the uptake of ions falls, particularly under hypoxic conditions (Hopkins et al., 1950; Hopkins, 1956). However maintenance of ion uptake by submerged roots of trees depends on the ion studied, and time of root acclimation to hypoxic conditions.

The O_2 dependent nature of ion influx in unacclimated or hypoxic intolerant root tissues suggest that fermentative respiration does not produce sufficient levels of ATP to drive the plasmalemma H^+ -ATPase (Drew, 1988). Investigations with wheat suggest that xylem loading of K is more sensitive to O_2 deficiency than influx at the plasmalemma (Thompson et al., 1989). Recent investigations by Topa and Cheeseman (1993) concluded that ^{32}P transport to shoots of pond pine seedlings may be more sensitive to O_2 deficient soil conditions than uptake at the epidermal or cortical plasmalemma of pine roots, possibly because stelar tissue may experience O_2 deficiency before and more frequently than outer cortical cells. From the point of view of mineral nutrition of trees under field conditions, it is not the ion transport mechanism that is important, but the way in which the transport mechanism is controlled by the tree. Clarkson et

al. (1978) stated that the nutrient uptake effectiveness of a root system depends on the differential response of the absorbing surfaces to environmental variables.

Concluding Remarks

From the concepts presented above I conclude that uptake and transport of ions takes place in a complex structure of various root tissues (eg., apoplastic and symplastic pathway); at different stages of growth and development (e.g. cell division, elongation and cambial growth); and along the length of roots. Thus anatomical, as well as physiological characteristics of both young and old roots of perennial plants deserve to be studied under field conditions. The overall objective of the research described in the following four chapters was to investigate the absorption of phosphorus (P) and potassium (K) by the root surface area of twelve-year-old slash pine trees under field conditions. Chapter 3 is the description and testing of a field method that I developed to measure nutrient depletion by roots of trees under field conditions. Chapter 4 addresses the effect of low oxygen in the uptake of K and P by roots of pine trees under field conditions. Chapter 5 is a study of P and K depletion by fine woody roots of pine trees under field conditions.

CHAPTER 3
A METHOD FOR MEASURING NUTRIENT
DEPLETION BY ROOTS OF MATURE TREES IN THE FIELD

Introduction

Current understanding of water and ion uptake by roots generally comes from studies of transport phenomena using new, white roots of young plants (Bowen and Rovira, 1967; Bhat and Nye, 1973; Ginsburg and Ginzburg, 1970). Much less is known about nutrient absorption in older roots of perennial plants, yet their investigation is fundamental for a complete description of the "effective" nutrient absorbing surface area of a root system. "Effective root surface" is defined, in water and nutrient uptake models, as the fraction of the total surface area contributing to water and ion uptake (Baldwin et al., 1972). Root surface area can be the single most important factor determining nutrient uptake by plants (Barber, 1984). However, a root's ability to absorb nutrients can change with age and stage of growth development (Fusseder, 1987; Nayakekorala and Taylor, 1990). Types of root surface areas have been categorized as (i) white roots (with and without root hairs), (ii) mycorrhiza, (iii) other nonwoody roots (nonwoody roots undergoing root browning which may be due to metaculization, suberization, or cortical cell decay, and iv) woody-roots (those containing secondary xylem)

(Chung and Kramer, 1975; Sutton and Tinus, 1983; Comerford et al., 1994a). Nutrient uptake by roots has, in most cases, been studied in solution culture. It has been described by relating the rate of ion uptake by the root to the external ion solution concentration. Net ion influx has been described by equations derived from the Michaelis-Menten equation (Claassen and Barber, 1974). Parameters of these equations may vary according to the type of nutrient, plant species (Clarkson, 1985; Foehse et al., 1988), genotype (Nielsen and Barber, 1978), and age of plants (Jungk and Barber, 1975). Therefore, it is reasonable to believe that uptake characteristics also change with different types of root surface area. Determining the kinetics of ion uptake by roots is of fundamental importance to the study of plant mineral nutrition, yet field studies of root uptake tend to be complicated due to access to plant roots below the soil, plant age and plant size. This is especially true when considering mature trees.

Uptake kinetic studies have been accomplished either by using excised roots (Noggle and Fried, 1960) or intact plants (Loneragan and Asher, 1967). Uptake studies using excised roots may not be relevant because (i) roots are not attached to the sink (the shoot), (ii) a steady state condition might not be established in the short uptake periods used (e.g. 20 minutes) and, (iii) changes in root metabolic processes may occur due to injury stress (Bloom and Caldwell, 1988).

Longer-term depletion studies by intact roots have been conducted under (i) steady-state conditions, or (ii) transient conditions. Steady-state conditions occur when the influx of the ion into the system occurs at a rate equal to root uptake of the ion, so the quantity of the monitored ion remains steady over time, even though ion uptake is on-going. The amount of the ion that must enter or leave the system per unit time to maintain a steady-state becomes a measurement of the rate of the reaction. Nutrient flow systems that monitor steady-state ion absorption by intact plants were described by Bloom (1989), Bloom and Chapin (1981), and Glass et al. (1987). The systems are sophisticated, and have been used under greenhouse conditions. Cost and technical requirements are a constraining for implementing a steady-state system that provides field measurements of ion uptake for roots of mature trees. Also, the steady-state ion absorption method has received some criticism in that the procedure may not adequately represent soil solution conditions in the rhizosphere of plants growing under field conditions.

Transient conditions to measure root uptake by intact plants are based on the time required to deplete the ion concentration in a solution; hence they are called depletion methods. A depletion method can be accomplished by either a) exposing root systems to a graded series of initial ion concentrations (Nye and Tinker, 1977); or, b) measuring rates of ion depletion from an initial ion concentration (Claassen and Barber, 1974). A major requirement for the first case is

to have enough root systems and replicates for the chosen graded series of initial ion concentrations. Uniformity of root systems could be a major constraint in measuring *in situ* ion uptake on roots of mature trees using this approach. The second case is less laborious than the first one, requiring only a system to maintain a constant solution volume during the whole experiment. In this fashion, the ion uptake rate can be calculated from changes in the ion concentration in the solution. The few ion uptake studies using forest tree roots have employed the depletion method (Beck, 1979; Van Rees and Comerford, 1990).

In order to measure ion uptake by intact roots of mature trees in the field, two major requirements must be met (i) to grow the root system in a way that allows access and minimizes disturbance; and (ii) to enclose the root system in a chamber where environmental conditions can be controlled. Meeting these two conditions in the field is challenging. Not surprisingly, I found only two useful examples of nutrient depletion studies in the field and a handful of related studies. The first example was published by Marschner et al. (1991). Non-mycorrhizal, intact long roots of Norway spruce (*Picea abies* (L.) Karst.) were carefully excavated, lifted from the soil, and placed into containers where a solution with nutrient concentrations similar to those in soil solution was supplied. The experiment was designed to study the preferential uptake of nitrogen ions (NH_4^+ vs. NO_3^-) from the nutrient solution. The second example was recently

published by Goutouly and Habib (1996). Whole root systems of 3 to 5 year-old, non-bearing peach (*Prunus persica* (L.) Batsch.) trees were excavated. Each root system was split in half and put into nutrient solution containers. The whole root system was maintained at 1 or 5 mM NO_3^- . Hourly nitrate uptake rates of each root subsystem were measured under natural climatic conditions. Besides these two, a few related studies include descriptions of techniques for measuring ion uptake by intact roots of mature trees under field conditions. Glavac and Ebben (1986) described a root chamber to test the reaction of roots to different nutrients and toxic chemicals. The system utilized a square container made of 1 mm² polyvinylchloride (PVC)-net, filled with washed sand, a ceramic plate, and a remote solution reservoir. Capillary action provided by the ceramic plate in equilibrium with the soil in the chamber resulted in a constant supply of nutrients and chemicals to the root system. This technique was designed to investigate root morphological changes following four weeks of exposure to different chemical environments. As described, however, the system was inappropriate for nutrient depletion studies. Niederholzer et al. (1994) described a method for measuring root development of field-grown, mature peach trees. They developed a novel system to measure root regeneration and elongation from the cut ends of roots placed into root-impenetrable cloth bags. Sisson (1983) described in detail a system for determining

respiration rates of intact *Yucca elata* Engelm. roots. The system is a good example of meeting aeration requirements.

There does not appear to be a system appropriate for the study of nutrient depletion by roots of mature trees in the field. The objectives of this study were (i) to present the design and construction of a methodology for nutrient uptake by lateral root systems of mature trees in the field, and (ii) to test the procedure under laboratory and field conditions.

Materials and Methods

Site Description

Twelve-year-old slash pine trees (*Pinus elliotii* Engelm. var. *elliotii*) averaging 16.7 m (std=3.7) tall and 19.4 cm (std=2.7) dbh (diameter-base-height) were selected from a research plot in north Florida that received complete weed control. The study area is a long-term field experiment, initiated in 1983 by the Intensive Management Practices Assessment Center (IMPAC) of the USDA Forest Service, to assess potential biological productivity of pine and the processes controlling it (Colbert et al., 1990). A complete description of the site is given in Swindel et al. (1988) and Neary et al. (1990). Weeds were controlled with a combination of herbicide and mechanical means. The study site is about 10 km north of Gainesville, FL (29° 80' N, 82° 20' W). The climate is warm, temperate-subtropical with a mean annual

precipitation of 1332 mm, most of which occurs in summer, with the least falling in autumn and spring. Mean annual temperature is 21 °C. The soil is a poorly-drained Pomona fine sand (sandy, siliceous, hyperthermic Ultic Alaquod).

Root Regeneration System

The root regeneration system was a variation of the method used by Lyford and Wilson (1966). Four lateral roots 1-1.5 cm in diameter were carefully excavated at the base of a tree so that the fine root system was preserved as much as possible. Each lateral root was pruned to a length that varied from 20 to 40 cm. If a ramification of the lateral root was present, the cut was made after the branching. A five-cm inside diameter (i.d.) PVC cap was then threaded over each root through a 2.2 cm hole drilled in the middle of the cap, until the cap rested near the base of the root where it was attached to the tree. Each root was then placed in a 50 x 25 x 6 cm black plastic tray and the trays were filled with sieved soil from the A horizon of the study plot (Figure 3-1). The PVC cap was placed outside the tray at the point of entry for the root. Black plastic mesh was placed over the tray and covered with pine litter from the plot. The root system was irrigated with nutrient solution every other day to keep the soil moist. The nutrient solution consisted of 110 μM nitrogen (90 μM as ammonium-N and 20 μM as nitrate-N), 20 μM phosphate-P, 77 μM potassium, 65 μM calcium, 10 μM magnesium, 10 μM sulfate-S, 0.24 μM borate-B, 0.20 μM iron,

Figure 3-1. The root regeneration system. Each root system was covered with moistened wiper paper sheets. A PVC cap (A) was threaded over each root system, before entering the root tray (B). (C) Tree trunk

0.02 μM manganese, 0.02 μM zinc, 0.005 μM molybdate-Mo, and 0.005 μM copper. These nutrient concentrations are typical of soil solutions of a Florida Spodosol (Van Rees and Comerford, 1990). Excess drained through holes drilled in the bottom of each tray. Watering was suspended when rain was plentiful. The PVC cap was used as one end of the "nutrient uptake root chamber". The design of the chamber is described in detail in the following section. Because this cap part of the chamber is installed proximal to new root growth, it was convenient to install it before root growth occurred, thus preventing damage to the root system at the time of the nutrient depletion study. This method was applied to twenty trees (80 trays) between July 24 to July 28, 1994. Fourteen of these trees were used for this study.

Nutrient Uptake Root Chamber Design and Construction

Root uptake chambers were constructed from PVC (ASTM-D-2665) with a 5 cm i.d. and walls 4 mm thick (Figure 3-2). Each column consisted of 3 parts: (a) the main column, (b) L-type PVC connector with both ends made to fit 5 cm i.d. PVC pipes, and (c) a 10. cm long PVC pipe to fit into part (b). Root chambers varied in length from 30-40 cm to accommodate various root system sizes. Figure 3-2 is a schematic representation of the nutrient uptake root chamber. Part (a) has a 3-cm diameter hole, sealed with a No 6.5 solid neoprene rubber stopper (1). The hole was used to completely drain the nutrient solution from the chamber. Parts (b) and (c) were

Figure 3-2. Nutrient uptake root chamber. (a, c) PVC pipes; (b) PVC elbow; (d, 2) PVC cap; (e) cross section of the root chamber; (1, 3, 4) rubber stoppers; (5) plastic L connectors; (6, 9) plastic syringe; (7) one way stopcock valve; (8) disposable syringe filter.

used to raise the level of the nutrient solution so the root system could be completely immersed within the root chamber. Part (c) was attached to a 5 cm i.d. PVC cap (2) with a 2.2 cm hole drilled in the middle (3). The hole was used to insert a tube that gassed the solution with either compressed air, or N_2 gas. Parts (a), (b), and (c) were attached with silicone lubricant (Dow Corning). Modeling clay (Rose Arts Industries, Inc., Livingston NJ) was used to externally seal the chamber. By using silicone lubricant and modeling clay I was able to assemble and disassemble the chamber in the field. The root chamber could then be connected to the root system by fitting it to the 5 cm i.d. PVC cap (d) which was previously threaded over each root system (as described in the root regeneration system section). Sealing the root-PVC hole intersection was achieved by using a No. 4 solid neoprene rubber stopper (4). A hole, slightly larger than the lateral root diameter (3-5 cm) was drilled in the center of the rubber stopper, then radially cut, so that the stopper could be inserted around the lateral root. The rubber stopper was used to seal the PVC cap hole. Epoxy putty, a two-part semisolid resin that cures in one hour (Cole Parmer H-08785-10) was used to seal externally the root-stopper junction.

Diagram (e), Figure 3-2, is a cross-sectional representation of the assembled root chamber. It consisted of two polypropylene elbow connectors (i.d. 4 mm) attached to main PVC column (5). They were inserted in the middle of the chamber opposite each other. One of the elbow connectors was

attached to a 10 mL plastic burette (6). The plastic burette was constructed by inserting the barrel of a 10 mL polypropylene syringe into one end of the elbow connector. This burette was used to maintain a constant volume in the root chamber utilizing a Mariotte flask system. The other elbow connector was attached to a one-way stopcock valve, with a male Luer lock (7). The male lock end was attached to a 25-mm cellulose acetate disposable syringe filter (8) connected to a 10-mL disposable polypropylene syringe (9). This system was used to retrieve or add a known amount of solution to the chamber. When needed, connections were sealed with a hot glue gun. A complete list of parts and materials for constructing a nutrient uptake chamber is in Table 3-1.

In-situ Nutrient Uptake System

A view of the *in situ* nutrient uptake system is shown in Figure 3-3. Prior to lab and field experiments the whole system was flushed with NaOCl (5%) and diluted sulfuric acid and then rinsed well with distilled water. The three components of the system were (a) the gas system, (b) the root uptake chamber and, (c) the Mariotte flask system. The system was used as follows. The root chamber was gassed with compressed gas tanks (air, or N₂ gas) (1) and the gas was delivered from the tanks to a controlling board (2). The controlling board had a main flowmeter (3) that, through the use of a needle valve (4), controlled the gas pressure delivered to each tree. Trees were 1-2 m apart, with four

Figure 3-3. *In situ* nutrient uptake system. (a) aeration system; (b) root chamber; (c) Mariotte flask system; (1) gas tank; (2) controlling board; (3) main needle valve; (4) needle valves for each tree; (5) needle valves for each root.

Table 3-1. List of parts and materials for constructing a nutrient uptake chamber.

<u>Material Description</u>	<u>Qty.</u>	<u>Refer to Figure</u>	<u>Suggested supplier (Catalog number/description)</u>
PVC cap (5 cm i.d.)	2	3-2.2 3-2.d	Hardware stores (ASTM-D 2665)
PVC pipe (5 cm i.d.)	2	3-2.a 3-2.c	"
L-PVC connector (5 cm i.d.)	1	3-2.b	"
Rubber stopper No. 6.5, 4)	3	3-2.1 3-2.3 3-2.4	Fisher/Cole-Parmer (Solid Neoprene)
Polypropylene elbow connectors (i.d. 4 mm)	2	3-2.5	Cole-Parmer (H-06285-10)
One-way male stopcock	1	3-2.7	Cole-Parmer (H-06464-71)
Disposable cellulose acetate syringe filter (25 mm)	1	3-2.8	Cole-Parmer (H-02915-62)
Disposable, polypropylene syringe Luer-tip (10 mL)	2	3-2.6 3-2.9	Fisher (14-823-2A)
Epoxy putty		sealant	Cole-Parmer (H-08785-10)
Plastaline		sealant	Handicraft stores (modeling clay)
Hot-glue for guns		sealant	Handicraft stores
Silicone lubricant (Dow corning grease)		grease	Fisher (14-635-5D)

root chambers each. The gas delivered to each root chamber was controlled by another needle valve (5). Aeration to each root chamber was checked by measuring dissolved oxygen in root chambers with a Yellow Springs Instrument (YSI) portable oxygen meter and a Clark type membrane-covered probe (accuracy $0.1 \text{ mg L}^{-1} \text{ O}_2$).

Details of the root uptake chamber and the Mariotte flask system are presented in Figure 3-4. The root uptake chamber was placed in a shallow pit excavated around the lateral roots. A plywood (1) stand for the chamber was inserted into the ground to stabilize each chamber. The whole chamber was covered with a black plastic mesh to avoid direct sunlight.

The Mariotte flask system was built using a 250-mL straight-sided glass digestion tube (2), a rubber stopper (3), nalgene-VI grade tubing (i.d. 0.31 mm) (4), a 1-mL polystyrene disposable pipette (5), and two one-way stopcock valves, with a male Luer lock (6). The glass digestion tube flask system was held by a burette support stand (7). When needed, connections were sealed with a hot glue gun and Teflon tape. As the solution level dropped in the chamber, the solution in the glass tube moved into the chamber due to atmospheric pressure. The glass digestion tube (2) was marked with milimetric paper and calibrated, so that the volume of water used to replace the root chamber solution level was known. The glass tube can be refilled by connecting it to a

Figure 3-4. Mariotte Flask system. (1) plywood stand; (2) glass digestion tube; (3) rubber stopper; (4) Nalgene tubing; (5) polystyrene pipette; (6) one way stopcock valve; (7) burette support stand.

reservoir. A complete list of parts and materials for constructing the Mariotte flask system is in Table 3-2.

Testing of the Nutrient Uptake System

Laboratory studies

All laboratory tests of the nutrient uptake chamber system were performed without roots in the chambers. Four nutrient uptake chambers were assembled in the laboratory, and sealed as described above. A Mariotte flask system was assembled for each chamber, and gassing was provided using compressed air (Medical Grade). Each chamber was filled with 1 L of nutrient solution containing $6.2 \mu\text{M}$ P (ca. 0.20 mg L^{-1}), $25.6 \mu\text{M}$ K (ca. 1.00 mg L^{-1}), $110 \mu\text{M}$ nitrogen ($90 \mu\text{M}$ as ammonium-N and $20 \mu\text{M}$ as nitrate-N), $65 \mu\text{M}$ calcium, $10 \mu\text{M}$ magnesium, $10 \mu\text{M}$ sulfate-S, $0.24 \mu\text{M}$ borate-B, $0.20 \mu\text{M}$ iron, $0.02 \mu\text{M}$ manganese, $0.02 \mu\text{M}$ zinc, $0.005 \mu\text{M}$ molybdate-Mo, and $0.005 \mu\text{M}$ copper. The nutrient solution was adjusted to pH 4.5.

Solution samples (8 mL) were collected from each chamber every 8 hours for 48 hours using the syringe described in Figure 3-2, Label 9. Mariotte flasks were filled with distilled water (pH 4.5), and were connected to each chamber to maintain a constant solution volume. The volume of water replenished by the Mariotte flasks between sampling intervals was recorded. Dissolved oxygen saturation, and temperature were measured for each chamber at each sampling interval with

Table 3-2. List of parts and materials for constructing a Mariotte flask system.

<u>Material Description</u>	<u>Qty.</u>	<u>Refer to Figure</u>	<u>Suggested supplier (Catalog number/description)</u>
Glass digestion tube (250 mL)	1	3-4.2	Fisher (TC1000-0155)
Rubber stopper (No. 4)	1	3-4.3	Fisher (Solid Neoprene)
PVC tubing (Nalgene i.d. 0.31 mm)		3-4.4	Fisher (180-clear VI grade)
Polystyrene pipette (1 mL)	1	3-4.5	Fisher (Disposable)
One-way male stopcock	2	3-4.6	Cole-Parmer (H-06464-71)
Burette stand	1	3-4.7	Fisher
Teflon tape		sealant	Fisher (14-831-300A)
Hot-glue for guns		sealant	Handicraft stores

a Yellow Springs Instrument (YSI) portable oxygen meter, and a Clark type membrane-covered probe (accuracy $0.1 \text{ mg L}^{-1} \text{ O}_2$).

In-situ field studies

Root regeneration system. Nine months after the installation of the root trays, thirty-six intact lateral roots from 9 trees were gently uncovered and washed with distilled water from soil particles. The root regeneration system was evaluated based on the presence or absence of new root growth. Eleven months later, twenty lateral roots from another 5 trees were also uncovered and evaluated as described previously.

Nutrient uptake system installation. Chambers were fitted to seventeen lateral roots on 5 trees as described above. Sealing the root-stopper junction was the most critical part. The junction was checked for leaks before connecting the cap to the root uptake chamber. During this procedure the root system was wrapped in several layers of soggy sheets of delicate task paper wiper (38 x 43 cm 2-ply), and covered with a black plastic mesh to avoid direct sunlight. The chamber was connected to the root as described above. Lastly, the Mariotte flask system was placed in the shallow pit as shown in Figure 3-4. The setting was accomplished by a two-person team. In order to minimize the risk of breaking the lateral root, I left enough length of lateral root between the tree trunk and the root-stopper-cap junction to allow easy flexible movement of the root. This

length facilitated the movement needed when assembling the chamber system. After installation, the portion of root external to the chamber was covered with soil.

Pre-depletion treatment. The chamber was filled with the nutrient solution described in the laboratory study section, but lacking P and K. Roots in the chambers were continuously gassed with compressed air for 24 hours. During that time the system was monitored for leaks. Modeling clay was used to seal leaks. The aeration and Mariotte flask systems were also monitored for proper performance. Each chamber was covered with a black plastic mesh to avoid direct sunlight. This prevented the softening of the modeling clay by sunlight and kept the nutrient solution temperature uniform. The chamber was drained before the nutrient depletion study and then flushed with distilled water.

Nutrient depletion study with air. The root chambers containing roots were filled with known amounts of nutrient solution (800 to 1200 mL). Initial P and K concentrations for the study were $6.25 \mu\text{M P}$ (ca. 0.20 mg L^{-1}) and $25.66 \mu\text{M K}$ (ca. 1.00 mg L^{-1}), plus other nutrients as described in the previous section. The nutrient solution was adjusted to pH 4.5. Solution samples (8 mL) were collected every 6 hours for a period of 72 hours. An equal volume of distilled water (8 mL (pH 4.5)) was replaced. Water uptake was measured by calculating the volume of water replenished by the Mariotte flasks between sampling intervals. The system was continuously aerated with pressurized air. Dissolved oxygen

saturation and temperature were measured for each root chamber at each sampling interval.

Nutrient depletion study with N_2 gas. After completion of the previous experiment, all root chambers were drained and flushed with distilled water. They were then subjected to the pre-depletion treatment for 24 hours. Afterwards, the root chambers were filled with known amounts of nutrient solution (c.a. 800-1200 mL). Nutrient concentrations for the study were the same as for the air study. The nutrient solution was purged with N_2 gas for 2 hours before the depletion study experiment. Solution samples (8 mL) were collected from each chamber every 8 hours for 48 hours. An equal volume of distilled water (8 mL pH 4.5) was added back after taking each sample. Root water uptake was measured by calculating the volume of water replenished by the Mariotte flasks between sampling intervals. The system was continuously gassed with N_2 gas (ultra-high purity grade) to decrease the oxygen solution concentration (Steward et al., 1936). Dissolved oxygen saturation, and temperature were measured for each root chamber at each sampling interval.

Chemical analyses

Nutrient solution samples were kept on ice until the depletion study was completed. They were analyzed within 24 hours of the completion of each depletion study. Concentration of P was determined by the method of Murphy and

Riley (1962). Potassium concentration was determined by atomic absorption spectroscopy.

Data Analyses

Laboratory studies

Statistical comparisons of P and K concentrations, volume of water replenished by the Mariotte flask system and oxygen saturation were made using a repeated measures analysis (SAS Institute, 1985). The chambers (without roots) were the measurement units and the repeated measures were the time intervals.

Field studies

Periodic P and K uptake were calculated by expressing the P and K concentration at each sampling interval as a fraction of the initial nutrient concentration. Corrections were made for the dilution caused by sample withdrawal. Statistical comparisons for P and K concentrations, oxygen saturation, and nutrient solution temperature were made using a repeated measures analysis (SAS Institute, 1985). Treatments were the aeration environments caused by air or N₂ gas. The blocks were trees while the replicates were the roots from each tree. The measurement unit was the chamber (with root) and the repeated measures were the time intervals.

Results

Laboratory Studies (No Roots Present In Chambers)

Phosphorus and K solution concentrations in the chamber were not significantly different at sampling intervals, nor among chambers. Average solution concentrations of the nutrient solution were $6.09 \mu\text{M}$ (c.a. 0.195 mg L^{-1}) for P ($\text{SE}=1.16 \times 10^{-3}$), and $26.28 \mu\text{M}$ (c.a. 1.024 mg L^{-1}) for K ($\text{SE}=7.49 \times 10^{-2}$).

The Mariotte flask system accurately replenished the water removed by sampling. The average volume of water replenished by the Mariotte flask system was 8.4 mL ($\text{SE}=0.15 \text{ mL}$). The volume of solution withdrawn from each chamber was 56 mL (7 samples $\times$ 8 mL). The average volume of solution replenished by the Mariotte flask system was 58.8 mL (7 $\times$ 8.4 mL). This represents a 5% error per volume sampled. The dilution of the solution concentration in the chamber was 5.8%.

Oxygen saturation in the chambers was not significantly different at sampling intervals, nor among chambers. Nutrient solution temperature was 25°C and the average oxygen saturation was 98.3%.

Field Studies

Root regeneration system

A total of 56 lateral roots from 14 trees were uncovered after 9 and 20 months from the initial installation of root trays. From this total, thirty-two (57%) lateral roots had new white roots. Sixteen (29%) more lateral roots had new fine roots that were brown in color. Two lateral root systems did not have fine roots but the lateral was still alive (4%). Six lateral roots (10%) were dead, apparently broken during the initial excavation. Therefore, 86% of the fifty-six lateral roots that were evaluated had new growth.

Nutrient depletion with air

Phosphorus and K solution concentrations (expressed as a fraction of the initial) were significantly different at sampling intervals ($p < 0.001$), but were not significantly different among root chambers. The percentage of nutrient depleted was different for P than for K. It was as high as 92% for P while for K only 25% of the initial K concentration was depleted. (Figure 3-5).

Nutrient depletion with N_2 gas

Potassium depletion was effectively inhibited by N_2 gas. The chamber K concentrations for the sampling intervals were not significantly different (Figure 3-6). In contrast, the

Figure 3-5. Phosphorus and Potassium depletion by seventeen root systems of *Pinus elliotii* var. *elliotii*. Each symbol represent an average with standard error of seventeen root chambers. Values are expressed as fraction of initial phosphorus (●) and potassium (■) concentration in the nutrient uptake chamber. The roots were gassed with air during the experiment.

Figure 3-6. Phosphorus and Potassium depletion by seventeen root systems of *Pinus elliotii* var. *elliottii*. Each symbol represent an average with standard error of seventeen root chambers. Values are expressed as fraction of initial phosphorus (●) and potassium (■) concentration in the nutrient uptake chamber. The roots were gassed with N₂ gas during the experiment.

initial P concentrations in the roots chambers gassed with N_2 were depleted as much as 92% (Figure 3-6), and were depleted in a similar trend as when roots were gassed with air (Figure 3-5).

Oxygen saturation with air and N_2 gas

The oxygen saturation and the nutrient solution temperature in the root chambers during the depletion experiment with air and N_2 gas are presented in Figure 3-7. For either air or N_2 gas treatment the oxygen saturation values were not significantly different at sampling intervals, nor among root chambers (Figure 3-7b and 3-7c). However the N_2 gas treatment decreased the oxygen saturation significantly as compared with the air gas treatment ($p < 0.001$). The average oxygen saturation was effectively reduced from 96.3% (SE 1.69 %) to 4.2 % (SE= 2.7 %) when the gassing was changed from air to N_2 gas. The average nutrient solution temperature when roots were gassed with air was 25.9 °C. (SE= 5.67 °C), and was not different from nutrient solution temperature when roots were gassed with N_2 gas (average=23.9°C, SE= 5.7 °C). Diurnal air temperature fluctuations influenced the nutrient solution temperature. They were as low as 18 °C at the 6:00 a.m. samplings and as high as 35 °C at the 12:00 a.m. (Figure 3-7a).

Figure 3-7. Nutrient solution temperature (A) and Oxygen saturation (B and C), in root chambers with lateral root systems of *Pinus elliottii* var. *elliottii*. The roots were gassed with air (■) and N₂ gas (□). Each symbol represent an average with standard error of seventeen root chambers

Discussion

I have described a method of measuring ion depletion by intact roots of mature trees in the field by (i) detailing a root regeneration system that allows access and minimizes disturbance of roots, and by (ii) designing and testing a chamber that effectively encloses the root system under controlled aeration conditions.

In order to implement the root regeneration system described here, one must take into account the ability of a root system to regenerate after damage. In this study I had an 86% success rate in acquiring new root growth. For *Pinus*, lateral roots often extend great distances and are among the least branched of the important forest species but have abundant short roots. (Pritchett, 1979).

The laboratory study showed that the chambers were not a source of P or K and that biotic or abiotic immobilization of P or K was occurring. All components of the nutrient uptake system that were in direct contact with the nutrient solution were made from inert material such as PVC, nalgene tubing, polyethylene, rubber, and glass. Tygon tubing has been reported to contain a plastilizer which supports microbial growth and interferes with ion analysis (Bloom, 1989). Microorganisms such as *Pseudomonas* sp. have been shown to colonize the interior surface of PVC water distribution pipes (Vess et. al, 1993). However, since there was no change in

the P and K solution concentrations over time, I infer that neither microbial growth, ion sorption to the chamber nor ion release from the chamber occurred in our system. In previous studies, microbial growth on surfaces has been reported after 3-7 weeks. Our experiments lasted just five days.

This nutrient uptake system has many advantages for repetitive measurements of ion depletion. The root system in each chamber can be subjected to different treatments, allowing observations of the response of the same root system to different conditions. For example, in my study, I illustrated the effect of different gases (air and N_2) on P and K depletion for the same root system. N_2 has been commonly used to deoxygenate solutions resulting in low O_2 treatment. Dissolved oxygen concentrations of $<0.5 \text{ mg L}^{-1}$ ($<50 \text{ }\mu\text{M}$) could result in anoxia/hypoxia of root tissue, known as hypoxic conditions. Nitrogen gas has been used to demonstrate inhibition of active ion uptake (Hopkins, 1956; Fisher and Stone, 1990a). Its mode of action is to decrease the oxygen solution concentration to hypoxic conditions where active ion uptake is inhibited or stopped (Steward et al., 1936). I achieved hypoxic conditions in our N_2 gas treatment study (4.2 % oxygen saturation, c.a. $=0.35 \text{ mg L}^{-1}$). The net efflux of K from roots in the N_2 gas treatment has been previously reported (Rosen and Carlson, 1984). Similarly, P depletion by roots in the N_2 gas treatment has been also previously reported (Topa and Cheeseman, 1993). The difference in K and P depletion by roots under hypoxic conditions seems to be

associated with the ion uptake mechanism. K^+ as an unmetabolised cation is transported across the plasma membrane into the root symplast by a transport mechanism different from that of P. For instance K^+ ions are transported actively mainly through the symplast (Lauchli et al., 1973). Although P-phosphate ions are also transported through the symplast (Clarkson and Sanderson, 1969), starving roots first take up P-phosphate by diffusion into the apoplast. After the saturation of the apoplast and when the steady-state of the external solution-tissue system is exceeded, the transport proceeds actively (symplastic) (Ulrich-Eberius et al., 1981).

Estimation of ion uptake rate by depletion experiments has been criticized due to transient conditions. During the depletion period, the total composition of the nutrient solution may vary significantly, inducing an ionic shock (Bloom, 1989). Moreover as the ion solution concentration decreases, the internal nutrient status of the roots may shift, resulting in immediate and long term effects (Clarkson and Hanson, 1980). Roots with a low nutrient status generally have a higher capacity and a lower affinity for the limited nutrient (i.e., higher V_{max} and lower K_m) (Drew et al., 1984). Therefore, under changing nutrient levels, the response of the plant is in transition. Measurements conducted under steady-state conditions avoid some of the uncertainties associated with transient conditions. Steady-state systems require a constant nutrient flow system and require

monitoring the ion concentration between the solution entering and leaving the root-chamber. Goutouly and Habib, (1996) recently described a steady-state nutrient uptake system in a peach orchard. However, cost and technical requirements can constrain the use of the same system in forests. Instead of our Mariotte flask system they used an electric detector gauge to detect changes in the nutrient solution level (Honeywell "LL 102007", precision ± 1 mm). This device was connected to a pressure balance to measure root water uptake. Instead of sampling manually from the nutrient solution, they used an automatic fraction collector. The complexity of their system only allowed measurement of nitrate uptake rates from one tree at a time. Peach trees were 2 m in height, with 3-4 main branches. The whole root system was split and immersed in a 30 L plastic containers filled with nutrient solution. Only one tree could be transported to the nutrient uptake system described above. Therefore, another advantage of the system I describe is its relative simplicity and low cost. Forest are usually devoid of electrical outlets and water faucets commonly found in managed orchards.

Despite the criticism of depletion studies to measure root nutrient uptake, measurements in the transient state are still useful. In practice, the issue of whether steady-state versus transient measurement conditions should be used in nutrient uptake by roots depends upon the system and the time scale involved in the experiment.

Fruit trees under managed orchards are subjected to seasonal fertilizer applications. The focus is to match nutrient supply from fertilizers with nutrient demand by the trees. Therefore, root systems from fruit trees are more likely to be exposed to a more constant supply of nutrients from the soil solution. Hence steady-state versus transient measurement conditions could be more useful in nutrient uptake studies. In comparison, management of pine plantations is much less intensive with few, if any, fertilizer applications. Therefore, transient measurement conditions may represent soil solution conditions in the rhizosphere and the depletion method described here could be more representative of pine trees in the field.

In practice, my nutrient uptake system can control aeration within the chamber and results are reproducible. By using this methodology, it should be possible to explore poorly documented aspects of nutrient uptake under field conditions, such as the nature of the uptake mechanism of woody fine roots.

CHAPTER 4
PHOSPHORUS AND POTASSIUM DEPLETION BY ROOTS OF FIELD-GROWN
SLASH PINE: AEROBIC AND HYPOXIC CONDITIONS

Introduction

Slash pine (*Pinus elliottii* Engelm. var. *elliotti*) and loblolly pine (*Pinus taeda* L.) are planted extensively on flatwood soils and coastal savannas of the Southeastern United States. Forested Spodosols of the Southeastern lower Coastal Plain typically have perched, fluctuating water tables. At any single location the saturated zone can range from near the soil surface to depths > 150 cm during a single year, and often during a single growing season (Phillips et al., 1989). Pine roots growing in those soils encounter shallow water tables which can rise close to the soil surface on flatwood soils (Comerford et al., 1996). The main problem for a tree associated with waterlogging is root anoxia. Anoxia is defined as the absence of O₂ in plant tissue, while hypoxia is defined as low but not zero oxygen in plant tissue (Drew, 1988). Waterlogging in soils results in reduced soil conditions which causes severe root tissue hypoxia. However, some pine trees cope with waterlogged or reduced soil conditions by strategies of avoidance. In these conifers, anatomical root modifications promote the internal transport

of O_2 to roots (Armstrong and Read, 1972; Philipson and Coutts, 1978). An internal gas exchange mechanism between the atmosphere and oxygen depleted tissues maintains the aerobic functions of flooded root systems of tree species (Carpenter and Mitchell, 1980). Roots of seedlings of pond pine (*Pinus serotina* Michx.) grown under O_2 -deficient conditions were well ventilated internally as a result of extensive air space formation from stem lenticels to root tips (Topa and McLeod, 1986a). Roots of loblolly pine seedlings grown under flooded conditions developed aerenchyma tissue, with large intercellular spaces present in the phellogen of woody roots. Also flooded stems exhibited lenticel hypertrophy (McKevlin et al., 1987). Fisher and Stone (1990a), demonstrated existence of a large volume of interconnected air filled pore space in the secondary xylem of slash pine taproots and associated sinker roots.

Low-oxygen concentration in the root environment strongly inhibits ion uptake. As soil oxygen tension decreases, the uptake of ions falls, particularly under hypoxic conditions (Hopkins et al., 1950; Hopkins, 1956; Drew, 1988). The term hypoxic is used to describe a low O_2 treatment that could result in hypoxia of root tissue. The link between O_2 supply and ion transport is principally through respiration and the generation of ATP to drive transport (Drew, 1988). Anaerobic metabolism does not maintain energy metabolism at a level that will drive primary active transport, presumably by the H^+ -translocating ATPase in

the plasma membrane. Nitrogen gas (N_2) has been commonly used to deoxygenate solutions resulting in dissolved oxygen concentrations of $<50 \mu M$ ($<1.6 \text{ mg L}^{-1}$) to demonstrate inhibition of active ion uptake for phosphorus (P) (Hopkins, 1956; Topa and McLeod, 1986b) and potassium (K) (Fisher and Stone, 1990b; Jalil and Carlson, 1993). Its mode of action is to decrease or deplete the oxygen solution concentration to levels where active ion uptake is inhibited or stopped (Steward et al., 1936). Maintenance of ion uptake by roots of trees subjected to N_2 gas treatments depends on the ability of the tree to maintain an internal gas-exchange mechanism between the atmosphere and oxygen-depleted tissue. Therefore it depends on the duration of root acclimation to hypoxic or anaerobic conditions. It also depends on the ion studied and initial concentration of the ion.

For K uptake studies, plum roots grown in aerated solutions with $100 \mu M$ K, leaked K into bathing solutions within 1 hour of changing the solution sparging gas from air to N_2 (Rosen and Carlson, 1984). After 18 h in the deoxygenated solution (short-term hypoxic conditions), the same root then resumed net uptake of K immediately after air sparging resumed. For slash pine roots grown under long-term hypoxic conditions (5 months), K did not leak into bathing solutions containing $50 \mu M$ K, when changing sparging gas from air to N_2 (Fisher and Stone, 1990b). However net efflux of K occurred when N_2 gas replaced air in enclosures surrounding the lower stem and basal roots. This effect was reversible

and K uptake resumed soon after enclosures were removed or when N_2 gas was temporarily interrupted.

For P uptake studies, short-term hypoxia (1-2 days) reduced ^{32}P accumulation by 50% in roots of pond pine seedlings (*Pinus serotina*) grown under aerobic conditions. In comparison ^{32}P influx in roots of pond pine seedlings grown under long term hypoxic conditions (5.3 weeks) and subjected to short term hypoxia was 4 times higher than ^{32}P influx of aerobically grown seedlings (Topa and McLeod, 1986b). In both cases the initial P concentration was 100 μM . This has similarly been reported for some herbaceous species (John et al., 1974). Furthermore earlier studies by Hopkins (1956), and Larkum and Loughman (1969) suggested that P uptake sensitivity to hypoxic conditions is related to the level of P in the solution. Root P uptake of barley seedlings subjected to short-term hypoxic conditions was reduced 45% from the aerobic treatment at external P concentration of 100 μM . However, no inhibition of P uptake was observed under short-term hypoxic conditions at external P concentration of 10 μM . Thus, the anaerobic mechanism for P uptake by roots becomes more efficient with increasing dilution of external P, which indicates that the carrier level for P uptake at concentrations below 10 μM can be maintained solely by energy from anaerobic metabolism. P concentrations below 10 μM , are commonly encountered in soil solutions. In contrast, the sensitivity for K uptake to hypoxic conditions as related to levels of K in the solution, has not been rigorously tested.

Most studies relating K uptake by roots under hypoxic conditions range between 60-100 μM K in the nutrient solution; these are K concentrations commonly encountered in soil-solutions. In all cases K uptake was inhibited when roots were subjected to hypoxic conditions. The difference in K and P uptake by roots under either aerobic or hypoxic conditions seems to be associated with the ion uptake mechanism. Ion transport across the plasmalemma of epidermal and cortical cells of plant roots is an energy-dependent process.

For K, at low external K concentrations ($< 100 \mu\text{M}$) the high affinity K uptake system operates against the prevailing electrochemical potential differences (e.g., 10 μM K^+ outside, 80 μM K activity in the cytoplasm) thus an energized K transport is required (Maathuis and Sanders, 1993). Although an early report advanced the notion of a close coupling between K uptake and H^+ extrusion in plant roots (Pitman et al., 1975), further studies by Newman et al., (1987), and Kochian et al., (1989) have suggested that net H^+ efflux is an electrically compensatory response to K influx. At present the mechanism of energization of K transport can only be speculated. Mechanisms suggested are carrier-mediated K proton-antiport (counter-transport) or, more likely as 1:1 K proton-symport, or cotransport (Kochian et al. 1989; Maathuis and Sanders, 1993). A possible mechanism is a K influx directly powered by ATP (adenosine triphosphate) with the involvement of an ATPase (Kochian et al., 1989).

For P, more than for K, root uptake has to be a metabolically driven process. The soil solution immediately outside the root cells is likely to contain $1\ \mu\text{M}$ P-phosphate or less, while in the cytoplasm the concentration will be 10^3 to 10^4 times greater (Clarkson and Grignon, 1991). Therefore a steep gradient must be overcome to transport P-phosphate into the interior of a typical cell. A minimum of 25-40 kJ of energy is required to transport 1 mole of phosphate which is roughly equivalent to the maximum free energy released by the hydrolysis of 1 mole of ATP ($50\text{--}60\ \text{kJ Mol}^{-1}$) (Clarkson and Grignon, 1991). Active phosphate influx into the roots does not appear to be directly related to the H^+ efflux pump discussed for K transport, but rather to an electrogenic phosphate pump across the plasma membrane. It operates using steep electrical (potential differences) and chemical (pH difference) gradients for protons as the driving force (Dunlop, 1989). For either cation (K^+) or anion transport (phosphate) the plasma membrane H^+ -ATPase plays a key role in both the cytoplasmatic pH and the driving force for cation and anion uptake (Serrano, 1990).

From the point of view of mineral nutrition of trees under field conditions, it is not the ion transport mechanism that is important, but the way in which the transport mechanism is controlled by the tree. Clarkson et al., (1978) stated that the nutrient-uptake effectiveness of a root system depends on the differential response of the absorbing surfaces to environmental variables. Under hypoxic conditions

the material referenced above showed that while P uptake by roots can adjust within relatively wide limits of aeration, K intake is more sensitive to hypoxic conditions. If we consider that the fine root system of pine trees growing in the surface 20-40 cm of a flatwood soil encounter short-term reduced soil conditions then we must document studies of P and K uptake by roots of pines trees under hypoxic field conditions. It is therefore reasonable to ask whether P and K uptake occurs under short-term reduced soil conditions, and if so, to what degree. Yet most studies of ion uptake under hypoxic or low O_2 treatment have been carried out on root systems of trees under greenhouse conditions. Moreover, with the exception of a few studies with roots of cereals, there are few reports simultaneously comparing the behavior of uptake of more than one ion (Hopkins et al., 1950; Larkum and Loughman, 1969). Until recently, there were no methods to study ion uptake by root systems of trees under field conditions (Goutouly and Habib, 1996). Therefore, the purpose of our study was to investigate the ability and resilience of P and K absorption by roots of 12-year-old slash pine under changing soil aeration. Our objectives were (i) to compare P and K uptake by lateral roots of slash pine roots subjected to short term hypoxic treatment, and (ii) to document the response of K and P uptake by lateral roots of slash pine following the removal of the hypoxic treatment.

Materials and Methods

Site Description

Twelve-year-old slash pine trees (*Pinus elliotii* Engelm. var. *elliotii*) were selected from a research plot in north Florida that received complete weed control. A description of the site is given in Swindel et al. (1988) and Neary et al. (1990). Weed control was accomplished using a combination of herbicide and mechanical measures. The soil is a poorly-drained Pomona fine sand (sandy, siliceous, hyperthermic Ultic Alaquod). The study site is about 10 km north of Gainesville, FL (29° 80' N, 82° 20' W). The climate is warm, temperate-subtropical with a mean annual precipitation of 1332 mm. Most of the precipitation occurs in summer, with the least falling in autumn and spring. Mean annual temperature is 21 °C.

Root Regeneration System

Four lateral roots (1-1.5 cm in diameter) were excavated at the base of a tree, preserving the fine root system as much as possible and a root regeneration system was established as described in Chapter 3. Briefly, each lateral root was pruned, then a five-cm inside diameter (i.d.) PVC cap was threaded over each root through a 2.2 cm hole drilled in the middle of the cap until the cap rested near the base of the root where it was attached to the tree. Roots were

then placed in 50 x 25 x 6 cm black plastic trays and filled with sieved soil from the A horizon of the study plot. Black plastic mesh was placed over the tray and covered with pine litter from the plot. The root system was supplied with a nutrient solution typical of soil solutions of a Florida Spodosol (Van Rees and Comerford, 1990). This method was applied to twenty trees (80 trays) between July 24 to July 28, 1994. Twenty months after the installation of the root trays twenty intact lateral roots from five trees were gently uncovered and washed with distilled water from soil particles. The root regeneration system was evaluated based on the presence or absence of new root growth. Seventeen lateral roots from these five trees were used for this study. These root systems were grown in soil under aerobic conditions.

Field Nutrient Uptake System Installation

The seventeen intact lateral roots from 5 trees mentioned above were inserted (while still attached to the tree) into nutrient-uptake root chambers. The construction and assembly of the nutrient uptake root chamber is fully described in Chapter 3. The root chamber is part of an *in situ* nutrient uptake system which includes (a) the gas system, (b) the root uptake chamber and, (c) the Mariotte flask system. In short, the root chamber was gassed using compressed gas tanks with either air (medical standard) or N_2 gas (99.9%). The Mariotte flask system was used to maintain a

constant volume in the root chamber. The root uptake chamber was placed in a plywood stand for mechanical support. . Together with the Mariotte flask system, they were placed in a shallow pit. Aeration to each root chamber was checked by measuring dissolved oxygen in root chambers with a Yellow Springs Instrument (YSI) portable oxygen meter and a Clark type membranecovered probe (accuracy $0.1 \text{ mg L}^{-1} \text{ O}_2$).

Depletion Studies

Pre-depletion treatment

The chamber was filled with a nutrient solution containing $110 \text{ } \mu\text{M}$ nitrogen ($90 \text{ } \mu\text{M}$ as ammonium-N and $20 \text{ } \mu\text{M}$ as nitrate-N), $65 \text{ } \mu\text{M}$ calcium, $10 \text{ } \mu\text{M}$ magnesium, $10 \text{ } \mu\text{M}$ sulfate-S, $0.24 \text{ } \mu\text{M}$ borate-B, $0.20 \text{ } \mu\text{M}$ iron, $0.02 \text{ } \mu\text{M}$ manganese, $0.02 \text{ } \mu\text{M}$ zinc, $0.005 \text{ } \mu\text{M}$ molybdate-Mo, $0.005 \text{ } \mu\text{M}$ copper but lacking P and K. The nutrient solution was adjusted to pH 4.5. The roots were continuously gassed with compressed air and kept in this system for 24 hours. The purpose of the pre-depletion treatment was to starve the root system from P and K and to allow roots to recover from any physical manipulation between treatments. The chamber was covered with a black plastic mesh to avoid direct sunlight. The chamber was drained previous to the nutrient depletion study and flushed with distilled water.

Experiment 1: Nutrient depletion study with air

The root chambers were filled with known amounts of nutrient solution (c.a. 800-1200 mL). Initial P and K concentrations for the study were $6.25 \mu\text{M P}$ (ca. 0.20 mg L^{-1}) and $25.66 \mu\text{M K}$ (ca. 1.00 ug mL^{-1}), plus other nutrients as described above. The nutrient solution was adjusted to pH 4.5. Solution samples (8 mL) were collected at 6-hr interval up to 72 hours. An equal volume of distilled water at pH 4.5 (8 mL) was replaced. Water uptake was measured by calculating the volume of water replenished by the Mariotte flasks between sampling intervals. The system was continuously aerated with pressurized air. Dissolved oxygen saturation, and temperature were measured for each root chamber at each sampling interval.

Experiment 2: Nutrient depletion study with N_2 gas

After completion of the previous experiment, all root chambers were drained and flushed with distilled water. They were then subjected to the pre-depletion treatment for 24 hours. Afterwards, the root chambers were filled with equal amounts of nutrient solution (c.a. 800-1200 mL). Initial P and K concentrations for the study were the same as previously described. The nutrient solution was adjusted to pH 4.5. Water (8 mL, pH 4.5) was added to the chamber following the removal of each sample. Root water uptake was measured by calculating the volume of water replenished by

the Mariotte flasks between sampling intervals. The system was continuously gassed with N_2 gas (ultra-high purity grade). The nutrient solution was previously purged with N_2 gas for 2 hours before the depletion study experiment. Dissolved oxygen saturation, and temperature were measured for each root chamber at each sampling interval.

Experiment 3: Nutrient depletion study with air after N_2 gas

After completion of the N_2 gas treatment, all root chambers were drained and flushed with distilled water. They were then subjected to the pre-depletion treatment for 24 hours. Afterwards, the root chambers were filled with nutrient solution containing the same amount of P and K concentrations as in previous experiments. The system was continuously aerated with pressurized air. Solution sampling, and water uptake measurements were done similarly as in Experiment 1. Oxygen saturation and temperature measurements were also measured for each root chamber in a similar fashion as described in Experiment 1 and 2.

Experiments 4, 5 and 6: Nutrient depletion study with air/ N_2 gas/ air

After completion of the air experiment, all root chambers were drained and flushed with distilled water. Then, six of the seventeen root chambers were then subjected to the pre-depletion treatment for 24 hours. Experiments 4, 5, and 6 are repeats of experiment 1, 2, and 3, respectively, with these six roots.

Chemical Analyses

Nutrient solution samples were kept on ice until each experiment was completed. They were analyzed within 24 hours of the experiment's completion. Concentration of P was determined by the method of Murphy and Riley (1962). Potassium concentration was determined by atomic absorption spectroscopy.

Data Analyses

Periodic P and K uptake were calculated by expressing the nutrient content at each sampling interval as a fraction of the initial nutrient concentration. Corrections were made for the dilution caused by sample withdrawal. Statistical comparisons for P, K, O_2 saturation, and nutrient solution temperature were made using a repeated measures analysis (SAS Institute, 1985). Treatments were the aeration environments caused by air or N_2 gas. The blocks were trees while the replicates were the roots from each tree. The measurement unit was the root chamber and the repeated measures were the time intervals.

Results

Oxygen Concentration and Temperature in the Chambers

For both the air and N₂ gas treatments the oxygen-saturation values were not significantly different at sampling intervals, nor among root chambers. Therefore mean values of oxygen saturation (%) for each experiment, were converted to mg L⁻¹ and μM , based on the average nutrient solution temperature. They are presented in Table 4-1.

The average temperature of solutions gassed with air was not different from the temperature of those gassed with N₂. Diurnal temperature fluctuations outside of the chamber influenced the nutrient solution temperature. They were as low as 18 °C at 6:00 a.m. samplings and as high as 35 °C at 12:00 a.m. (See Figure 3-7A, Chapter 3). Oxygen concentrations in root chambers gassed with N₂ (Experiments 2 and 5) indicated hypoxic conditions ($\text{O}_2 < 50 \mu\text{M}$), and were statistically lower than the air treatment ($p < 0.001$). For root chambers gassed with air (Experiments 1,3,4 and 6), all were aerobic ($\text{O}_2 > 50 \mu\text{M}$); however, during Experiment 4, the oxygen concentrations were slightly lower than oxygen concentrations during experiments 1 and 3 ($p < 0.001$), (Table 4-1). Although it could be argued that Experiment 4 had different levels of oxygen than experiments 1,3 and 6, the oxygen concentration is within the aerobic range, and both K

Table 4-1. Temperature and dissolved oxygen concentration in nutrient solution, during consecutive experiments of P and K depletion by pine roots.

<u>Experiment</u>	<u>Treatment</u>	<u>n</u>	<u>Temperature</u> °C	<u>Dissolved Oxygen</u> mg L ⁻¹	<u>Oxygen</u> uM
1. Air 1	Aerobic	17	26.0 (5.6)a	7.82 (0.14)a	244 (4.3)a
2. N ₂ gas 1	Hypoxic	17	24.0 (5.8)a	0.35 (0.23)b	11 (7.3)b
3. Air 2	Aerobic	17	25.5 (5.3)a	7.76 (0.17)a	242 (5.2)a
4. Air 3	Aerobic	6	29.7 (5.4)a	6.69 (0.38)c	209 (11.9)c
5. N ₂ gas 2	Hypoxic	6	24.2 (4.7)a	0.45 (0.16)b	14 (5.0)b
6. Air 4	Aerobic	6	25.2 (5.3)a	7.73 (0.19)a	241 (5.8)a

Temperature and dissolved Oxygen values followed by a different letter are significantly different between experiments ($P < 0.001$). A experiment were the dissolved oxygen in root chambers was < 50 uM was considered hypoxic. Values of temperature and dissolved oxygen are means, where numbers in parentheses are standard deviations with n replicates.

depletion and P depletion were similar to the first aerobic treatment.

Potassium Depletion with Alternating Aerobic and Hypoxic Conditions

Potassium concentration changed little after 66 hours under aerobic conditions in Experiment 1. However, when the same roots were gassed with N_2 (Experiment 2), K depletion ceased (Figure 4-1). This was true both times the N_2 gas was used (Figure 4-2). The initial K concentrations in chambers gassed with N_2 gas were not significantly different across the sampling intervals. This effect was reversible and K uptake resumed after the pine roots were removed from N_2 gas and air was re-introduced (Experiment 3- Air 2). Yet the K depletion of the pine roots was not as strong as the first experiment (Air 1 vs. Air 2, Figure 4-1; $p < 0.01$). While K was depleted to 35% of the initial K concentration in Experiment 1 (Air 1), K was only depleted about 15% after air was re-introduced. After experiment 3 (Air 2, after N_2 gas), root chambers were gassed with air again (Air 3, experiment 4), and K depletion resumed a similar depletion trend to the first air depletion experiment. The average K depletion in aerated solution after a second exposure to N_2 gas ion (Air 4) was only 10% (Figure 4-3). This represents about 70% reduction in K uptake resulting from residual effects of N_2 gas exposure.

Potassium Depletion Under Hypoxic Conditions

Potassium depletion ceased in the presence of N_2 gas in both experiments where hypoxic conditions were achieved (Figure 4-2). On both occasions, there was a K efflux from the pine roots and the K efflux was not significantly different between the two experiments.

Phosphorus Depletion with Alternating Aerobic and Hypoxic Conditions

Phosphorus concentration changed little after 42 hours (data not shown) when pine roots were gassed with air or N_2 gas (Figure 4-4 and 4-5). P depletion in the presence of N_2 gas (Experiment 2) was not significantly different from that in the presence of air. Initial P concentrations were depleted about 90% when root chambers were gassed with either air (Experiment 1 and 3) or N_2 (Experiment 2). Phosphorus depletion of pine roots under aerobic conditions was not different between four experiments (Experiments 1,3,4, and 6), even after the effect of intervening N_2 gas treatment (Experiment 3 and 6) (Figure 4-5).

Phosphorus Depletion Under Hypoxic Conditions

While P depletion in a N_2 environment was not curtailed in two separate experiments, the amount of P depleted and the trend of depletion was significantly different ($p < 0.001$)

Figure 4-1. Potassium depletion by seventeen root systems of *Pinus elliotii* var. *elliotii*. under aerobic and hypoxic conditions. The root chambers were gassed with air (■), followed by N₂ gas (●) and then with air again (□). Refer to experiments 1, 2 and 3 in the text. Each symbol represent an average with standard error. Values are expressed as fraction of initial concentration in the nutrient uptake chamber.

Figure 4-2. Potassium depletion by root systems of *Pinus elliotii* var. *elliotii*. under hypoxic conditions. Experiment 2; N₂ gas 1 (●) is the result from seventeen root chambers. Experiment 5; N₂ gas 2 (○) is the result from six root chambers. In both cases the root chambers were gassed with air before and after the N₂ gas treatment. Each symbol represent an average with standard error bars. Values are expressed as fraction of initial concentration in the nutrient uptake chamber.

Figure 4-3. Potassium depletion by seventeen root systems of *Pinus elliottii* var. *elliottii*. under series of aerobic conditions. The root chambers were gassed with air (■) (Experiment 1; air 1), followed by N_2 gas (data not shown). Then root chambers were gassed with air again (□) (Experiment 3; Air 2). After Experiment 3, six root chambers were gassed with air (●) (Experiment 4; Air 3), followed by N_2 gas (data not shown). Then root chambers were gassed with air again (○) (Experiment 6; Air 4). Each symbol represent an average. Standard error bars are shown for Exp. 1 and Experiment 3 only. Values are expressed as fraction of initial concentration in the nutrient uptake chamber.

(Figure 4-6). Only 65% of the initial P concentration was depleted by pine roots subjected to hypoxic conditions for the second time (Experiment 5). In comparison 90% of the initial P concentration was depleted by the same pine roots when subjected to hypoxic conditions for the first time (Experiment 2). Therefore P depletion decreased about 28% when the same roots were exposed to hypoxic conditions for the second time (Experiment 5).

Discussion

Sudden O₂ deprivation (hypoxic conditions) resulted in net efflux of K from roots of trees within 6 hours. This response has been previously reported by others (Fisher and Stone, 1990b; Rosen and Carlson, 1984; Jalil and Carlson, 1993). Active membrane transport maintains high K concentrations inside the cells against electrochemical gradients that would otherwise cause K diffusion out of the roots (Cheeseman and Hanson, 1979). With hypoxia, active transport ceases and K diffuses from the concentrated internal cell solutions into the considerably more dilute bathing solution. After 72 hours in the deoxygenated solution, the same roots then resumed depletion of K after air sparging resumed. This response has also been observed in seedlings of slash pine (Fisher and Stone, 1990b) and seedlings of plum (Jalil and Carlson, 1993). It is attributable to changes in cell energy levels with are

Figure 4-4. Phosphorus depletion by root systems of *Pinus elliottii* var. *elliottii*. under aerobic and hypoxic conditions. Experiment 2; N₂ gas 1 (●) is the result from seventeen root chambers. Experiment 5; N₂ gas 2 (○) is the result from six root chambers. In both cases the root chambers were gassed with air before and after the N₂ gas treatment. Each symbol represent an average with standard error bars. Values are expressed as fraction of initial concentration in the nutrient uptake chamber.

Figure 4-5. Phosphorus depletion by seventeen root systems of *Pinus elliotii* var. *elliotii*. under series of aerobic conditions. The root chambers were gassed with air (■) (Experiment 1; air 1), followed by N_2 gas (data not shown). Then root chambers were gassed with air again (□) (Experiment 3; Air 2). After Experiment 3, six root chambers were gassed with air (●) (Experiment 4; Air 3), followed by N_2 gas (data not shown). Then root chambers were gassed with air again (○) (Experiment 6; Air 4). Each symbol represent an average. Standard error bars are shown for Exp. 1 and Experiment 3 only. Values are expressed as fraction of initial concentration in the nutrient uptake chamber.

Figure 4-6. Phosphorus depletion by root systems of *Pinus elliotii* var. *elliotii*, under hypoxic conditions. Experiment 2; N₂ gas 1 (●) is the result from seventeen root chambers. Experiment 5; N₂ gas 2 (○) is the result from six root chambers. In both cases the root chambers were gassed with air before and after the N₂ gas treatment. Each symbol represent an average with standard error bars. Values are expressed as fraction of initial concentration in the nutrient uptake chamber.

directly related to O_2 availability rather than to more fundamental damage of cell membranes. The fact that these pine roots resumed depletion of K after two consecutive experiments with hypoxic nutrient solution (each lasting 3 days) further suggests a lack of membrane damage. However a previous treatment with N_2 gas (hypoxic conditions) did reduce K depletion under aerobic conditions by 70%. This response could be attributed to either a root recovery time due to membrane depolarization and/or decrease in root O_2 supply after the N_2 gas treatment. However evidence supporting either possibility was beyond the scope of this study. Nevertheless if one considers that studies with plum seedlings documented the same level of K depletion both before and after hypoxic treatment (Jalil and Carlson, 1993). Root systems of seedlings are easier to saturate with O_2 after N_2 gas treatment as compared to root systems of trees. Root structural differences such as cortical breakdown and periderm formation due to secondary growth, might account for a retarded response in root saturation with O_2 after the N_2 gas treatment in our experiment.

Unlike K, sudden O_2 deprivation (hypoxic conditions for 3 days) did not inhibit P uptake of pine roots in the first hypoxic treatment, but did reduce it by 28% in the second treatment. Our results from the first hypoxic treatment are consistent with previous studies by Larkum and Loughman (1969) which suggested that P uptake sensitivity to hypoxic

conditions is related to the level of P in the solution. P uptake of roots of barley seedlings was not inhibited under short term hypoxic conditions at external P concentration of 10 μM . They concluded that P uptake was maintained solely by energy from anaerobic metabolism. Yet in pine trees grown under field conditions, O_2 movement through roots within intercellular gas-filled spaces has been shown to account for some internal root O_2 (Fisher and Stone 1990b; Topa and Mcleod, 1986b), even in root tissues of pine roots grown under aerobic soil conditions. Nevertheless a root O_2 gradient is likely to be found with outer root cortical tissue experiencing O_2 deficiency. If a O_2 gradient existed in the root tissues during the hypoxic treatments, it was still too low to maintain K depletion by the pine roots in our experiment.

Implications of P and K Uptake by Roots of Pines in Reduced Soil Conditions in the Field

Pine root that developed primarily under aerobic conditions in the surface soil of a common flatwood Spodosol, should experience shortages of O_2 , since periodic water table rise to near the soil surface will induce reduced soil conditions that results in anaerobiosis. As much as 80% of the total fine root length of slash pine occurs above the 30 cm depth (Gholz et al., 1986; Van Rees and Comerford, 1986; Adams et al., 1989). Gholz et al., (1986) observed seasonal

water tables reaching the soil surface in both young (7-year-old) and old (27-year-old) pine stands, with fine roots showing no major die backs. Therefore they survived the short term inundation (2 days). Modeling efforts summarizing the role of belowground dynamics in a Florida pine plantation at the ecosystem-level, concluded that high water tables often encountered in these plantations had no significant effect on root respiration or mortality (Ewel and Gholz, 1991). Comerford et al, (1996) concluded that soil 15 cm above a water table will have reduced soil conditions, documenting as much as 47% of the total area of a flatwood landscape could have reduced soil conditions at the soil surface for short time periods. It is, therefore, reasonable to ask to what degree P and K uptake differences by pine roots under short-term reduced soil conditions are relevant to forest plantations of the southeastern United States.

Slash pine roots grown in aerobic soil conditions, and subjected to hypoxic treatments remain functional for P uptake but not for K. However for either P or K these roots are still active for both K and P uptake when the hypoxic soil conditions are removed. These differences in ion depletion are relevant to Michaelis-Menten kinetics parameters and the modeling of nutrient uptake by roots. If one wants to incorporate water table fluctuations when modeling K uptake one should account for changes in $I_{\max}$ values. For example $I_{\max}$ for K will be zero under hypoxic conditions. Most likely these values will also be a function

of time since hypoxic conditions were evident. Therefore one could estimate that a time cycle of aerobic-reduced-aerobic soil conditions where the reduced phase last 3 days will result in only 40 to 50 % of the potential K uptake (Figure 4-7).

Besides the physiological mechanism involved in P and K uptake by pine roots under hypoxic conditions, our findings are remarkable if we take into account that soil P limits pine productivity (Neary et al., 1990a). Documenting the effectiveness of pine roots to acquire P under reduced soil conditions has been necessary to evaluate mechanisms of nutrient uptake which regulate the productivity of forest.

Conclusions

In summary, intact slash pine roots grown under aerobic soil conditions, and from twelve-year-old pine trees, responded to levels of O₂ availability in the nutrient solution. The response was different for K than for P. Under hypoxic conditions, K depletion by pine roots ceased and resulted in net K efflux. Phosphorus depletion was not eliminated under hypoxic nutrient solution conditions. My results can be viewed in the context of fluctuating water tables under field conditions. They suggest that pine roots grown in aerobic soil conditions of surface horizons are capable of P depletion even when reduced soil conditions are present.

Figure 4-7. Potassium uptake potential of slash pine roots when subjected to cycles of aerobic and hypoxic conditions. (a) Under a time cycle of three consecutive aerobic soil conditions. (b) Under a time cycle of aerobic-hypoxic-aerobic soil conditions.

CHAPTER 5
PHOSPHORUS AND POTASSIUM DEPLETION BY WOODY ROOTS OF TWELVE-
YEAR-OLD SLASH PINE TREES

Introduction

There is no question that white roots of trees are active zones of nutrient absorption (George and Marschner 1996). But of continuing interest to plant physiologists is the effect that root development (i.e., changing root morphology from white to brown to woody) may have on a root's capacity to absorb ions.

A fully developed endodermis in brown roots, which normally inhibits the flux of water and nutrients through the apoplasm, can be compromised by short and lateral root development. The lateral roots leaves a pathway for mass flow where they exit the pericycle and break the endodermis. When secondary xylem growth occurs (woody roots) it can cause corruption of the endodermis with the cortex progressively sloughing away (Troughton, 1957). A cork tissue with a cork cambium will eventually develop, becoming another possible barrier to apoplastic flow.

Nutrient uptake by "woody roots" of trees has been reported (Chung and Kramer, 1975; Van Rees and Comerford, 1990). However, the classification of these roots as woody

was based only on the external root coloration. *Citrus* sp. and *Pinus taeda* L. have both been shown to have brown roots that absorb PO_4 (Crider, 1933; Chung and Kramer, 1975). The work by Chung and Kramer even suggested that an effective ion barrier existed allowing selective ion absorption. Kramer (1946) suggested that the selective barrier was either the cork cambium, the vascular cambium, or both. Uptake of PO_4 by brown root of *Prunus* has also been reported (Atkinson and Wilson, 1980). Van Rees and Comerford (1990) showed that brown roots from pine seedlings can absorb K and Rb from nutrient solutions. Yet all claims of nutrient uptake by woody roots, based on studies of nutrient uptake by brown roots, are still questionable. This is because brown roots have traditionally been considered woody (e.g., Blake and Hoogenboom, 1988), and are often described as suberized (Addoms, 1946; Kramer, 1946; Van Rees and Comerford, 1990). Terms such as "brown root" and "suberized root" are used imprecisely or incorrectly when used to describe root morphology and anatomy related to secondary growth (Richards and Considine, 1981). The brown coloration of roots is a poor indicator of anatomical attributes that define secondary growth of loblolly pine roots (nonwoody vs. woody) (unpublished data). McKenzie and Peterson (1995b) concluded that roots from pine trees were divided into three anatomically distinct zones. Beginning at the root tip these are 1) the white zone, 2) the tannin zone in which the cells external to the endodermis are dead and their walls are

modified by tannin deposits, and 3) the cork zone in which the process of secondary growth results in the formation of cork cells. Microscopic investigations of jack pine (*Pinus banksiana* Lamb) (McKenzie and Peterson, 1995a) revealed that white roots were not colored internally, despite the presence of suberin in the endodermis. The transition from white to brown along the root axis was due to the deposition of tannins in cell walls. Therefore the term "suberized root" should not be used because all zones of the root contain suberin, both in the Casparian bands and suberin lamellae. It is also an inappropriate term for the tannin as the outer cortical cells are tannified and may not necessarily be suberized.

Still, because of their abundance, woody roots have the potential to play a major role in nutrient uptake of trees. For example, in a twelve-year-old loblolly pine (*Pinus taeda* L.) stand, woody fine root tissues constituted from 62 to 80% of the fine root surface area (SA) available for nutrient uptake (Comerford et al., unpublished data). Furthermore, the research on nutrient uptake by brown root comes principally from experiments which used either excised roots (e.g. Chung and Kramer, 1975) or intact roots from tree seedlings grown under laboratory or greenhouse conditions (e.g. VanRees and Comerford, 1990). Nothing is known about nutrient absorption by intact, woody roots of perennial plants under field conditions. Only a very few attempts have been made to develop techniques to study the nutrient uptake of different

root types under field conditions. These studies included nitrogen uptake by long roots of Norway spruce (*Picea abies* (L.) Karst) (Marschner et al., 1991), and by whole root systems of peach trees (*Prunus persica* (L.) Batsch) (Goutouly and Habib, 1996). In both cases the nutrient uptake capacity of brown or woody root zones was not evaluated.

Productivity of southern pine stands of the southeastern lower coastal plain is limited by nutrient availability (Neary et al., 1990). Comerford and Neary (1991) used a nutrient uptake model to show that, for a semi-mature slash pine stand, the only way to accurately predict root uptake of phosphorus (P) was to either adjust mineralization rates or to limit P uptake to a small percentage of the fine root system. The latter suggests that a significant portion of the fine-root (brown-woody) system is not functional in absorbing P. Consequently, a major question regarding the process of nutrient uptake by pine trees is to ascertain whether woody roots function in nutrient uptake. Again, the importance of this question for southern pine is obvious when one considers 80% of the total fine root length and surface area falls in the woody category (Comerford et al., unpublished data).

This study documented P and K depletion of fine roots for twelve-year-old slash pines growing under field conditions. The objectives of this study were (i) to compare P and K depletion between whole fine root systems and woody fine root systems of slash pine roots and (ii) to compare P and K depletion between the whole fine root system and woody

fine root systems when N_2 gas was used as a metabolic inhibitor of ion absorption.

Materials and Methods

Root System Description

Twelve-year-old slash pine trees (*Pinus elliotii* Engelm. var. *elliotii*) were selected from a research plot in north Florida that was being treated with complete weed control. The soil is a poorly-drained Pomona fine sand (sandy, siliceous, hyperthermic Ultic Alaquod). The study site is about 10 km north of Gainesville, FL (29° 80' N, 82° 20' W). The climate is warm, temperate-subtropical with a mean annual precipitation of 1332 mm most of the precipitation which occurs in summer, with the least falling in autumn and spring. Mean annual temperature is 21 °C.

Four lateral roots (1-1.5 cm in diameter) were previously excavated at the base of a tree and the fine-root system was preserved as much as possible. A root regeneration system was established as described in Chapter 3. Briefly, lateral roots were pruned, placed in 50 x 25 x 6 cm black plastic trays, and covered with sieved soil from the A horizon of the study plot. Black plastic mesh was placed over the tray and the tray was covered with pine litter from the plot. The root system was supplied with a nutrient solution typical of soil solutions of a Florida Spodosol (Van Rees and Comerford, 1990). Nine months after the installation of the

root regeneration system, and prior to initiating these studies, thirty-six lateral roots from 9 trees were gently uncovered and washed with distilled water. Thirty-three lateral roots from these 9 trees were used for the 1995 studies. Twenty months after the installation of the root trays and prior to the studies, twenty intact lateral roots from 5 trees were uncovered and washed with distilled water. Seventeen lateral roots from these 5 trees were used for the 1996 study.

Nutrient Uptake System Installation

The lateral roots were inserted (while still attached to the tree) into uptake chambers. The construction and assembly of the nutrient uptake root chamber is fully described in Chapter 3. The root chamber is part of an in-situ nutrient uptake system which includes (a) the gas system, (b) the root uptake chamber and, (c) the Mariotte flask system. The root chamber was gassed with compressed gas tanks filled with either air (medical standard) or N_2 (99.9%). The Mariotte flask system was used to maintain a constant solution volume in the root chamber. Aeration of each root chamber was monitored by measuring root-chamber dissolved oxygen with a Yellow Springs Instrument (BSI) portable oxygen meter and a Clark type membrane-covered probe (accuracy $0.1 \mu g \text{ mL}^{-1} O_2$).

Depletion Studies

Pre-depletion treatment

This pre-depletion treatment was performed prior to each depletion experiment. The chambers were filled with a nutrient solution containing 110 μM nitrogen (90 μM as ammonium-N and 20 μM as nitrate-N), 65 μM calcium, 10 μM magnesium, 10 μM sulfate-S, 0.24 μM borate-B, 0.20 μM iron, 0.02 μM manganese, 0.02 μM zinc, 0.005 μM molybdate-Mo, 0.005 μM copper but lacking P and K . The nutrient solution was adjusted to pH 4.5. The roots were continuously gassed with compressed air and kept in this system for 24 hours. The chamber was covered with a black plastic mesh to avoid direct sunlight. Prior to the nutrient depletion studies, the chamber was drained and flushed with distilled water.

Previous to each pre-depletion treatment, a photograph of every root system was taken. The root systems were carefully extended on top of a 30 cm x 100 cm clear acrylic plastic sheet (0.6 cm thick). The acrylic plastic sheet was marked with a 1 cm x 1 cm grid for reference. The photo was used to account for any new root growth between depletion experiments.

To protect roots from dessication, root chambers were opened for root manipulation only before sunrise or after sundown. All root systems were shaded using a black umbrella and kept moist by continuously spraying with distilled water.

Study #1. P and K depletion by brown roots: Isolation of white roots by waxing

Experiment 1.a: Nutrient depletion study with air:
whole root systems. Fifteen lateral roots from 4 trees were used for this experiment. Root chambers were filled with known amounts of nutrient solution (c.a. 800-1200 mL) . Initial concentrations of P and K for the study were $6.25 \mu\text{M}$ P (ca. $0.20 \mu\text{g mL}^{-1}$) and $25.66 \mu\text{M}$ K (ca. $1.00 \mu\text{g mL}^{-1}$), plus other nutrients as described in the previous section. The nutrient solution was adjusted to pH 4.5. Solution samples (8 mL) were collected at 0, 8, 24, 32, 48, and 66 hours. At the time of sampling an equal volume of distilled water at pH 4.5 (8-mL) was replaced in order to maintain a constant volume. Water uptake was measured by calculating the volume of water replenished by the Mariotte flasks between sampling intervals. The system was continuously aerated with pressurized air. Dissolved oxygen saturation, and temperature were measured for each root chamber at each sampling interval.

Experiment 1.b: Nutrient depletion study with air: waxed roots. After completion of the previous experiment, all root chambers were drained and flushed with distilled water. From each tree, 2 root chambers were disassembled in the field. The white root portion, along with ≤ 1 cm of the adjacent brown roots, were covered with wax by submerging the roots for several seconds in melted embedding medium grade paraffin wax (Oxford Labware, St. Louis MO, USA; ASTM melting point

50-54 °C). The purpose of waxing brown roots next to white roots was to render nonwoody portions inactive in ion uptake. After the waxing, the root chambers were re-assembled. Eight roots were subjected to this treatment. The other 7 roots were not waxed. Afterwards, all 15 root chambers were subjected to a pre-depletion treatment. The root chambers were then filled with nutrient solution containing the P and K concentrations as previously described. The system was continuously aerated with pressurized air. Solution sampling and water uptake measurements were made as in Experiment 1.a. Oxygen saturation and temperature were measured throughout the experiment.

Experiment 1.c: Nutrient depletion study with N₂ gas.

After completion of the previous experiment, all root chambers were drained, flushed with distilled water, and subjected to the pre-depletion treatment for 24 hours. The root chambers were then filled with fresh nutrient solution (as above), but purged with N₂ gas for 2 hours prior to the experiment. A depletion study was run as before, but chambers were continuously gassed with N₂ (ultra-high purity grade) rather than air. Dissolved oxygen saturation, and temperature were measured for each root chamber at each sampling interval.

Experiment 1.d: Nutrient depletion study with air after N₂ gas. After completion of treatment with N₂ gas, all root chambers were drained, flushed with distilled water, and subjected to the pre-depletion treatment. A further depletion

experiment was run in the manner described for experiment 1a, gassing all root chambers with compressed air.

After completion of this experiment, the root system inside each chamber was cut from the tree at the point where it entered the chamber. The whole root chamber, with the intact root system, was transported immediately to the laboratory for root length measurement and sectioning.

Study #2 (1995) and study #3 (1996). P and K depletion by brown roots: Isolation of white roots by cutting

Experiments 2a-d and 3.a-d were identical to those described above, with the following exceptions. Fourteen lateral roots from 4 trees were used for study #2, while 17 laterals from 5 trees were used for study #3. In experiment 2b and 3b, the white roots and their adjacent brown roots were prevented from absorbing P and K by removing them from the tree. This was accomplished by cutting them from the lateral root with a pair of scissors. The cut end was then sealed with Vaseline. In experiment 2, 9 roots were cut, and 5 left intact. In experiment 3, 11 roots were cut and 6 were left intact.

Study #4. P and K depletion by whole root systems subjected to NaOCl solution

Sodium hypochlorite (NaOCl) solution was used for root surface sterilization to render microorganisms growing on the root surface inactive in ion immobilization. Seven lateral roots from 2 trees were used for this experiment. The root

chambers were filled with known amounts of nutrient solution (c.a. 800-1200 mL). Initial P and K concentrations for the study were $6.25 \mu\text{M P}$ (ca. 0.20 mg L^{-1}) and $25.66 \mu\text{M K}$ (ca. $1.00 \mu\text{g mL}^{-1}$), plus other nutrients as described in previous sections. The system was continuously aerated with pressurized air. Solution sampling was done similarly as described in previous experiments. After completion of this experiment, the whole root system was soaked for 5 minutes in 1% NaOCl solution, then rinsed thoroughly five times with distilled water and subjected to the pre-depletion treatment. Afterwards, the root chambers were filled with nutrient solution containing the same amount of P and K concentrations as in previous experiments. The system was continuously aerated with pressurized air. Solution sampling, and water uptake measurements were similar to the first experiment.

After completion of the above depletion experiment, the whole root system was rinsed with distilled water and again subjected to the pre-depletion treatment. Afterwards the nutrient depletion experiment was repeated.

Laboratory Incubation Studies

Inoculation with root scrapings

After experiment 2.d.(nutrient depletion study with air after N_2 gas) root chambers with whole root systems were detached from the tree and disassembled before being transported to the lab. The root surface was gently scraped

with a wire loop to collect rhizodeposition, including loose cortical root cells invaded by microorganisms. The purpose was to collect root surface microorganism to assess their role in K or P immobilization. Surface scraping was done five times per root, for 8 roots and used to inoculate 50 mL of fresh nutrient. The blank consisted of 50 mL of nutrient solution brought to the field, and subjected to the same procedure, but without receiving root scrapings. Aseptic conditions were maintained by immersing the wire loop in an ethanol solution (70%) between root samples. All samples were immediately transported to the laboratory. At the laboratory, two 10 mL subsamples were taken from each 50 mL sample. One subsample was autoclaved, and the other one received no treatment. The two subsamples were incubated at 30 °C for 48 hours. Solution samples were taken before and after incubation for measurement of differences between P and K concentration before and after incubation.

Inoculation with nutrient solution

After experiment 2.d., the bulk nutrient solution from 7 randomly selected root chambers was collected and transported to the laboratory where a 12 mL aliquot from each chamber was added to a 50 mL volumetric flask. This was brought to volume with freshly made nutrient solution. The purpose was to collect microorganism from the nutrient solution to assess their role in K or P immobilization. Two 20 mL subsamples were taken from each 50 mL volumetric flask. One subsample

was autoclaved, and the other one received no treatment. The two subsamples were incubated at 30 °C for 48 hours. Solution samples were taken before and after incubation for measurement of differences between P and K concentration before and after incubation.

Ion Mass in the Root Free Space

Upon completion of experiment 3.f., 6 intact root systems from the uncut treatments were transferred to a walk-in cooler and soaked in 0.8 L of a nutrient solution (8 °C, pH 4.5) lacking P and K. Two, 10 mL subsamples were taken from each flask using a syringe, one immediately after immersion in the nutrient solution and the other 8 hours later. Immediately following the cold treatment, roots from each container were placed in plastic bags and stored in a freezer for further root length and root section analysis.

Root Measurements

Once in the laboratory, root systems were removed from the root chambers and separated into coarse and fine-roots. Coarse-roots were operationally defined as regenerated lateral roots from which the fine roots regenerated. They were > 2 mm in diameter (3-5 cm range). Fine roots were < 2 mm in diameter. The waxed root systems were separated into waxed and non-waxed portions. The waxed portions were carefully sandwiched between moist pieces of Parafilm (American Can Company). The whole root systems (never cut or

waxed) were separated in white and brown fine roots. The white roots were also put between layers of Parafilm. Whole root system is operationally defined as the fine root system that was always kept intact during the experiment. Root lengths were calculated from digitized images using a flat-bed scanner. Root lengths were measured from the digitized images using the program "BranChing" (Berntson, 1992). Root lengths corresponding to coarse and fine-roots were recorded separately. Fine-root categories included brown, white, waxed and cut.

Anatomical Observations of Root Sections

Roots were hand-sectioned in the manner described by Frohlich (1984). Roots were sandwiched between pieces of Parafilm and placed on a plastic Petri dish while being sectioned. Multiple cross-sections were made within 10 mm of the distal end of the root. Cuts were easily oriented as the roots were visible through the semi-transparent layers of the Parafilm. All detached fine roots (brown, white and waxed) from each root system were sampled. For each detached fine root, 3 cross sections were selected randomly and observed under light microscopy. Root developmental stage was determined using bright-field or polarized light. Roots were considered nonwoody (primary) or woody (secondary) based on descriptions developed by McCrady and Comerford (unpublished data). Briefly, roots were nonwoody if no vascular cambium or secondary xylem elements were present external to the

centrally located metaxylem. Roots with secondary xylem elements located external to the metaxylem were classified as woody. Root sections were only characterized based on developmental stage (primary or secondary growth).

Chemical Analyses

Samples of nutrient solution were kept on ice until each experiment was completed and analyzed within 24 hours of the experiment's completion. Inorganic P was measured colorimetrically by the procedure of Murphy and Riley (1962). P concentration was not determined for Experiments 1a-d., as preliminary studies under greenhouse conditions had demonstrated interference due to binding of P by the wax surface. Potassium concentration was determined by atomic absorption spectroscopy.

Data Analyses

Oxygen saturation, and nutrient solution temperature

Statistical comparisons of O_2 saturation and nutrient solution temperature were made using a repeated measures analysis (SAS Institute, 1985). Treatments were the aeration environments caused by air or N_2 gas. The blocks were trees while the replicates were the roots from each tree. The measurement unit was the chamber (with root) and the repeated measures were the time intervals between samplings. There was no difference in time sampled for both O_2 saturation and

temperature in the chambers. Comparisons between treatments were made using Tukey's multiple comparison procedures (Ott, 1988).

Phosphorus and K uptake and mass flow

Estimates of mass flow influx of P and K were used to evaluate the maximum amount of each nutrient that could be acquired by passive uptake. Nutrient influx was calculated as the product of the mean P or K concentration in solution and water consumption. "Passive" uptake, as defined here, is used to describe potential nutrient uptake through the apoplastic pathway, requiring no metabolic energy. The difference between total ion uptake and mass flow ion influx is equivalent to the minimum "active" uptake (Van Rees and Comerford, 1990). Total uptake was calculated by expressing the nutrient concentration at each sampling interval as a fraction of the initial nutrient concentration. Eight lateral roots in study #1 (where whole lateral root systems were subjected to waxing), and 11 root systems in study #3 (where whole lateral roots were subjected to cutting) were compared for total depletion. Potential uptake was calculated for the same roots in the manner described earlier.

Phosphorus and K influx

To calculate influx rates of P and K ($\mu\text{mol cm}^{-1} \text{h}^{-1}$) I plotted Q_L for each time interval. Q_L was the total pool of P or K in solution per unit root length ($\mu\text{mol cm}^{-1}$), and time

was in hours. Depletion curves from studies #1, #2 and #3, were expressed as $\mu\text{mol cm}^{-1}$ (Q_L) for each time interval as described by Classen and Barber (1974). Q_L vs. time, was fit with a non-linear least squares program (SAS Institute, 1985) to the equation

$$Q_L = Q_i \exp (-rt^{1/2}) \quad (4)$$

where t is time in hours, Q_L is the amount of P or K in nutrient solution at a given time ($\mu\text{mol cm}^{-1}$), Q_i is the initial amount of P and K in solution ($\mu\text{mol cm}^{-1}$), $\exp$ is the base of the natural logarithm (i.e. 2.7182), and r is a constant. The net influx (In) ($\mu\text{mol cm}^{-1} \text{ h}^{-1}$) of P and K per root system is given by $-dQ_L/dt$. Influx (In) per unit root length per unit time is given by:

$$In = -dQ_L/dt = -r/(2t^{1/2}) Q_i \quad (5)$$

Influx of P and K were estimated at a fixed nutrient solution concentration for each root system. For P, the fixed nutrient concentration was $5.0 \times 10^{-3} \mu\text{mol cm}^{-3}$ P (ca 0.16 mg L^{-1}), while for K was $2.0 \times 10^{-2} \mu\text{mol cm}^{-3}$ K (ca. 0.77 mg L^{-1}). These estimates were transformed to natural logarithms to achieve a normal distribution. When efflux was observed (a negative value), I transformed as $\ln |In|$. $\ln (In)$ data was compared by a one-way analysis of covariance (SAS Institute, 1985). Treatments were the aeration environments caused by air or N_2 gas and method of isolating roots (cutting or waxing, vs. uncut). The blocks were trees while replicates were the roots from each tree. The measurement unit was the

chamber (with root). Treatments were looked upon as 2 x 2 factorial [Whole/Brown (due to cutting or waxing) x Air/N₂ gas] plus a Control. The goal was to determine if influx of P or K was affected by the method of isolating brown roots (cutting or waxing vs. whole), and if the gassing of the nutrient solution (Air/N₂ gas) affects P or K influx. The covariant was the nutrient influx of a root system before a given treatment. The variant analyzed was $\ln |In|$ of a root system at a given treatment. The experimental layout is presented in Table 5-1 for studies 1,2 and 3. When a covariant was significant and treatment differences were significant, mean comparisons were made using Least square means (SAS Institute, 1985). The adjusted treatment means of the covariance analysis were analyzed further with orthogonal contrasts comparing "control vs. treatments", "whole vs. woody (either waxed or cut)", "air vs. N₂ gas", and "(whole/woody)*(air/ N₂ gas)".

Phosphorus and K uptake, NaOCl solution study

Samples from study #4 were compared in a similar manner to those described for total P and K uptake in the mass flow section. Briefly, the nutrient content at each sampling interval was expressed as a fraction of the initial nutrient concentration, and its relationship with time was fit to equation 1. Values of r for individual root systems were compared for analysis of variance (ANOVA) (SAS Institute, 1985). Treatments were considered to be (1) prior to

Table 5-1. Experimental design of Covariance analysis used for P and K Influx data obtained from depletion experiments of studies 1,2 and 3.

Treatment	Study 1		Study 2 and 3	
	Covariate	Variate	Covariate	Variate
Whole air	a.Whole Air 1	b.Whole Air 2	a.Whole Air 1	a.Whole Air 2
Whole N ₂	b.Whole Air 2	c.Whole N ₂ gas	b.Whole Air 2	b.Whole N ₂ gas
Brown air	a.Whole Air 1w*	b.Waxed air	a.Whole Air 1c*	a.Cut air
Brown N ₂	b.Waxed air	c.Waxed N ₂ gas	b.Cut air	b.Cut N ₂ gas
Brown Control	a.Whole Air 1w*	d.Waxed Air after N ₂ gas	a.Whole Air 1c*	d.Cut Air after N ₂ gas
Whole control	a. Air 1	d.Whole Air after N ₂ gas	a.Air 1	d.Whole Air after N ₂ gas

* roots to be waxed (w) or to be cut (c)

treatment with NaOCl solution, and (2) after the treatment with NaOCl solution. When treatment differences were significant, comparisons between treatments were made using Tukey's multiple comparison procedures (Ott, 1988).

Laboratory incubation studies

For each sample, differences were calculated between P and/or K concentration before and after incubation. Differences between the paired measurements were compared between the autoclaved and non-autoclaved treatments using paired t-test (SAS Institute, 1985).

Results

Anatomical Observations and Root System Descriptions

All sections of white roots were primary roots (n= 252), while all brown root sections from the cutting and waxing experiments had secondary growth (woody roots; n=738). Therefore, all roots previously described as brown (wax and cut experiments) will be referred to as woody fine roots throughout the following results and discussion sections.

Description of root systems used for the 3 field studies are presented in Tables 5-2, 5-3 and 5-4. The volume of nutrient solution in the root chambers varied from 675 mL to 1250 mL, due to differences in root length (tap and fine). The percentage of woody roots as a portion of the total root systems ranged from 24% to 100%. The portion of the fine-root

Table 5-2. Description of root systems used in study one, where roots were subjected to waxing (1995 depletion experiments.)

Tree-root	Volume of Root chamber (mL)	Treatment [†]	Coarse [‡] R.L. (cm)	Fine [‡] R.L. (cm)	Brown [§] R.L. (% fine)	Waxed R.L. (% fine)
1-1	1000	Waxing	70.35	136.90	75.57	24.43
1-2	900	None	35.68	1511.95	74.43	-----
1-3	850	Waxing	66.15	506.87	48.87	51.13
1-4	1000	None	29.26	851.62	100.0	-----
2-1	1200	Waxing	128.03	220.85	11.20	88.80
2-2	1200	None	83.41	85.31	100.0	-----
2-3	1200	None	78.70	125.45	100.0	-----
2-4	1200	Waxing	62.01	114.68	19.44	80.56
3-1	850	None	28.56	162.92	39.48	-----
3-2	820	Waxing	63.98	447.50	34.82	65.18
3-3	1250	Waxing	46.88	1071.93	16.30	83.70
3-4	950	None	47.88	87.53	37.93	-----
5-1	1250	Waxing	19.07	283.20	66.24	33.76
5-2	1100	None	15.75	525.11	36.73	-----
5-3	890	Waxing	66.84	70.14	50.00	50.00

[†] Refers to the treatment of isolating brown surface areas. See text.

[‡] R.L = Root Length, Coarse roots (Lateral root). Fine root < 2 mm in diameter.

[§] based on external color.

Table 5-3. Description of root systems used in study two, where roots were subjected to cutting (1995 depletion experiments.).

Tree-root	Volume of Root chamber (mL)	Treatment [†]	Coarse [‡] R.L. (cm)	Fine [‡] R.L. (cm)	Brown [§] R.L. (% fine)	White R.L. (% fine)	Cut R.L. (% fine)
6-1	1000	Cut	45.30	279.46	46.62	45.07	53.88
6-2	1200	None	78.60	1816.81	76.54	23.46	-----
6-3	850	Cut	39.63	159.11	20.90	55.47	79.10
6-4	1100	None	63.56	269.99	76.79	23.21	-----
7-1	900	None	123.08	31.01	69.64	30.36	-----
7-2	850	Cut	20.65	161.15	92.57	6.00	7.43
7-3	1150	Cut	51.66	699.53	51.15	30.65	48.85
7-4	1200	None	44.42	1116.55	65.37	34.63	-----
8-1	800	Cut	25.47	15.30	100.00	0.00	100.00
8-2	900	Cut	48.03	11.60	100.00	0.00	100.00
8-4	800	Cut	19.84	5.25	0.00	100.00	100.00
9-1	800	Cut	41.92	4.50	0.00	100.00	100.00
9-2	1100	Cut	48.14	291.63	92.44	5.73	7.56
9-3	800	None	36.61	308.12	89.43	10.57	-----

[†] Refers to the treatment of isolating brown surface areas. See Text

[‡] Coarse roots (lateral root). Fine root < 2 mm in diameter.

[§] based on external color.

Table 5-4. Description of root systems used in study three, where roots were subjected to cutting. 1996 depletion experiments.

(Tree- root)	Volume. of Root chamber (mL)	Treatment†	Coarse‡ R.L. (cm)	Fine‡ R.L. (cm)	Brown\$ Root R.L. (% fine)	White R.L. (% fine)	Cut Root (% fine)
10-1	900	Cut	20.05	976.23	100.00	0.00	74.64
10-2	700	Cut	17.55	37.55	100.00	0.00	51.45
10-3	975	Cut	58.90	767.16	99.20	0.80	50.69
10-4	875	Cut	68.56	405.55	99.80	0.20	43.64
11-1	750	Cut	29.11	13.52	100.00	0.00	100.00
11-2	825	Cut	52.13	197.09	95.51	4.49	56.61
11-3	850	Cut	85.61	1022.97	100.00	0.00	45.86
12-1	825	Cut	54.17	642.49	24.31	75.69	85.09
12-2	850	Cut	100.21	755.11	81.74	18.26	42.88
12-3	850	Cut	76.08	171.59	100.00	0.00	42.25
12-4	900	Cut	105.73	38.27	75.16	24.84	52.79

† Refers to the treatment of isolating brown surface areas.

‡ Coarse roots (lateral root), fine root < 2 mm in diameter.

\$ based on external color. ¶ see text for details.

Table 5-4--continued

(Tree- root)	Volume. of Root chamber (mL)	Treatment [†]	Coarse [‡] R.L. (cm)	Fine [‡] R.L. (cm)	Brown [§] R.L. (% fine)	Root R.L. (% fine)	Cut Root (% fine)
13-1	1100	None	112.35	820.22	84.16.	15.84	-----
13-2	800	None	16.47	105.69	100.00	0.00	-----
14-1	675	None	141.44	3110.69	100.00	0.00	-----
14-2	700	None	118.79	3994.36	99.51	0.49	-----
14-3	800	None	28.95	1794.51	99.58	0.42	-----
14-4	500	None	91.64	1133.47	77.51	22.49	-----

† Refers to the treatment of isolating brown surface areas.

‡ Coarse roots (Lateral root), fine root < 2 mm in diameter.

§ based on external color. ¶ see text for details.

system that was waxed varied from 24% to 88%, while the portion that was cut ranged from 7% to 80% of the total, fine-root length.

O₂ Concentration and Temperature in the Chambers

The average temperature of solutions gassed with air was not significantly different from the temperature of those gassed with N₂ (Table 5-5 and 5-6). Therefore, mean values of oxygen saturation (%) for each experiment, were converted to μM , based on the average nutrient solution temperature. Results are presented in Tables 5-5 and 5-6. O₂ concentrations in root chambers gassed with N₂ indicated hypoxic conditions (O₂ < 50 μM), and were significantly lower than the air treatment ($p < 0.001$). All root chambers gassed with air were aerobic (O₂ > 50 μM).

Microbial Interferences

There was no evidence of uptake of K or P by microorganisms during the depletion studies (Table 5-7). Initially, treatment with 1% NaOCl solution totally inhibited K uptake (Table 5-7). However, in a second uptake experiment following the NaOCl treatment, K uptake resumed with a significant increase when compared to K uptake before the NaOCl treatment (Table 5-7). Similarly, P uptake was initially suppressed after treatment with NaOCl but resumed with a second uptake experiment (Table 5-7). When root

Table 5-5. Temperature and Dissolved Oxygen concentrations[†] in the nutrient solution during consecutive experiments of P and K depletion by pine roots. 1995 studies.

Study One (Experiments)	Treatment	n	Temperature °C	Dissolved Oxygen µM
1.a. Air 1	Whole	15	27.0 (4.6)a	238 (5.3)a
1.b. Air 2	Whole and Waxed	8 and 7	25.0 (5.7)a	235 (4.2)a
1.c. N ₂ gas	Whole and Waxed	8 and 7	28.7 (4.4)a	11 (7.3)b
1.d. Air after N ₂ gas	Whole and Waxed	8 and 7	28.2 (3.3)a	241 (5.8)a

Study Two (Experiments)	Treatment	n	Temperature °C	Dissolved Oxygen µM
2.a. Air 1	Whole	14	29.1 (4.6)a	240 (3.4)a
2.b. Air 2	Whole and Cut	5 and 9	28.2 (4.2)a	238 (6.2)a
2.c. N ₂ gas	Whole and Cut	5 and 9	27.8 (5.3)a	14 (8.1)b
2.d. Air after N ₂ gas	Whole and Cut	5 and 9	29.2 (3.2)a	242 (7.1)a

Table 5-6. Temperature and Dissolved Oxygen concentrations[†] in the nutrient solution during consecutive experiments of P and K depletion by pine roots. 1996 study.

Study Three (Experiments)	Treatment	n	Temperature °C	Dissolved Oxygen µM
3.a Air 1	Whole	17	25.5 (5.3)a	242 (5.2)a
3.b Air 2	Whole and Cut	6 and 11	29.7 (5.4)a	209 (11.9)c
3.c N ₂ gas 2	Whole and Cut	6 and 11	24.2 (4.7)a	14 (5.0)b
3.d Air after N ₂ gas 2	Whole and Cut	6 and 11	25.2 (5.3)a	241 (5.8)a

[†] Temperature and dissolved Oxygen values followed by a different letter are significantly different between experiments ($P < 0.001$). Values of temperature and dissolved oxygen are means. Numbers in parentheses are standard deviations of n replicates.

scrapings and nutrient solution from the growth chambers were incubated with a fresh nutrient solution, there was also no evidence of microbial uptake for both P and K (data not shown).

Potassium and Phosphorus Influx

For the 6 root systems tested, P and K concentrations in the root free space were negligible (data not shown). Therefore, no corrections to the depletion estimates for the storage of ions in the free space were made. Two methods were used to determine if active uptake was occurring. The first estimated the maximum amount of mass flow that could be accounted for by considering the water influx to the root and the concentration of the ion in the solution. For K, I estimated that a maximum of 30% of the total K absorbed by aerated roots could have been absorbed by mass flow (Figure 5-1). Using the second method I attempted to use N_2 to inhibit the active uptake mechanism. N_2 significantly inhibited K uptake for both whole root systems and woody roots (Tables 5-8 and 5-9).

Mass flow had a maximum of 25% of the total depletion of P from the nutrient solution due to non-active uptake (Figures 5-2,5-3). In general, N_2 did not have a significant effect on P absorption (Table 5-8 and 5-9). However, there was some evidence that hypoxic conditions did affect P influx but only for one study and only for the whole root system (Table 5-8). Rates of P and K uptake for woody roots were

Table 5-7. Comparison of constant rate of depletion (r)[†] and C [‡] for P and K depleted by pine roots treated with 1% NaOCl. Study four.

Study Four (Experiments)	Treatment	n	Potassium		Phosphorus	
			r †	C ‡	r †	C §
4.1. Air 1	Before NaOCl	7	0.0298 b	0.78	0.3611 a	0.08
4.2. Air 2	After NaOCl First	7	-0.0218 c	1.19	0.0576 c	0.67
4.3. Air 3	After NaOCl Second	7	0.1773 a	0.23	0.2397 ab	0.19

† based on the equation $C = \exp(-rt)^{1/2}$ where r is a constant, and C is the fraction of initial K or P concentration at final time (see text for details).

‡ Fraction of final K concentration was calculated at time 66 hours.

§ Fraction of final P concentration was calculated at time 48 hours.

† Numbers are replicate means, within each column differences are significant at $P = 0.05$ if followed by different letters.

Figure 5-1. Calculation of potassium depletion due to mass flow by eight root systems of *Pinus elliotii* var. *elliotii*. The root chambers were gassed with air. Solid symbols (● ■) represent the total K depleted, Open symbols (○ □) represent the K depleted not accounted by mass flux (active). Circles (● ○) represent K depleted by the whole root system. Squares (■ □) represent the K depleted by the woody root systems. Each symbol represent an average with standard error. Values are expressed as fraction of initial concentration in the nutrient uptake chamber.

similar to rates of ion uptake of whole (woody and nonwoody) roots (Table 5-9).

Discussion

These results demonstrate that woody fine roots of twelve-year-old slash pine actively absorb P and K. However, this conclusion assumes that the root manipulations have not influenced uptake. Possible criticisms of the methodology are that (i) some uptake could be ascribed to microorganism activity on the root surface and that (ii) cutting and waxing of roots can result in mechanical shock, hence affecting uptake.

Concern over microbial uptake of P and K during the depletion experiments was shown to be unfounded. When root scrapings and nutrient solution from a root chamber study were added to a nutrient solution, there was no evidence of a significant microbial effect between autoclaved and non-autoclaved samples. Likewise, less than 1% of the depleted P can be explained by potential P immobilization by microorganisms. Here, I assumed that soluble C released by roots ranges from 10-100 mg C g⁻¹ root (Newman, 1985) to 143-455 mg C g⁻¹ root (Lambers, 1987). Using the upper and lower ends of these values and a pine root wood density of 1 g cm⁻³ with a root radius of 0.05 cm, then the potential C released is between 0.01 -0.45 g C cm⁻¹ root. With a C:P ratio of 16:1 in microbial tissue (Stevenson, 1986) and assuming all C released is used for microbial growth, P

Table 5-8 Covariance analysis for P and K influx (Ln(Influx)) by pine root systems of studies 1,2 and 3.

Source of Variation	Study 1 1995 (Waxed)	Study 2 1995 (Cut)		Study 3 1996 (Cut)	
	K	K	P	K	P
Covariate	0.45	0.14	0.07	0.07 (*)	0.07 (*)
Control vs Treatment	0.03 (**)	0.24	0.08 (*)	0.04 (**)	0.34
Whole vs Woody	0.24	0.68	0.82	0.07(*)	0.13
Whole air vs Woody Air	0.12	0.74	0.51	0.43	0.75
Whole N ₂ vs. Woody N ₂	0.86	0.35	0.33	0.00 (***)	0.07 (*)
Air vs. N ₂	0.00 (***)	0.01(***)	0.38	0.01 (***)	0.10 (*)
Whole air vs Whole N ₂	0.01 (***)	0.20	0.84	0.00 (***)	0.06 (*)
Woody air vs. Woody N ₂	0.10 (*)	0.01(***)	0.15	0.01 (***)	0.74
(Whole/Woody) * (Air/N ₂)	0.03 (**)	0.38	0.25	0.12	0.21

*** significant at 0.01 level

** significant at 0.05 level

* significant at 0.10 level

n.s. non significant

Table 5-9. Mean P and K influx for woody roots and whole roots of 12-year-old slash pine calculated at standard concentration¹. These untransformed values ($\mu\text{Mol cm}^{-1} \text{ h}^{-1}$) are a summary of the three separate studies performed in 1995 and 1996. Cut refers to the studies when white roots were removed by cutting while waxed refers to the study where white roots were covered with wax.

ROOT TYPE	Study 1 1995 (Waxed)		Study 2 1995 (Cut)		Study 3 1996 (Cut)	
	WOODY	WHOLE	WOODY	WHOLE	WOODY	WHOLE
POTASSIUM						
AIR	6.7×10^{-3} Aa ²	2.2×10^{-4} Aa	2.0×10^{-4} Aa	8.6×10^{-4} Aa	5.3×10^{-7} Aa	1.8×10^{-5} Aa
N ₂ GAS	-2.2×10^{-1} Ab	-1.1×10^{-1} Ab	-1.2×10^{-1} Ab	2.4×10^{-1} Aa	-2.6×10^0 Aa	-3.5×10^6 Bb
AIR after	1.3×10^{-3} Aa	5.2×10^{-4} Aa	8.4×10^{-4} Aa	1.7×10^{-3} Aa	1.9×10^{-5} Aa	7.1×10^{-6} Aa
N ₂ GAS						
PHOSPHORUS						
AIR	-----	-----	2.5×10^{-3} Aa	1.3×10^{-3} Aa	4.8×10^{-3} Aa	3.0×10^{-5} Aa
N ₂ GAS	-----	-----	5.6×10^{-4} Aa	1.6×10^{-3} Aa	3.0×10^{-3} Aa	1.2×10^{-4} Bb
AIR after	-----	-----	4.9×10^{-4} Aa	3.4×10^{-4} Aa	9.3×10^{-3} Aa	1.4×10^{-3} Aa
N ₂ GAS						

¹ $5.0 \times 10^{-3} \mu\text{Mol cm}^{-3}$ P and $2.0 \times 10^{-2} \mu\text{Mol cm}^{-3}$ K.

² Capital letters represent comparisons between columns within a study, while small letters represent comparisons within a column for each nutrient. Different letters signify a statistically difference at the 0.10 level.

Figure 5-2. Calculation of phosphorus depletion due to mass flow by eleven root systems of *Pinus elliotii* var. *elliotii*. The root chambers were gassed with air. Solid symbols (● ■) represent the total P depleted. Open symbols (○ □) represent the P depleted not accounted by mass flux (active). Circles (● ○) represent P depleted by the whole root system. Squares (■ □) represent the P depleted by the woody root systems. Each symbol represent an average with standard error. Values are expressed as fraction of initial concentration in the nutrient uptake chamber.

Figure 5-3. Calculation of phosphorus depletion due to mass flow by eleven root systems of *Pinus elliottii* var. *elliottii*. The root chambers were gassed with N₂ gas. Solid symbols (● ■) represent the total P depleted. Open symbols (○ □) represent the P depleted not accounted by mass flux (active). Circles (● ○) represent P depleted by the whole root system. Squares (■ □) represent the P depleted by woody root systems. Each symbol represent an average with standard error. Values are expressed as fraction of initial concentration in the nutrient uptake chamber.

immobilization would be between to 2×10^{-11} and 9.1×10^{-10} $\mu\text{mol P cm}^{-1}$, less than 1% of the concentrations, even at the end of the depletion studies. This approach is consistent with those of Darrah (1993).

Physical manipulation of roots has been shown to affect subsequent nutrient uptake. For example, just dipping hydroponically-grown roots in and out of a solution disturbed P absorption (Gronewald and Hanson, 1980). Bloom (1989) suggested waiting 24 hours after manipulation before commencement of measurements in order to allow for recovery of roots. This suggestion was adapted in these studies. However, dipping in hot wax and pruning roots is likely to cause a significant degree of heat and mechanical shock. In particular, cutting has the potential to open breaks in the roots and allow for more water influx and hence mass flow. Root pruning has been shown to initially affect K uptake in barley (Bloom and Caldwell, 1980), and has influenced both root respiration and N uptake (Bloom, 1989). In the case of K uptake, this effect appeared to last for 6 hours after incision (Bloom and Caldwell, 1988), while for pruning of corn roots, P uptake was relatively unaffected (Gronewald and Hanson, 1980). Therefore, it appears that the effect of physical manipulation is variable and a concern when trying to estimate ion uptake. The major question addressed in this study is whether woody fine roots function in uptake and whether there is evidence that the uptake can be classified as active. While physical manipulation may affect the actual

uptake rate and the magnitude of the effect is unknown, there is no evidence to suggest that the inhibition of uptake in the presence of N_2 gas will be affected. Therefore, these data are useful for answering the major questions of the study, while recognizing that the magnitude of the actual influx rates of the manipulated roots may be affected. I conclude then, that woody fine roots of these 12-year-old trees functioned in P and K uptake in this experiments, and that they appeared to have active uptake.

This conclusion is consistent with studies of other pine species, with the major difference being that previous studies worked with seedlings (Van Rees and Comerford, 1990; slash pine) or young trees under greenhouse conditions (Chung and Kramer, 1975; loblolly pine) rather than large trees. Further, these earlier studies had no evidence to suggest that roots were woody, only that the roots were brown.

We conclude, that not only do woody roots function in ion uptake, but that active uptake is implicated. Both the use of N_2 as a metabolic inhibitor and the estimates of the potential maximum mass flow influx demonstrate that an oxygen-requiring mechanism for uptake is responsible for the majority of K uptake. The mass flow evaluation suggested active uptake of P, while gassing with N_2 did not affect uptake. The results of the N_2 treatment could be interpreted against active uptake of P, but in combination with the results presented in Chapter 4 and the mass flow evaluation a better interpretation is that the mechanism of P uptake is

insensitive to conditions of low O_2 . The anatomical pathway, whereby woody roots absorb P and K, were not addressed in this study. They are uncertain and invite further study. Woody roots have some secondary xylem development. In some of these roots, the casparian band is still functional. In others it is disrupted. Consequently there is a range of root anatomies in woody roots that are likely to be associated with a range of uptake pathways.

Assuming that woody fine roots actively function in P and K uptake, then the next question is how important are these roots to a forest stand? Woody fine roots may constitute approximately 60-80% of the total fine-root surface area at this study site (McCrary and Comerford, unpublished data). Therefore, the total uptake of nutrients by the tree would be greatly underestimated if this surface area was not included in uptake models. Estimates of P uptake previously made for this study site (data not shown), found that predictions of P using total root length were greater, by about a factor of 2, than the stand actually absorbed. It was hypothesized that the lack of uptake by woody roots might account for this discrepancy. However, the data presented here suggests that this hypotheses is erroneous, and that woody roots are indeed likely to contribute to overall nutrient uptake. Other studies showed that root spatial pattern, promoting inter-root competition, could reduce predicted uptake by about 50% (Comerford et al., 1994b) making this a possible important consideration in modeling of

uptake of natural ecosystems and the primary explanation of uptake overestimates. The description of other important processes, still need to be adequately evaluated.

In summary, woody, fine-roots of field grown pine trees absorb P and K, and appear to do so in an active manner. Since they are a large portion of the total fine-root system, they are an important source of nutrient uptake by pine trees and need to be included in estimates of the effective root surface area.

CHAPTER 6 OVERALL DISCUSSION

Nutrient management of forest plantations in the southeastern United States is focusing on matching nutrient supply to tree demand. These nutrient management efforts can be aided through the use of nutrient uptake models. P and K uptake by field-grown slash pine seedlings have been successfully predicted using nutrient uptake models (Van Rees et al., 1990; Smethurst and Comerford, 1993). However, nutrient uptake models have overestimated P uptake when used for mature slash pine plantations. Factors that could account for overprediction of P uptake by older pine trees growing under field conditions are (i) the ability to define what is the functional or effective root absorbing surface; (ii) lack of knowledge about the relative amounts of different absorbing surfaces present and the nutrient absorption rates of each type of surface; (iii) poor understanding of the differential response of the absorbing surfaces to environmental variables; and (iv) the effect of the spatial distribution of roots at the scale of inter-root competition.

From the above mentioned list, spatial root pattern is the only factor tested that could account for overprediction of P uptake by older pine trees growing under field conditions. A random spatial pattern at a scale of inter-root

competition can reduce P uptake efficiency by as much as 50% (Escamilla et al., 1991; Comerford et al., 1994b).

Estimation of different root absorbing surfaces have documented that under field conditions, brown roots may contribute over 90% of the fine root system of slash pine (*Pinus elliotii* Engelm. var. *elliotii*) and up to 80% of those fine brown roots are woody (McCrary and Comerford, unpublished data). However all claims of nutrient uptake by woody roots are based on studies of nutrient uptake by brown roots and they come from experiments which used either excised roots (e.g. Chung and Kramer, 1975) or intact roots from tree seedlings grown under greenhouse conditions (e.g. Van Rees and Comerford, 1990). The brown coloration of roots is a poor indicator of anatomical attributes that define secondary growth of loblolly pine roots (nonwoody vs. woody) (McCrary and Comerford, unpublished data). Nothing is known about nutrient absorption by intact, woody fine roots of perennial plants under field conditions. Of continuing interest to plant physiologists is the effect that root development (i.e, changing root morphology from white to brown to woody) may have on a root's capacity to absorb ions. Moreover, because of their abundance, woody roots have the potential to play a major role in nutrient uptake of trees.

Field studies of root uptake tend to be complicated due to access to plant roots below the soil, plant age and plant size. This is especially true when considering mature trees. Not surprisingly, I found only two useful examples of

nutrient depletion studies in the field and a handful of related studies (Marschner et al., 1991; Goutouly and Habib, 1996; Glavac and Ebben, 1986). Therefore I had to implement a nutrient uptake system that can be used for trees growing under field conditions. The design included (i) a root chamber; (ii) a system for controlling the O_2 level of the root chamber and; (iii) a Mariotte flask system. I used this system to clarify if woody fine roots of trees, growing under field conditions, function in the absorption of P and K. I showed that woody roots actually do function in P and K uptake, since the hypothesis that woody fine roots do not play a role in nutrient uptake is refuted.

Under field conditions absorbing surface areas of trees can be exposed to changing soil environmental variables. An important characteristic of many forest soils of the southeastern United States is a seasonally high water table that reduces O_2 supply to a large portion of the root system. Pine roots growing in the top 16 cm can account for 50% of the total fine root system (Van Rees and Comerford, 1986). I documented that nutrient uptake of slash pine roots was affected by levels of oxygen availability but that the response differed for K and P. Under hypoxic nutrient solution conditions ($O_2 < 50 \mu M$), K depletion by pine roots was totally inhibited and even resulted in net efflux of K from roots. In contrast, P depletion was not inhibited under hypoxic nutrient solution conditions. Since soils that support slash pine often have fluctuating water tables, this

sensitivity to K is significant. If I also take into account that soil P limits pine productivity (Neary et al., 1990a) documenting the effectiveness of pine roots to acquire P and K under low-O₂ soil conditions is necessary to evaluate mechanisms of nutrient uptake which regulate the productivity of forest.

From my results I can conclude that, in order to improve predictions of nutrient uptake under field conditions future research efforts should focus on the inclusion of (i) root spatial patterns, and (ii) temporal changes in root uptake kinetics due to changing soil aeration in nutrient uptake models.

LIST OF REFERENCES

- Adams, M.B., Pennell, K.D. and Campbell, R.G. 1989. Fine root distribution in a young loblolly pine (*Pinus taeda* L.) stand: effects of preplant phosphorus fertilization. *Plant Soil* 113:275-278.
- Addoms, R.M. 1946. Entrance of water into suberized roots of trees. *Plant Physiol.* 21:109-111.
- Andersen C.P., and P.T. Rygielwicz. 1991. Stress interactions and mycorrhizal plant response: understanding carbon allocation priorities. *Environ. Pollut.* 73:217-244.
- Anderson, W.P. 1975. Long-distance transport in roots. p231-265. In D.A. Baker and J.L. Hall (ed.). *Ion transport in plant cells and tissues*. North-Holland Publ. Comp., Amsterdam Oxford.
- Armstrong, W., and D.J. Read. 1972. Some observations on oxygen transport in conifer seedlings. *New Phytol.* 71:55-62.
- Ashford, A.E., W.G. Allaway, C.A. Peterson and C.J. Cairney. 1989. Nutrient transfer and the fungus-root interface. *Aust. J. Plant. Physiol.* 16:85-97.
- Ashford A.E., C.A. Petersen., J.L. Carpenter., J., W.G. Cairney and W.G. Allaway. 1988. Structure and permeability of the fungal sheath in the *Pisonia* mycorrhiza. *Protoplasma.* 147:149-161.
- Atkinson, D. and S.A. Wilson. 1979. The root-soil interface and its significance for fruit tree roots of different ages. p. 259-271. In J.L. Harley and R.S. Russell (eds.) *The soil-root interface*. Academic Press, London.
- Atkinson, D. and S.A. Wilson. 1980. The growth and distribution of fruit tree roots: some consequences for nutrient uptake. p. 137-150. In D. Atkinson, J.E. Jackson, R.O.L. Sharples and W.M. Waller (eds.) *Mineral nutrition of fruit trees*. Butterworths, London.
- Ayling, S.M. 1993. The effect of ammonium ions on membrane potential and anion flux in roots of barley and tomato. *Plant Cell Environ.* 16:297-303.

- Baldwin, J.P., P.B. Tinker, and P.H. Nye. 1972. Uptake of solutes by multiple root systems from soil. II. The theoretical effects of rooting density and pattern on uptake of nutrients from soil. *Plant Soil* 38:621-635.
- Barber S.A. 1984. Soil Nutrient bioavailability: a mechanistic approach. John and Wiley and Sons, New York.
- Barber, S.A. and J.H. Cushman. 1981. Nitrogen uptake model for agronomic crops. p.382-409. In I.K. Iskandar (ed.) *Modeling waste-water renovation*. Wiley, New York.
- Beck, R.H. 1979. Potassium and ammonium uptake kinetics of slash pine and corn. Ph.D. Purdue Univ. Diss. Abst. 7926349.
- Bernston, G.M. 1992. A computer program for characterizing root system branching patterns. *Plant and Soil* 140:145-149.
- Bhat, K.K.S. 1981. Nutrient inflow into apple roots. I. ^{32}P -orthophosphate uptake from solution by M.9 rootstocks and Worcester Pearmain seedlings. *Plant Cell Environ.* 4:297-302.
- Bhat, K.K.S. 1983. Nutrient inflows into apple roots. *Plant Soil* 71:371-380.
- Bhat, K.K.S. and P.H. Nye. 1973. Diffusion of phosphate to plant roots in soil. I. Quantitative autoradiography of the depletion zone. *Plant Soil* 38:161-175.
- Blake, J.I. and G. Hoogenboom. 1988. A dynamic simulation of loblolly pine (*Pinus taeda* L.) seedling establishment based upon carbon and water balances. *Can. J. For. Res.* 18:833-850.
- Bloom, A.J. 1989. Continuous and steady-state nutrient absorption by intact plants. p. 147-163. In J. G. Torrey and L.J. Winship (eds.). *Applications of continuous and steady-state methods to root biology*. Kluwer Academ. Press, Dordrecht-Netherlands.
- Bloom, A.J. and R.M. Caldwell. 1988. Root excision decreases nutrient absorption and gas fluxes. *Plant Physiol.* 87:794-796.
- Bloom, A.J. and F.S. Chapin III. 1981. Differences in steady-state net ammonium and nitrate influx by cold and warm adapted barley varieties. *Plant Physiol.* 68:1064-1067.

- Bloom, A.J., L.E. Jackson, and D.R. Smart. 1993. Root growth as a function of ammonium and nitrate in the root zone. *Plant Cell Environ.* 16:199-206.
- Bowen, G.D. 1969. The uptake of orthophosphate and its incorporation into organic phosphates along roots of *Pinus radiata*. *Aust. J. Biol. Sci.* 22:1125-1135.
- Bowen, G.D. and A.D. Rovira. 1967. Phosphate uptake along attached and excised wheat roots measured by an automatic scanning method. *Aust. J. Biol. Sci.* 20:369-378.
- Bowling, D.J.F. 1981. Release of ions into the xylem in roots. *Physiol. Plant.* 53:392-397.
- Brouwer, R. 1965. Ion absorption and transport in plants. *Annu. Rev. Plant Physiol.* 16:241-266.
- Caldwell, M.M., L.M. Dudley and B. Lilieholm. 1992. Soil solution phosphate, root uptake kinetics and nutrient acquisition: implications for a patchy soil environment. *Oecologia (Berlin)* 89:305-309.
- Caradus, J. R. 1979. Selection of root hair length in white clover (*Trifolium repens* L.). *Euphytica* 28:489-494.
- Carpenter, J.R. and C.A. Mitchell. 1980. Root respiration characteristics of flood tolerant and intolerant tree species. *J. Am. Soc. Hort. Sci.* 105:684-687.
- Cheeseman, J.M. and J.B. Hanson. 1979. Energy-linked potassium influx as related to cell potential in corn roots. *Plant Physiol.* 64:842-845.
- Chung, H.H. and P.J. Kramer. 1975. Absorption of water and ³²P through suberized and unsuberized roots of loblolly pine. *Can. J. of For. Res.* 5:229-235.
- Claassen, N. and S.A. Barber. 1974. A method for characterizing the relation between nutrient concentration and flux into roots of intact plants. *Plant Physiol.* 54:564-568.
- Claassen, N. and S.A. Barber. 1976. Simulation model for nutrient uptake from soil by a growing plant root system. *Agron. J.* 68:961-964.
- Clarkson, D.T. 1985. Factors affecting mineral nutrition acquisition by plants. *Annu. Rev. Plant. Physiol.* 36:77-115.

- Clarkson, D.T. and C. Grignon. 1991. The phosphate transport system and its regulation in roots. p. 49-62. In C. Johnsen, K.K. Lee, and K.L. Sahrawat (eds.). Phosphorus nutrition of grain legumes in the semi-arid tropics. ICRIAT Patancheru, India.
- Clarkson, D.T. and B. Hanson. 1980. The mineral nutrition of higher plants. *Annu. Rev. Plant Physiol.* 31:239-298.
- Clarkson, D.T., A.W. Robards, J. Sanderson, and C.A. Peterson. 1978. Permeability studies on epidermal-hypodermal sleeves isolated from roots of *Allium cepa* (onion). *Can. J. Bot.* 56:1526-1532.
- Clarkson D.T. and J. Sanderson. 1969. The uptake of polyvalent cation and its distribution in the root apices of *Allium cepa*: tracer and autoradiographic studies. *Planta* 84:136-154.
- Clarkson D.T. and J. Sanderson. 1974. The endodermis and its development in barley roots as related to radial migration of ions and water. p. 87-100. In Kolek (ed.) *Structure and Function of Primary Root Tissues*. Veda, Bratislava.
- Colbert, S.S., E.J. Jokela, and D.G. Neary. 1990. Effects of annual fertilization and sustained weed control on dry matter partitioning, leaf area, and growth efficiency of juvenile loblolly and slash pine. *For. Sci.* 36:905-1014.
- Comerford, N.B., A. Jerez, A.A. Freitas and, J. M. Montgomery. 1996. Soil water table, reducing conditions and hydrologic regime in a Florida flatwood landscape. *Soil Science* 161:194-199.
- Comerford, N.B. and D.G. Neary. 1991. Plant nutrient uptake sensitivity to changes in plant root parameters: implications for genotype performance. Report for the pine productivity research program. University of Florida. Soil Science Department.
- Comerford, N.B., P.S. Porter and J.A. Escamilla. 1994b. Use of theissen areas in models of nutrient uptake in forested ecosystems. *Soil. Sci. Soc. Am. J.* 58:210-215.
- Comerford, N.B., P.J. Smethurst, and J.A. Escamilla. 1994a. Nutrient uptake by woody root systems. *New Zealand Journal of Forestry Science.* 24(2/3):195-212.
- Coutts, M.P. 1982. The tolerance of tree roots to waterlogging. V. Growth of woody roots of *Sitka spruce* and lodgepole pine in waterlogged soil. *New Phytologist.* 90:467-476.

- Crider, F.J. 1933. Selective absorption of ions confined to young roots. *Science* 78:169.
- Curl, E. A., and B. Truelove. 1986. The structure and function of roots. In B. Yaron (ed.) *The rhizosphere "Chap. 2", Advanced series in agricultural sciences, Vol. 15* Springer-Verlag, New York.
- Darrah, P.R. 1993. The rizosphere and plant nutrition: a quantitative approach. *Plant and Soil* 155/156:1-20.
- Dighton, J., and A.F. Harrison. 1990. Changes in phosphate status of Sitka spruce plantations of increasing age, as determined by root bioassay. *Forest Ecol. Manag.* 31:35-44.
- Dittmer H.J. 1949. Root hair variations in plant species. *Amer. J. Bot.* 36:152-155.
- Drew, M. C. 1988. Effects of flooding and oxygen deficiency on plant mineral nutrition. p. 115-119. In B. Tinker and A. Läuchli (eds.). *Advances in plant mineral nutrition. Vol. III.* Praeger Publishing. New York.
- Dumbroff, E.B., and D.R. Pierson. 1971. Probable sites for passive movement of ions across the endodermis. *Can. J. Bot.* 49:35-38.
- Dunlop, J. 1989. Phosphate and membrane elctropotentials in *Trifolium repens* L. *J. Exp. Bot.* 40:803-807.
- Eissenstat, D.M., and K.C.J. Van Rees. 1994. The growth and function of pine roots. *Ecollogical Bulletins* 43:76-91.
- Epstein, E. 1972. *Mineral nutrition of plants. Principles and perspectives.* Wiley, New York.
- Escamilla, J.A., N.B. Comerford, and D.G. Neary. 1991. Spatial pattern of slash pine roots and its effects on nutrient uptake. *Soil Sci. Soc. Amer. J.* 55:1716-1722.
- Ewel, K.C., and H.L. Gholz. 1991. A simulation model of the role of below ground dynamics in a Florida pine plantation. *For. Sci.* 37:397-438.
- Fisher, H.M. and E.L. Stone. 1990a. Air conducting porosity in slash pine roots from saturated soils. *For. Sci.* 36:18-33.
- Fisher, H.M. and E.L. Stone. 1990b. Active potassium uptake by slash pine root from O₂ depleted solutions. *For. Sci.* 36:582-598.

- Foehse, D., N. Claassen, and A. Jungk. 1988. Phosphorus efficiency of plants: 1. External and internal P requirements of different plant species. *Plant Soil*. 110:101-109.
- Fox, T.R., and N.B. Comerford. 1990. Low-molecular weight organic acids in selected forest soils of the southern USA. *Soil Sci. Soc. Amer. J.* 54:1139-44.
- Fox, T.R., N.B. Comerford, and W. McFee. 1990. Phosphorus and aluminum release caused by low-molecular-weight organic acids. *Soil Sci. Soc. Amer. J.* 54:1139-1144.
- Frohlich, M. 1984. Freehand sectioning with Parafilm. *Stain technology*. 59:61-62.
- Fusseder, A. 1987. The longevity and activity of of the primary root of maize. *Plant Soil* 101:257-265.
- Goutouly, P., and R. Habib. 1996. Diurnal and spatial variations of nitrate uptake by peach trees. *Agronomie*. 16:337-345.
- George, E. and H. Marschner. 1996. Nutrient and water uptake by roots of forest trees. *Z. Pflanzenernahr. Bodenk.* 159:11-21.
- Gholz, H.L., L.C. Hendry and W.P. Cropper Jr. 1986. Organic matter dynamics of fine root plantations of slash pine (*Pinus elliottii*) in North Florida. *Can. J. For. Res.* 16:529-538.
- Ginsburg, H. and Ginzburg B Z. 1970. Radial water and solute flow in roots of *Zea mays*. II. Ion fluxes across root cortex. *J. Expt. Bot.* 21:593-604.
- Glass, A.D.M., M. Saccomani, G. Crookall, and M.Y. Siddiqi. 1987. A microcomputer-controlled system for the automatic measurement and maintenance of ion activities in nutrient solutions during their absorption by intact plants in hydroponic facilities. *Plant Cell Environ.* 10:375-381.
- Glavac, V., and U. Ebben. 1986. Die Wurzelkammer, eine einfache Einrichtung zur experimentellen Nachprufung der Bodentoxizität an ausgewachsenen Baumen im Freiland. *Angew. Bot.* 60:95-102.
- Gronewald, J.W. and J.B. Hanson. 1980. Sensitivity of the proton and ion transport mechanisms of corn roots to injury. *Plant Sci. Lett.* 18:143-150.

- Harley, J.L. and E.L. Harley. 1987. A check-list of mycorrhiza in the British flora. *New Phytol.* 105 Supplement: 1-102.
- Harley, J.L. and S.E. Smith. 1983. *Mycorrhizal symbiosis.* Academic Press, New York.
- Head, G. C. 1967. Effects of seasonal changes in shoot growth on the amount of unsubsized root on apple and plum trees. *J. Hort. Sci.* 42:169-180.
- Hendrick, R.L., and K.S. Pregitzer. 1992. The demography of fine roots in a northern hardwood forest. *Ecology.* 73:1094-1104.
- Higinbotham, N., B. Etherton, and R.J. Foster. 1967. Mineral ion contents and cell membrane electropotentials of pea and oat seedling tissue. *Plant Physiol.* 42:37-46.
- Hodges, T.K. 1973. Ion absorption by plant roots. *Advances in Agronomy* 25:163-207.
- Hodges, T.K., R.L. Darding and T. Weidner. 1971. Gramicidin-D stimulated influx of monovalent cations into plant roots. *Planta.* 97:245-256.
- Hopkins, H.T. 1956. Absorption of ionic species of orthophosphate by barley roots: Effects of 2,4-dinitrophenol and oxygen tension. *Plant Physiol.* 31:155-161.
- Hopkins, H.T., A.W. Spetch, and S.B. Hendricks. 1950. Growth and nutrient accumulation as controlled by oxygen supply to plant roots. *Plant Physiol.* 25:193-198.
- Itoh, S. and S.A. Barber. 1983. A numerical solution of whole plant nutrient uptake for soil-root systems with root hairs. *Plant Soil* 70:403-413.
- Jackson, R.B., and M.M. Caldwell. 1992. Shading and the capture of localized soil nutrients: nutrient contents, carbohydrates and root uptake kinetics of a perennial tussock grass. *Oecologia (Berlin)* 91:457-462.
- Jalil, A. and R.M. Carlson. 1993. Potassium uptake by mariantha plum under limited oxygen and elevated carbon dioxide levels in the root atmosphere. *J. of Plant Nut.* 16:723-737.
- Jaunin, F. and R.M. Hofer. 1986. Root hair formation and elongation of primary maize roots. *Physiol. Plant.* 68:653-656.

- John, C.D., V. Limpinuntana, and H. Greenway. 1974. Adaptation of rice to anaerobiosis. *Aust. J. Plant Physiol.* 1:513-520.
- Jungk, A., C.J. Asher, D.G. Edwards, and D. Meyer. 1990. Influence of phosphate status on phosphate uptake kinetics of maize and soybean. *Plant Soil* 124:175-182.
- Jungk, A., and S.A. Barber. 1975. Plant age and the phosphorus uptake characteristics of trimmed and untrimmed corn root systems. *Plant Soil* 42:227-239.
- Jungk, A. and N. Claassen. 1986. Availability of phosphate and potassium as the result of interactions between root and soil in the rhizosphere. *Z. Pflanzenernaehr. Bodenk.* 149:411-427.
- Kelly, J.M. and S.A. Barber. 1991. Magnesium uptake kinetics in loblolly pine seedlings. *Plant Soil* 134:227-232.
- Keyes, M.R. and C.C. Grier. 1981. Above and below-ground net production in 40-year-old Douglas-fir stands on low and high productivity sites. *Can. J. For. Res.* 11:599-605.
- Koch, A.S., and E. Matzner. 1993. Heterogeneity of soil and soil solution chemistry under Norway spruce (*Picea abies* Karst.) and European beech (*Fagus silvatica* L.) as influenced by distance from the stem basis. *Plant and Soil* 151:227-237.
- Kochian, L.V., J.E. Shaff, and W.J. Lucas. 1989. High affinity K⁺ uptake in maize roots. A lack of coupling with H⁺ efflux. *Plant Physiol.* 91:1202-1211.
- Kozlowsky, T.T., and W.H. Scholtes. 1948. Growth of roots and root hairs of pine and hardwood seedlings in the piedmont. *J. For.* 46:750-754.
- Kramer, P.J. 1946. Absorption of water through suberized roots of trees. *Plant Physiol.* 21:37-41.
- Kramer, P.J., and H.C. Bullock. 1966. Seasonal variations in the proportions of suberized and unsuberized roots of trees in relation to the absorption of water. *Am. J. Bot.* 53:200-204.
- Lambers, H. 1987. Growth, respiration, exudation and symbiotic associations: The fate of carbon translocated to the roots. In P.J. gregory, J.V. Lake and D.A. Rose (eds.) *Root development and function. Soc. Exp. Bio. Seminar Series 30.* Cambridge University Press, Cambridge, UK.

- Larkum, A.W.D. and B.C. Loughman. 1969. Anaerobic phosphate uptake by barely plants. *J. of Exp. Bot.* 20:12-24.
- Lauchli A, U. Luttge, and M. G. Pitman. 1973. Ion Uptake and transport through barley seedlings: Differential effect of cyclohexamide. *Z. Naturforsch.* 28:431-434.
- Lechowicz, M.J., and G. Bell. 1991. The ecology and genetics of fitness in forest plants. 2. Microspatial heterogeneity of the edaphic environment. *J. Ecol.* 79:687-696.
- Lehninger, A.L. 1970. Biochemistry: The molecular basis of cell structure and function. Worth, New York.
- Li, X., E. George, and H. Marschner. 1991. Phosphorus depletion and pH decrease at the root-soil and hyphae-soil interfaces of VA mycorrhizal white clover fertilized with ammonium. *New phytologist.* 119:397-404.
- Lodge, D.J. 1989. The influence of soil moisture and flooding on formation of VA endo- and ectomycorrhizae in *Populus* and *Salix*. *Plant and Soil* 117:243-253.
- Loneragan, J.F. and Asher, C.J. 1967. Response of plants to phosphate concentrations in solution culture. II. Rate of phosphate absorption and its relation to growth. *Soil Sci.* 103:311-318.
- Lyford, W. H. 1975. Rhizography of non-woody roots of trees in the forest floor. p. 179-196. *In* J. G. Torrey and D. T. Clarkson (eds.). *The Development and Function of Roots.* Academic Press, London.
- Lyford, W.H. and B.F. Wilson. 1966. Controlled growth of forest tree roots: technique and application. Harvard Forest Paper No. 16.
- Maathuis, F.J.M. and D. Sanders. 1993. Energization of potassium uptake in *Arabidopsis thaliana*. *Planta* 191:302-307.
- MacKenzie, B.E., and C.A. Peterson. 1995a. Root browning in *Pinus banksiana* Lamb. and *Eucalyptus pilularis* Sm. 1. Anatomy and permeability of the white and tannin zones. *Botanica Acta.* 108:127-137.
- MacKenzie, B.E., and C.A. Peterson. 1995b. Root browning in *Pinus banksiana* Lamb. and *Eucalyptus pilularis* Sm. 2. Anatomy and permeability of the cork zone. *Botanica Acta.* 108:138-143.

- Marschner, H. 1991. Plant-soil relationships: acquisition of mineral nutrients by roots from soils. p.125-155. In J.R. Porter and D.W. Lawlor (eds.). Plant growth: Interactions with nutrition and environment. Univ. Press, Cambridge.
- Marschner, H., M. Häussling and E. George. 1991. Ammonium and nitrate uptake rates and rhizosphere pH in non-mycorrhizal roots of Norway spruce [*Picea abies* (L.) Karst.] Trees 5:14-21.
- McCully, M.E. 1975. The development of lateral roots. p. 105-124. In J.G. Torrey and D.T. Clarkson (eds.). The Development and function of roots. Academic Press, New York.
- McKevlin, M.R., D.D. Hook, W.H., McKee Jr., S.U. Wallace, and J.R. Woodruff. 1987. Loblolly pine seedling root anatomy and iron accumulation as affected by soil waterlogging. Can. J. For. Res. 17:1257-1264.
- Mengel, K. 1974. Ion uptake and translocation. p. 83-100. In E.W. Carson (ed.). The plant root and its environment. Univ. Press, Charlottesville, VA.
- Mexal, J.G., C.C.P. Reid, and E.J. Burke. 1979. Scanning electron microscopy of lodgepole pine roots. Bot. Gaz. 140:318-323.
- Moon G.J., B.F. Clough, C. A. Peterson, and W.G. Allaway. 1986. Apoplastic and symplastic pathways in *Avicennia marina* (Forsk) Vierh. roots revealed by fluorescent tracer dyes. Aust. J. Plant Physiol. 13:637-648.
- Morris, L.A. and R.E. Miller. 1994. Evidence for long-term productivity changes as provided by field trials. p. 41-80. In W.J. Dyck, D.W. Cole and N.B. Comerford (eds.). Impacts of forest harvesting on long term site productivity. Chapman and Hall, London.
- Murphy, J. and J. P. Riley. 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chem. Acta 27:31-36.
- Nayakekoralala, H. and H.M. Taylor. 1990. Phosphorous uptake rate of cotton roots at different growth stages from different soil layers. Plant Soil 122:105-110.

- Neary, D.G., E. J. Jokela, N.B. Comerford, S.R. Colbert and T.E. Cooksey. 1990. Understanding competition for soil nutrients-the key to site productivity on southern Coastal Plain Spodosols. p. 432-450. In S.P. Gessél, D.S. Lacate, G.F. Weetman and R.F. Powers (eds.). Sustained productivity of forest soils. Proceedings of the 7th North American Forest Soil Conference. University of British Columbia, Vancouver, B.C.
- Newman, E. I. 1985. The rizosphere: Carbon sources and microbial populations. p. 107-122. In A.H. Fitter (ed.). Ecological interactions in Soil. Blackwell Scientific Publications, London.
- Newman, I.A., L.V. Kochian, M.A. Grusak, W. J. Lucas. 1987. Fluxes of H^+ and K^+ in corn roots. Plant Physiol. 84: 1177-1184.
- Niederholzer, F.J.A., S.A. Weinbaum, and T.M. Dejong. 1994. A novel method of growth and nutrient uptake by field grown tree roots. p.388. Agronomy Abstracts. ASA,CSSA,SSSA. Madison, WI.
- Nielsen, N.E., and S.A. Barber. 1978. Differences among genotype of corn in the kinetics of phosphorus uptake. Agron. J. 70:695-698.
- Nissen, P. 1980. Multiphasic uptake of potassium by barely roots of low and high potasium content: separate sites for uptake and transitions. Physiol. Plant. 48:193-200.
- Noggle, J.C. and M. Fried. 1960. A kinetic analysis of P absorption by excised roots of millet, barley, and alfalfa. Soil. Sci. Soc. Amer. Proc. 24:315-324.
- Nye, P.H. and P.B. Tinker. 1977. Solute movement in the soil-root system. Univ. California Press. Berkley.
- Oaks, A. 1992. A re-evaluation of nitrogen assimilation in roots. Biosci. 42:103-111.
- Omata, T., M. Ohmori, N. Arai, and T. Ogawa. 1989. Genetically engineered mutant of the *Cyanobacterium synechococcus* PCC 7942 defective in nitrate transport. Proc. Natl. Acad. Sci. (USA) 86:6612-6616.
- Ott, L. An introduction to statistical methods of data analysis. 1988. (Third ed). 835 p. PWS-Kent Publishing Company, Boston.

- Perumalla, C.J. and C.A. Peterson. 1986. Deposition of Casparian bands and suberin lamellae in the exodermis and endodermis of young corns and onion roots. *Can. J. Bot.* 64:1873-1878.
- Peters, R.L. 1990. Effects of global warming on forests. *For. Ecol. Manage.* 35:13-33.
- Peterson, C.A., M.E. Emanuel, and G.B. Humphreys. 1981. Pathway of movement of apoplastic fluorescent dye tracers through the endodermis at the site of secondary root formation in corn (*Zea mays*) and broad bean (*Vicia faba*). *Can. J. Bot.* 59:618-625.
- Pettersson, S. 1981. Passive uptake of K^+ ($^{86}R^+$) in sunflower roots at low external K^+ concentrations. *Physiol. Plant.* 52:431-436.
- Pettersson, S., and P. Jensen. 1979. Regulation of rubidium in sunflower roots. *Physiol. Plant.* 45:83-87.
- Philipson, J.J. and M.P. Coutts. 1978. The tolerance of trees to waterlogging. III. Oxygen transport in Sitka spruce and lodgepole pine roots of primary structure. *New Phytol.* 80:341-349.
- Phillips, L.P., N.B. Comerford, D.G. Neary and R.S. Mansell. 1989. Simulation of soil water table above a water table in a forested Spodosol. *Soil Sci. Soc. Am. J.* 53:1236-1241.
- Pitman, P.G., N.Schaefer, and R.A. Wildes. 1975. Stimulation of H^+ efflux and cation uptake by fusaric acid in barley roots. *Plant Sci. Lett* 4:323-329.
- Polglase, P.J., E.J. Jokela., and N.B. Comerford. 1992. Nitrogen and Phosphorus release from decomposing needles of southern pine plantations. *Soil. Sci. Soc. of Amer.* 56:914-920.
- Pritchett, W.L. 1979. Properties and Management of Forest Soils. John Wiley & Sons. New York, New York.
- Pritchett, W.L., and N. B. Comerford. 1982. Long-term response to phosphorus fertilization on southeastern coastal plain soils. *Soil Sci. Soc. Am. J.* 46:640-644.
- Richards, D. and J. A. Considine. 1981. Suberization and browning of grapevine roots. p. 111-115. In R. Brouwer, O. Gasparikova, J. Kolek, and B. C. Loughman (eds.) Structure and function of plant roots, Proc. of the 2nd International Symposium, Bratislava, Czechoslovakia. Martinus Nijhoff / DR W. Junk Publishers. The Hague/Boston/London.

- Robards A. W., and S. M. Jackson. 1976. Root structure and function: An integrated approach. p 413-422. In N. Sunderland (ed.). Perspectives in Experimental Biology. Vol. 2. Botany. Pergamon Press, Oxford.
- Rosen, C. J., and R. M. Carlson. 1984. Influence of root zone oxygen stress on potassium and ammonium absorption by Myrobalan plum rootstock. Plant Soil 80:345-353.
- Rousseau, J.V.D., D.M. Sylvia and A.J. Fox. 1994. Contribution of ectomycorrhiza to the potential nutrient-absorbing surface of pine. New Phytol. 128:639-644.
- Sanderson, J. 1983. Water uptake by different regions of the barley roots: pathways of radial flow in relation to development of the endodermis. J. Exp. Bot. 34:240-253.
- SAS Institute. 1985. SAS User's Guide. Statistics. Version 5 Ed. SAS Inst., Cary, NC.
- Serrano, R. 1990. Recent molecular approaches to the physiology of the plasma membrane proton pump. Bot. Acta. 103:230-234.
- Shone, M.G.T., and D.T. Clarkson. 1986. Rectification of radial water flow in the hypodermis of nodal roots of *Zea mays*. Plant Soil 111:223-229.
- Sisson, W.B. 1983. Carbon balance of *Yucca elata* Engelm during a hot and cool period in situ. Oecologia 57:352-360.
- Smethurst, P.J. and N.B. Comerford. 1993. Potassium and phosphorus uptake by competing pine and grass: observations and model verifications. Soil Sci. Soc. Am. J. 57:1602-1610.
- Smethurst, P.J. and E.K.S. Nambiar. 1990. Effects of slash and litter management on fluxes of nitrogen and tree growth in a young *Pinus radiata* plantation. Can. J. of For. Res. 20:1498-1507.
- Stevenson, F.J. 1986. Cycles of soil: Carbon, nitrogen, phosphorus, sulfur, micronutrients. John Wiley & Sons, New York.
- Steward, F.C., W.E. Berry, and T.C. Broyer. 1936. The absorption and accumulation of solutes by living plant cells. VIII. The effect of oxygen upon respiration and salt accumulation. Annals Bot. 50:345-366.

- Sutton, R.F., and R.T. Tinus. 1983. Root and root system terminology. Forest Science Monograph 24. Supplement to Vol. 29. 137 p.
- Swindel, B.F., D.G. Neary, N.B. Comerford, D.L. Rockwood, and G.M. Blakeslee. 1988. Fertilization and competition control accelerated early southern pine growth on flatwoods. South. J. Appl. For. 12:116-121.
- Thompson, C.J., B.J. Atwell, and H. Greenway. 1989. Response of wheat seedlings to low O_2 concentrations in nutrient solution. II. K^+/Na^+ selectivity of root tissues of different age. J. Exp. Bot. 40:993-999.
- Topa, M.A. and J.M. Cheeseman. 1993. ^{32}P uptake and transport to shoots in *Pinus serotina* seedlings under aerobic and hypoxic growth conditions. Physiol. Plant. 92:171-180.
- Topa, M.A. and K.W. McLeod. 1986a. Aerenchyma and lenticel formation in pine seedling: A possible avoidance mechanism to anaerobic growth conditions. Physiol. Plant. 68:540-550.
- Topa, M.A. and K.W. McLeod. 1986b. Effects of anaerobic growth conditions on phosphorus tissue concentrations and absorption rates of southern pine seedlings. Tree Physiol. 2:327-340.
- Troughton A. 1957. The underground organs of herbage grasses. Bull 44. Imp. Bur. of Plant Genetics. Com. Agric. Bur. Farnham Royal.
- Ullrich-Eberius, C.I., A. Novacky., E. Fischer, and U. Luttge. 1981. Relationship between energy dependent phosphate uptake and the electrical membrane potential in *Lemna gibba* Gl. Plant Physiol. 67:797-801.
- van Praag, H.J., S. Sougnez-Remy., F. Weissen, and G. Carletti. 1988. Root turnover in a beech and a spruce stand of the Belgian Ardennes. Plant and Soil. 105:87-103.
- Van Rees, K.C.J., and N.B. Comerford. 1986. Vertical root distribution and strontium uptake of a slash pine stand on a Florida Spodosol. Soil Sci. Soc. Am. J. 50:1042-1046.
- Van Rees K.C.J., and N. B. Comerford. 1990. The role of woody roots of slash pine seedlings in water and potassium absorption. Can. J. For. Res. 20:1183-1191.

- Van Rees, K.C.J., N.B. Comerford and W.W. McFee. 1990. Modeling potassium uptake by slash pine seedlings from low-potassium-supplying soils. *Soil Sci. Soc. Am. J.* 54:1413-1421.
- Vess, R.W., R.L. Anderson, J.H. Carr, W.W. Bond and M.S. Favero. 1993. The colonization of solid PVC surfaces and the acquisition of resistance to germicides by water micro-organisms. *J. of Appl. Bact.* 74:215-223.
- Waring, H.D. 1981. Forest fertilization in Australia: Early and Late. p. 201-217. In N.D. Turvey, (ed.). *Proceedings of the Australian Forest Nutrition Workshop, Productivity in Perpetuity*. CSIRO. Division of Forest Research, Canberra Australia.

BIOGRAPHICAL SKETCH

What? tell you my life story? Why?
who would be interested in that?
besides I got a very bad memory.
I am sure I forget a lot of important details.
I mean unimportant details.....
because I don't think anything very important
ever happened to me.
Of course I can tell you important things,
but what I mean is that,
how can I explain it to you?
It is like I have forgotten everything,
Of course I could tell you that.....

My name is José Armando Escamilla Bencomo. I was born on May 21, 1960, in Mérida, Yucatán, México, the second child of a family of ten. I graduated from Xmatkuil-Mérida High School in 1978. After graduation, I accepted a scholarship from "The Pan-American Agriculture School" (Zamorano), Honduras. In December of 1981, I completed a three year college degree in Zamorano with honors.

In the Fall of 1982, I transferred to the University of Florida, Gainesville, USA, and earned a Bachelor of Science in Agriculture with a soil science major in December of 1983. In January of 1984, I accepted a position at "The Pan-American Agriculture School" (Zamorano), Honduras. I worked there for two years as an instructor in soils and running a soil testing lab.

With an interest in soil fertility, I entered the "hidden half", the world of plant roots, in August of 1988, to pursue graduate studies with Dr. Nick Comerford of the

University of Florida, Gainesville, Fl. I completed my Master of Science program in August of 1990.

After receiving my MSc. degree, I worked for the Centro de Investigación Científica de Yucatán (CICY), México, for 3 years. In the summer of 1993, I was re-admitted to the Graduate School of the University of Florida to work toward my Ph.D. degree in rhizosphere dynamics of Forest Soils.

Of course I could tell you more,
you know, I realized that actually you can do
what you really want to do,
and that's happiness, don't you agree?
Now it is time to turn the page, right?

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Nicholas B. Comerford, Chair
Professor of Soil and Water
Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

P. Suresh C. Rao
Graduate Research Professor
of Soil and Water
Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Konda R. Reddy
Graduate Research Professor
of Soil and Water
Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

David M. Sylvia
Professor of Soil and Water
Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Kimberlyn Williams
Assistant Professor of
Botany

This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

December, 1997

Dean, College of Agriculture

Dean, Graduate School