ANATOMICAL CHARACTERISTICS OF ROOTS OF LOBLOLLY PINE SEEDLINGS

By

PREM KUMAR

Bachelor of Science
University of Calicut
Calicut, Kerala, India
1974

Master of Science
University of Calicut
Calicut, Kerala, India
1976

Master of Science
Oklahoma State University
Stillwater, OK, USA
1999

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Thesis adviser

Dean of the Graduate College

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TABLE OF CONTENTS

LIST OF TABLES

CHAPTER PAGE

LIST OF FIGURES

CHAPTER PAGE

I. Introduction

II. Environmental effects on the anatomy of first-order lateral roots (FOLR) of loblolly pine seedlings

III. Development of absorbing surface area of roots of loblolly pine seedlings

CHAPTER - I

INTRODUCTION

Significance of roots

Roots perform various mechanical as well as physiological functions. They spread out extensively in the soil and absorb water and minerals required for growth. They provide mechanical support to the growing shoot and store carbohydrates. Roots may send signals of rhizospheric conditions to the shoot to illicit water conservation responses. As a result, roots have an integral role in the soil-plant-atmosphere continuum that determines the amount of soil water accessed by the plant.

The wealth of information about expenditures on irrigation, fertilization, and other cultural operations relate mainly to crop production. An understanding of the physiology of plant roots will help to determine optimum input and reduce waste (Smit *et al.* 2000). Information on the allocation of resources to shoots and roots and to different categories of roots and ectomycorrhizae is very limited. To further understanding of the interactions of plants with their environment we need detailed knowledge about root functioning (Atkinson 1996). The root system

represents the major pathway for the flow of carbon to soil organisms in the rhizosphere involved in many key processes (Smit *et al.* 2000). Due to an increasing amount of carbon dioxide in the atmosphere, there may be changes in the response of plants and their interaction with soil microbes (Curtis *et al.* 1995). Moreover, roots and associated microflora have a major effect on soil structure and stability of aggregates (Miller and Jastrow 1992). The input of organic matter to soil will influence many properties of soil (Smit *et al.* 2000). In tropical production systems roots represent an important energy source as firewood and provide many root products to the pharmaceutical industry (Smit *et al.* 2000). The interactions of plants within and between species are viewed as means of improving the efficiency of resource use by roots, especially in multi-crop systems (Atkinson *et al.* 1976, Caldwell 1987, and Smit *et al.* 2000)

Roots perform many indirect functions that have ecological significance. Although plants survive in diverse conditions, all of them do not perform equally well on all sites. Some plants have specific requirements for environmental conditions. Why do some plants require a unique set of environmental conditions while others do not? (Smit *et al.* 2000). As roots are the link between aerial parts and soil, to answer such questions the physiology and functioning of roots must be examined in detail (Atkinson 1996). In the past much attention was focused on biomass partitioning between roots and shoots and many studies were focused on older trees in the forest ecosystem. Recently more attention has been directed towards adaptation of roots to environmental stresses and root-

shoot communication (Kramer and Boyer 1995). Nevertheless, little information is available on the anatomical changes that lead to morphological and physiological adaptations in plant roots.

Root morphology

The newly produced white roots of seedlings turn brown as they age and become bigger in size due to secondary growth. This change in color was used as the criterion to classify roots as 'suberized' or brown and 'unsuberized' or white (Chung and Kramer 1975, Kramer and Bullock 1966, Sands *et al.* 1982, and van Rees and Comerford 1990). A more reliable classification was suggested by McKenzie and Peterson (1995a, b). They identified three characteristic zones in the roots of pouch-grown jack pine (*Pinus banksiana* Lamb.) and a eucalyptus (*Eucalyptus pilularis* Sm.). The distal end of roots comprising young meristematic tissue was called the 'white zone' (Fig. 1). Proximal to this zone was the 'tannin zone', more appropriately called 'condensed tannin (CT) zone' that appears tan or brown colored as the name suggests. This was followed basipetally by the 'cork zone'. The same classification of three zones was applied to loblolly pine, *Pinus taeda* (Peterson *et al.* 1999). Each of these zones has its own characteristic anatomical feature that has a significant role in the absorption of water and nutrients (McKenzie and Peterson 1995a, b). Maturation of these zones is compressed towards the tip in slow growing roots that encounter adverse growth conditions (Peterson *et al.* 1999).

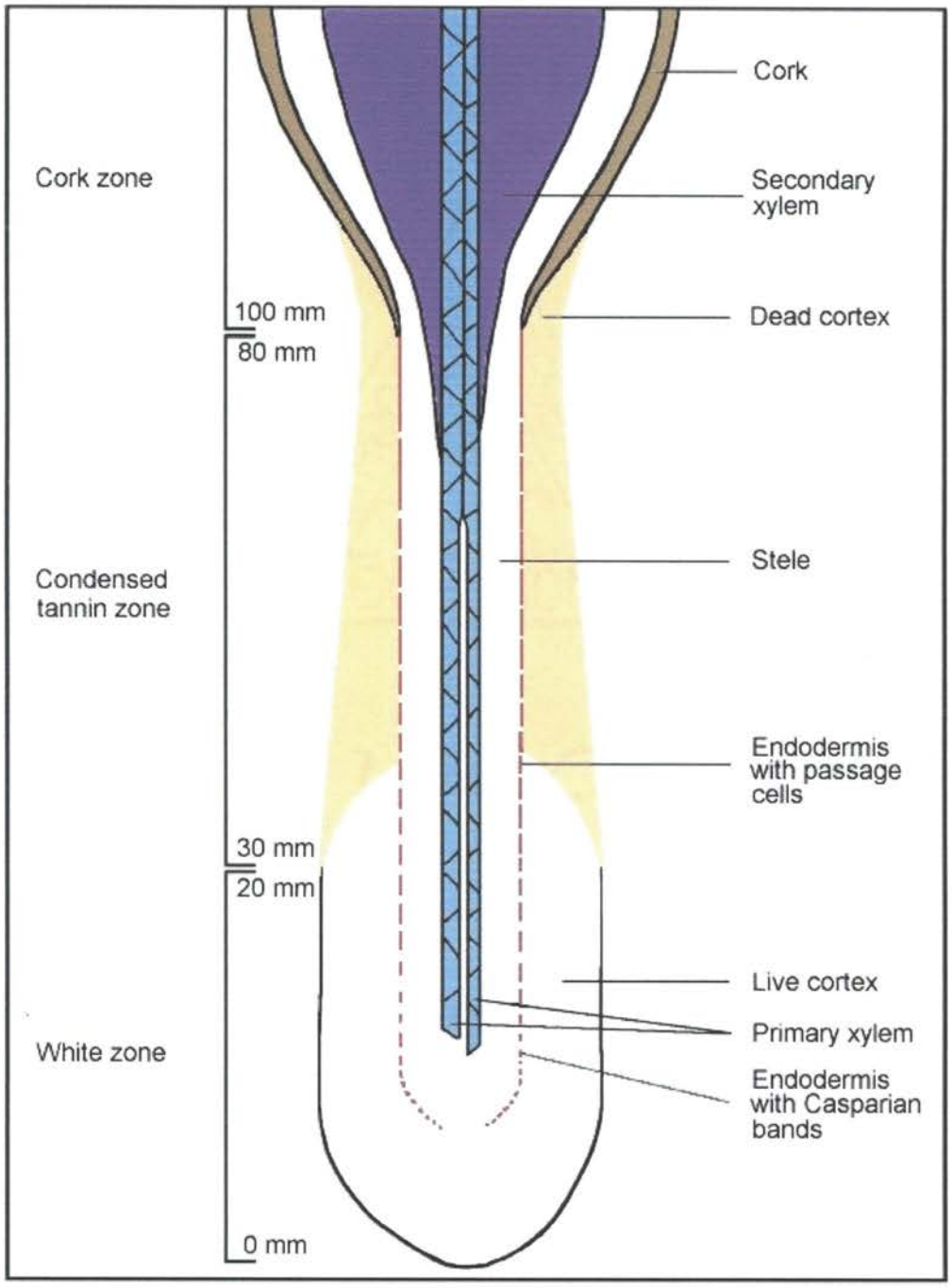

Fig. 1. Diagrammatic representation of a typical pine root showing different root zones and internal anatomy (Redrawn from Peterson *et al.* 1999).

Root anatomy

The cross section of a typical pine root contains different types of cells (Fig. 2). There is no well-developed epidermis in pine roots as it is sloughed off during early stages of root development (Mirov 1967). Therefore, the outer root is a multilayered cortex limited internally by a single layered endodermis. The cortex contains live cells that are circular in cross section and elongated in the axial direction. The outer cells are smaller in diameter and those adjacent to the endodermis are larger.

The innermost layer of the cortex is a single layered endodermis comprising cells that are more or less rectangular in cross section. The endodermis encloses a central stele comprising the vascular tissues of xylem and phloem. The radial and transverse or end walls of endodermal cells are thickened with Casparian bands, named after the German botanist, Robert Caspary (Sutton and Tinus 1983). The Casparian band consists of suberin, lignin, or both that infiltrate the spaces in the primary wall normally occupied by water (Peterson and Enstone 1996). On the inner tangential walls the endodermal cells are deposited with thin sheets of suberin lamellae (suberization) in a progressive manner. The Casparian bands and suberization prevent apoplastic movement of water and ions into or out of the stele (Peterson *et al.* 1999). The unsuberized cells are called passage cells. The development of endodermis occurs in three distinct stages (Clarkson and Robards 1975 and van Fleet 1961, Fig. 3). During the initial stage (State I), cells are considered

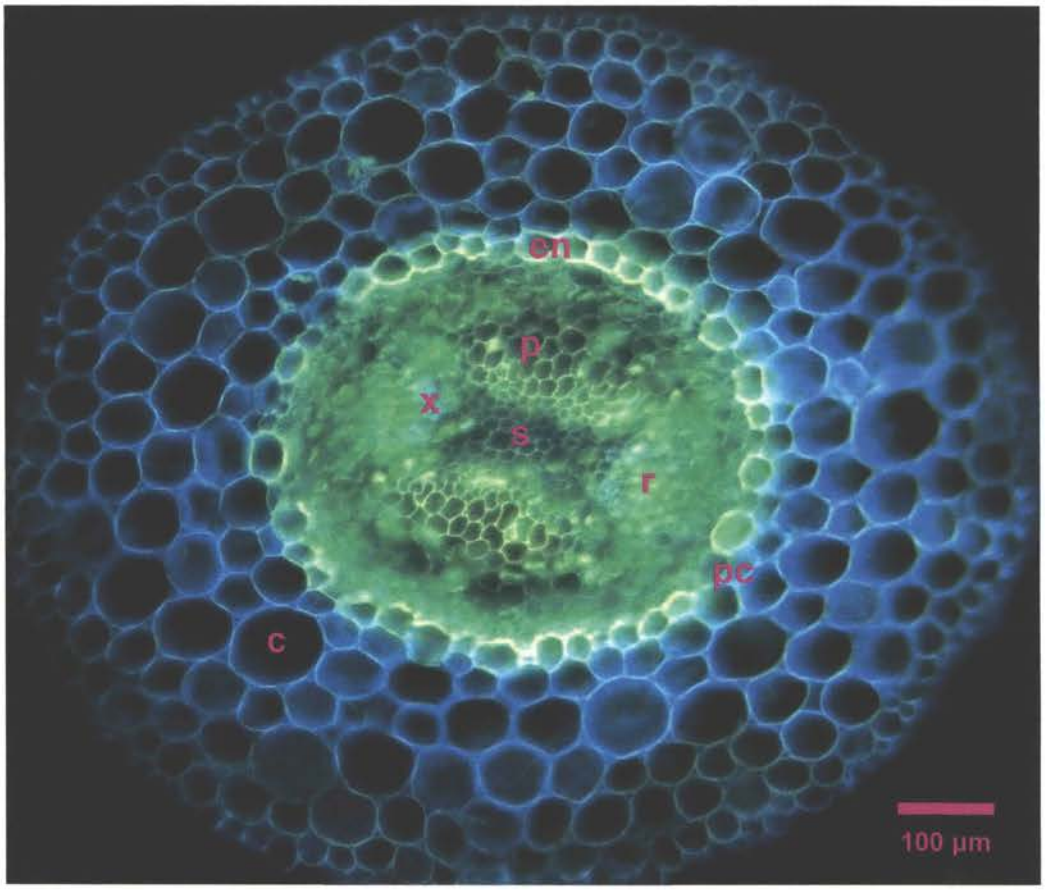

Fig. 2. Cross section at 20 mm from the tip of a typical pine root stained with Fluorol Yellow 088 (100x). The multilayered outer cortex (c) is distinguished from the central stele (s), by a single-layered endodermis (en) that contains suberized cells and unsuberized passage cells (pc). The central stele contains vascular tissues of xylem (x) associated with a resin canal (r) and phloem (p) alternating with it.

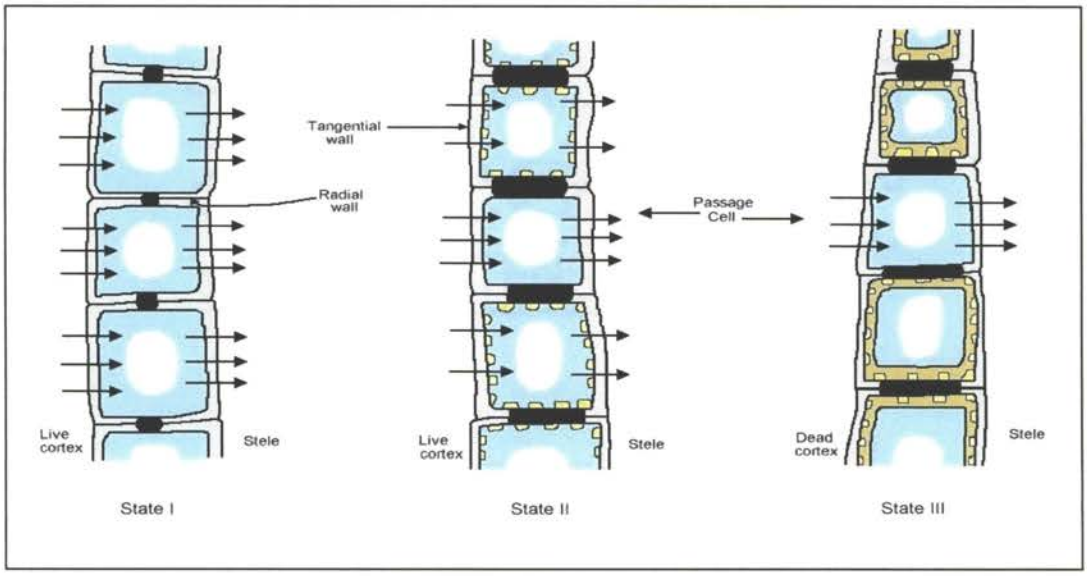

Fig. 3. Stages in the development of endodermal cells (⟶ = hypothesized water flow from cortex to stele through unsuberized cells, ■ = Casparian band, □ = cell wall, ▭ = primary suberin deposition, and ▭ = secondary suberin deposition. Endodermal cell cross sections were nearly square, 0.025 mm on each side).

mature when they develop a Casparian band on their radial and transverse or end walls. During the second stage (State II), endodermal cells continue maturation by the deposition of suberin lamellae on the inner tangential walls. The final stage (State III) of development is the production of a thick, cellulosic wall (sometimes called a tertiary wall) internal to the suberin lamellae. This may be suberized or lignified. Maturation of the cells from State I to State III is typically asynchronous, occurring first in the cells aligned radially with phloem. Although development of suberin lamellae and thick secondary walls prevent movement of soil solution through the apoplast, there is symplastic continuity. This is achieved through plasmodesmata connecting endodermis with live cortex on the outside and pericycle on the inside that remain intact during the deposition of suberin lamellae (Clarkson *et al.* 1971 and Robards *et al.* 1973).

A thin pericycle just beneath endodermis encircles the vascular tissues of xylem and phloem. When all cells of the endodermis become suberized, a layer of cork cambium begins to form in the pericycle. The cork cambium produces cork cells centrifugally crushing the endodermis. The xylem is a multicellular tissue that appears in the form of a crescent. It conducts water and nutrients to the shoot. The tips of the crescent face to the periphery and contain protoxylem elements while the apex is centripetal and contains metaxylem elements. The number of xylem poles usually varies from 2 to 5 in *Pinus taeda* (Enstone *et al.* 2001). A resin canal is usually seen within the space between the tips of each

crescent. The phloem alternates with tracheary elements and conducts photosynthates from the shoot to the roots.

White zone: As the term implies, this most distal portion of the root appears white to the unaided eye (Peterson *et al.* 1999). The tip of the root is swollen compared to the proximal portion and comprises meristematic tissue engaged in active cell division and multiplication (McCrady and Comerford 1998). The swollen tip tapers to form a pointed tip protected by a root cap that penetrates through soil.

A transverse section of a root at the white zone comprises a multilayered cortex limited by a single layered endodermis. The cortex cells in the white zone are alive and therefore, they contain large plasmalemma surfaces available for the absorption of water and nutrients (Taylor and Peterson 2000). The endodermis contains Casparian bands on their radial and transverse walls and hence it is fully matured. The deposition of suberin lamellae commences in the white zone on the inner tangential walls of endodermis aligned with phloem.

Condensed tannin zone: Basipetal to the white zone is the CT zone and it is generally tan or brown due to deposition of condensed tannins. The diameter of roots at the CT zone usually declines due to death and collapse of outer cortical cells. Taylor and Peterson (2000) reported that in one-year-old jack pine

seedlings most root length (74 %) existed in the CT zone and similar observations were made in loblolly pine seedlings.

The anatomy of the CT zone is different from that of the white zone. The cortex cells are dead and the death commences from the periphery (Peterson *et al.* 1999). They contain proanthocyanidins in their walls that give brown color to the root. In most cases cells lose their turgidity and some of them disintegrate depending on the age of the root and severity of rooting environment. As cortical cells die the symplastic pathway is eliminated and water can only pass through dead cell walls and spaces up to the endodermis. Although the death of cortical cells results in a reduction in resistance, there is a decline in the radial flow of water along the protoplasm due to elimination of the symplastic pathway (Enstone *et al.* 2001). The endodermis contains mostly suberized cells and the number of passage cells is limited. The passage cells are mostly aligned with xylem poles and their outer tangential walls become the only living membrane having direct access to soil solution. Therefore, there is a significant reduction in the absorptive surface of living cells in the CT zone compared to that in the white zone. At the time of secondary growth a vascular cambium arises at the proximal end of the CT zone (Enstone *et al.* 2001). As a result the development of xylem is more advanced in the CT zone compared to that in the white zone. The maturation of xylem progresses in a centripetal direction and metaxylem of each pole meets at the center.

Cork zone: The cork zone is oriented basipetal to the CT zone. It is darker in color and characterized by the presence of secondary growth. When all cells in the endodermis are suberized a layer of cork cambium arises just beneath the endodermis within the pericycle. This is the region where the CT zone becomes cork zone. As a result root diameter increases by the addition of more cork cells on the outside and tracheary elements at the center. The secondary growth occurs by the combined action of vascular cambium and cork cambium. According to Esau (1977) cork cells in roots typically arise in the outer stelar cells and thus they are produced interior to the endodermis. They are similar to the cells of shoot bark and comprise a multilayered tissue of dead cells that had suberin lamellae deposited on their walls (Sitte 1962). Studies have proved that cork cells of roots have a Casparian band-like structure in their primary walls (McKenzie and Peterson 1995b).

As there are no live cortex cells or endodermal passage cells in the cork zone, radial entry of water is inhibited. The capacity of cork roots to conduct water has been under debate because most roots in large plants contain a cork zone particularly during dormant periods. The possible pathways for radial entry of water may be through lenticels, openings created by lateral root emergence, and wounds in the roots (Addoms 1946).

Ectomycorrhizae

The term mycorrhiza, which literally means *fungus-root*, was first applied to fungus-tree associations described in 1885 by the German forest pathologist, A. B. Frank (Sylvia 1990). Mycorrhizal associations are characteristic of many plant roots and there are different types of mycorrhizal associations. In pine roots the symbiotic relationship is associated with ectomycorrhizal fungi belonging mostly to Basidiomycetes (Sylvia 1990, 1998). Propagules of fungi in the soil come in contact with young roots and on germination they produce fungal hyphae. The hyphae multiply within intercellular spaces of cortical cells without entering cell lumens or the stele (Clarkson and Robards 1975). Three components of ectomycorrhizal fungi are involved in enhancing the absorptive capacity of roots (Piché *et al.* 1983). Within the cortex hyphae multiply and form a labyrinthine structure called the Hartig net, named after Robert Hartig, the father of forest biology (Sylvia 1990, 1998). Peripheral to this the root surface is covered with a mantle or sheath. Many slender hyphae extend into the soil protruding from the mantle. Soil solution absorbed by the slender hyphae and mantle is transported to the Hartig net wherein exchange into cortical cells occurs. Due to activity of the fungus further elongation of the host root is retarded. The root becomes forked with two white tips containing abundant fungal hyphae protruding into the soil.

Ectomycorrhizae have a significant role in extending the capacity of roots to take up water (Mudge *et al.* 1987 and Parke *et al.* 1983) and nutrients (Vogt *et al.* 1991). The role of ectomycorrhizae in enhancing absorption of water and

nutrients by pine roots has been described variously. Although several researchers found a negative or no effect of ectomycorrhizae on pine roots, most authors found a positive relation between the abundance of mycorrhizae and growth of seedlings. Marschner (1986) reported that young roots with living epidermal cells and root hairs are responsible for most nutrient uptake. According to Harley and Smith (1983) the host plant receives increased amounts of mineral nutrients from the soil while the fungus obtains photosynthetically derived carbon compounds from host plants. The presence of ectomycorrhizae is usually higher in nutrient-limiting environments and the fungi have the potential to greatly increase the absorbing surface of roots (Rousseau *et al.* 1994). The hyphae must be distributed beyond the nutrient depletion zone to have effective absorption. A nutrient depletion zone develops when nutrients are removed from the soil solution more rapidly than they can be replaced by diffusion (Sylvia 1990). One of the factors contributing to the effective absorption of nutrients by mycorrhizae is their narrow diameter. The narrow hyphae reach into small soil pores inaccessible to roots or even root hairs (Sylvia 1990). Another advantage is the access of hyphal strands to pools of phosphorus not readily available to the plant (Fox *et al.* 1990).

Root dormancy

Roots are exposed to changes in the environment. During adverse environmental conditions roots undergo various changes and the most striking feature is the presence of a metacutized (metacutinized) layer. Metacutization is

the process by which the endodermis extends to the tip enclosing the apical meristem (Wilcox 1954). The metacutized layer undergoes lignification and suberization. The layers of cells outside the metacutized layer die and sometimes slough off. Then the color of the entire root becomes brown. Dormancy is induced by stimuli resulting from adverse environmental conditions in the rhizosphere. Taylor and Peterson (2000) reported that dormancy occurs through the development of CT zone closer to tip under unfavorable conditions and the number of dormant roots is inversely correlated with availability of soil moisture. Wilcox (1964) suggested root dormancy as a programmed event, as dormancy occurred even in roots maintained under constant and ideal conditions. Metacutized roots often appear totally brown under field conditions. Metacutization has a protective role in preventing water loss from the roots back into the soil (Wilcox 1954, 1968). When conditions become favorable the apical meristem resumes activity extending through the metacutized layer, and develops into a new white tip.

Role of passage cells in absorption

Root resistance to water uptake comprises root geometry or configuration and root hydraulic properties and it is the major limiting factor in the uptake of water from soil by plant roots (Boyer 1985 and Landsberg and Fowkes 1978). The passage cells have a significant role in the radial entry of water into roots especially in the CT zone (Peterson *et al.* 1999). Therefore, the presence and number of passage cells in the root may determine the amount of water it can

conduct. The variation in the number of passage cells in different root zones is dependent on the maturity and season of root growth.

During the development of the endodermis those cells that remain in State I are called passage cells, and they are mostly aligned radially with protoxylem poles to facilitate transport of water and ions into the stele. The passage cells also develop Casparian bands like other cells of the endodermis on their radial and transverse walls (Grymaszewska and Golinowski 1987 and Scott and Peterson 1979), but the development is delayed (Peterson and Enstone 1996). Therefore, the passage cells have a significant role in the transport of water and ions into the stele offering areas of low resistance (Peterson and Enstone 1996). The movement of phosphate or potassium ions is not inhibited by the development of suberin lamellae, indicating that they move symplastically either through suberized or unsuberized (passage) cells (Clarkson *et al.* 1968, 1971). In contrast, movement of calcium and magnesium declines markedly when suberin lamellae are deposited on the walls (Harrison-Murray and Clarkson 1973 and Robards *et al.* 1973). Clarkson (1993) suggested that calcium in the cytoplasm of passage cells could be transferred into the stele through plasmalemmae lining the inner tangential walls of the endodermis by calcium-ATPase pumps. When epidermal and cortical cells die due to adverse rhizospheric conditions, the endodermis becomes the outermost living layer of the root. Then the plasmalemmae lining the outer tangential walls of passage

cells are the only sites where soil solution contacts the symplast (Peterson and Enstone 1996).

Information on the function of the passage cells is limited to a few species. Taylor and Peterson (2000) studied the relationship between the plasmalemma surface area of cortex cells and mycorrhizhal association in the roots of jack pine. The authors found that mycorrhizae contained the most plasmalemma surface area followed by roots containing a white zone. In the CT zone plasmalemma surface belonged only to outer tangential surfaces of passage cells due to death of cortical cells. As there was no live cortex and the endodermis was crushed, there was no plasmalemma surface in the cork zone. Although the CT zone contained no live cortex, the presence of passage cells in this zone was important in absorbing water and nutrients. This was because the CT zone contained most of the root length compared to other root zones and this was particularly true during adverse conditions. Therefore, the presence of passage cells in the CT zone was more important than that in the white zone.

Pathways of ion and water absorption

Three distinct transport routes (apoplastic, symplastic, and transcellular) facilitate the radial transport of water and ions into roots (Steudle 1994, Fig. 4). The apoplastic transport occurs through the walls of living walls, skirting the protoplasts of cells. The symplastic transport occurs through cytoplasm of individual cells and plasmodesmata linking adjacent cells. The transcellular

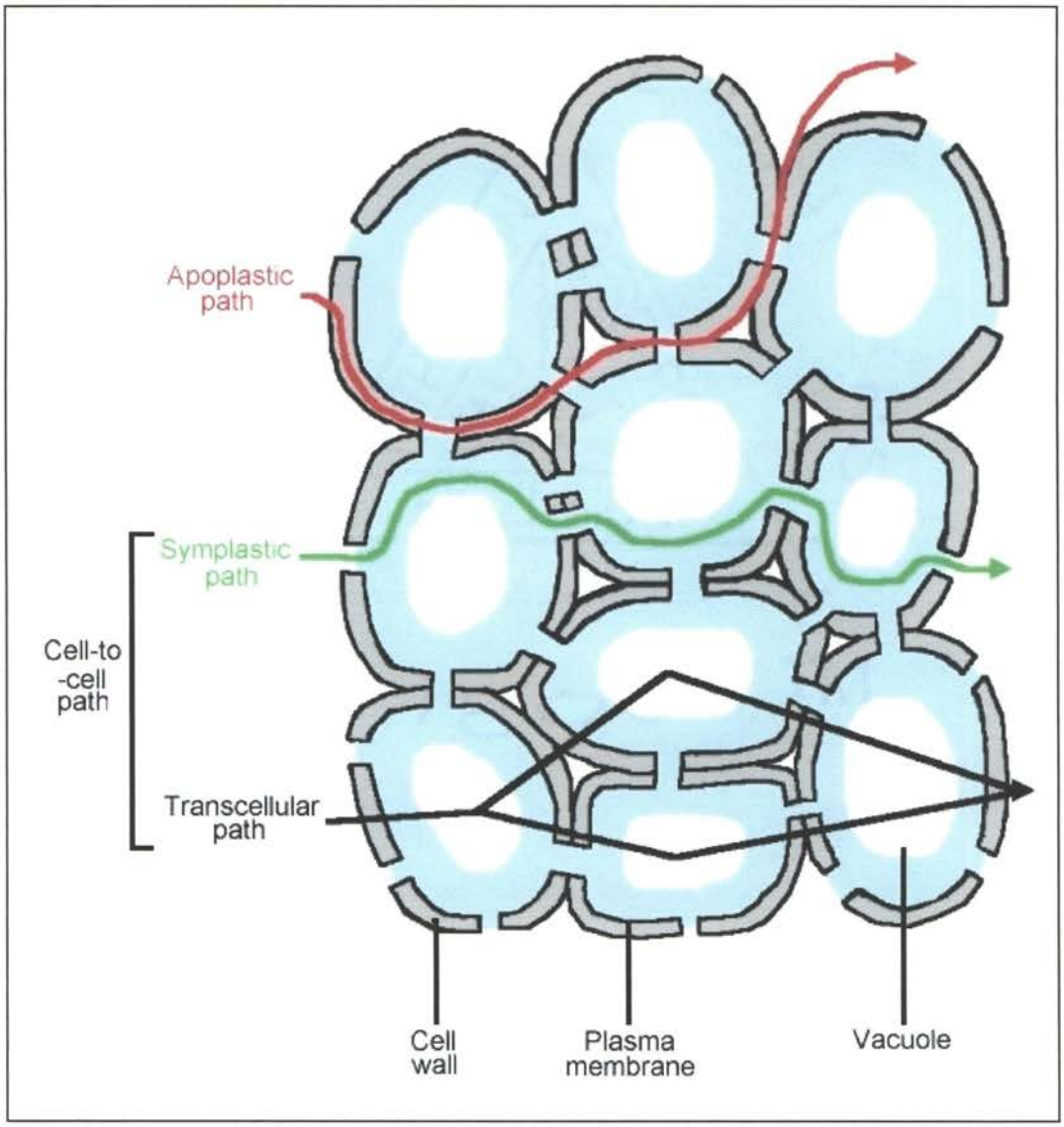

Fig. 4. Pathways of water transport through root cortical cells. The symplastic and transcellular pathways are collectively called the cell-to-cell pathway.

movement occurs when the substance passes through the plasmalemmae of cell walls, cytoplasm, and tonoplast lining the vacuoles, i.e. repeatedly through membranes. As a result, water crosses at least two cell membranes per cell layer. It must also include at least part of the apoplast as the substance moves from one cell to the next. Due to the difficulty in distinguishing between symplastic and transcellular movements, they are grouped together as cell-to-cell pathway. Several lines of evidence support the cell-to-cell pathway as the major route for the transport of water through unmodified parenchyma cells (Canny and Huang 1994 and Peterson *et al.* 1993). Therefore, the specific cells through which materials move in the radial path are cortical parenchyma, endodermis, pericycle and stelar parenchyma (Peterson *et al.* 1999). Ions initially diffuse into roots through walls of outermost cortex cells and then may be actively transported across the plasmalemmae. Ions are assumed to diffuse from the cytoplasm of one cell to the next through plasmodesmatal connections until reaching the pericycle or stelar parenchyma. Once in the walls of the pericycle and stelar parenchyma, ions diffuse along their concentration gradient to the lumens of tracheary elements. Movement of water in the roots is driven by gradients in water potential (Peterson *et al.* 1999). According to Maurel (1997) and Schäffner (1998) the resistance in the transcellular pathway is lowered by aquaporins (membrane spanning proteins). The opening and closing of plasmodesmata influence water flow in the symplastic path while Casparian bands and suberin lamellae control the apoplastic path.

The extreme tip of the root is hydraulically isolated (Melchior and Steudle 1993), and water enters most rapidly in a zone approximately 2 to 10 cm behind the tip depending on the species and season. Several studies have demonstrated the importance of new root growth in water and nutrient uptake (Ritchie and Dunlap 1980, Rose 1992, and Tinus *et al.* 2000). The amount of new root growth determined the survival of ponderosa pine, *Pinus ponderosa* (Stone and Schubert 1959) and Monterey pine, *Pinus radiata* (Nambiar *et al.* 1979). In loblolly pine root hydraulic conductivity was closely related to the number of new roots produced (Carlson 1986). Sands *et al.* (1982) found that conductivity in unsuberized roots of loblolly pine was 2.6 times greater than that in suberized roots. Despite the importance of white roots, suberized roots also are important in water and nutrient uptake as they make up the greatest part of the root system most of the year, and all of the root system part of the year (Reed 1939 and Wilcox 1968).

In order to achieve ion uptake, movement through symplast is indispensable especially in the endodermis. The white zone, with its living cortex, has a large plasmalemma surface containing space for many protein carriers, and thus has a large potential for ion uptake. As the deposition of suberin lamellae in the cell walls of endodermis does not sever the plasmodesmatal connections between neighboring cells, the symplastic diffusion of ions into the pericycle is not impeded (Peterson *et al.* 1999). In the CT zone, death of cortical cells drastically reduces the membrane surface available for ion

uptake. Therefore, presence of endodermal passage cells is critical for ion uptake in this root zone. During early secondary growth, cork cells form a continuous layer beneath the endodermis. Then the sites for ion uptake into the symplast are reduced to nil at the cork zone. In dormant roots the CT zone extends closer to the tip, but the endodermis retains some passage cells. Thus, even dormant roots have some potential for ion absorption via combined passive transport to interior living cells where active transport occurs.

The effects of root development on water uptake are even more difficult to predict than that for ions. In the white zone when the endodermis has only Casparian bands but no suberin lamellae, the major resistance to water uptake occurs in the living parenchyma of the cortex (Peterson and Steudle 1993). Progressive development of suberin lamellae in more endodermal cells would increase the resistance to water flow in the transcellular pathway in the older regions of white zone. In the CT zone, although the number of passage cells remains somewhat constant, progressive death of cortex and abrasion of outer layers decrease resistance to the inward flow of water. But deposition of condensed tannin in the cell walls may have an effect in reducing the entry of water. In the cork zone, cell maturation increases resistance in both apoplastic and cell-to-cell pathways, and water movement is drastically reduced. In dormant roots passage cells of the CT zone offer a low resistance region for the entry of water into stele (Peterson *et al.* 1999).

Loblolly pine

Loblolly pine (*Pinus taeda* L.) also called Arkansas pine, North Carolina pine, and Oldfield pine, is a widespread species in North America (Baker and Langdon 1990). It is a major component in southern forests and is very important to the forest products industry. It is an ideal tree species for site restoration and forest management (Schultz 1997). It is the most hardy and versatile of all southern pines in terms of its ability to reproduce and grow rapidly on diverse sites. It occurs naturally in 15 southern and mid-Atlantic states and has a wider geographic range than any other southern pine except shortleaf pine. It is the leading timber species in the United States, predominating on more than 13.7 million ha of southern forest land. It is an adaptable species that has been successfully planted along the periphery of its natural range (Baker and Langdon 1990). The natural range of loblolly pine extends from Texas eastward to Florida and northward to Delaware (Fig. 5). This is a continuous range with the exception of a disjunct population in Texas ('lost pines'). The western types are more drought hardy compared to the more moisture-loving eastern types. The area of commercial forest lands containing loblolly pine increased from 11.6 million ha in 1960 to 13.7 million ha in 1989 and the demand for timber is still increasing.

The climate over most of loblolly pine's range is humid, warm-temperate with long, hot summers and mild winters. The mean annual temperature ranges from 13 to 24 °C and average annual rainfall varies from 1020 to 1520 mm (Little

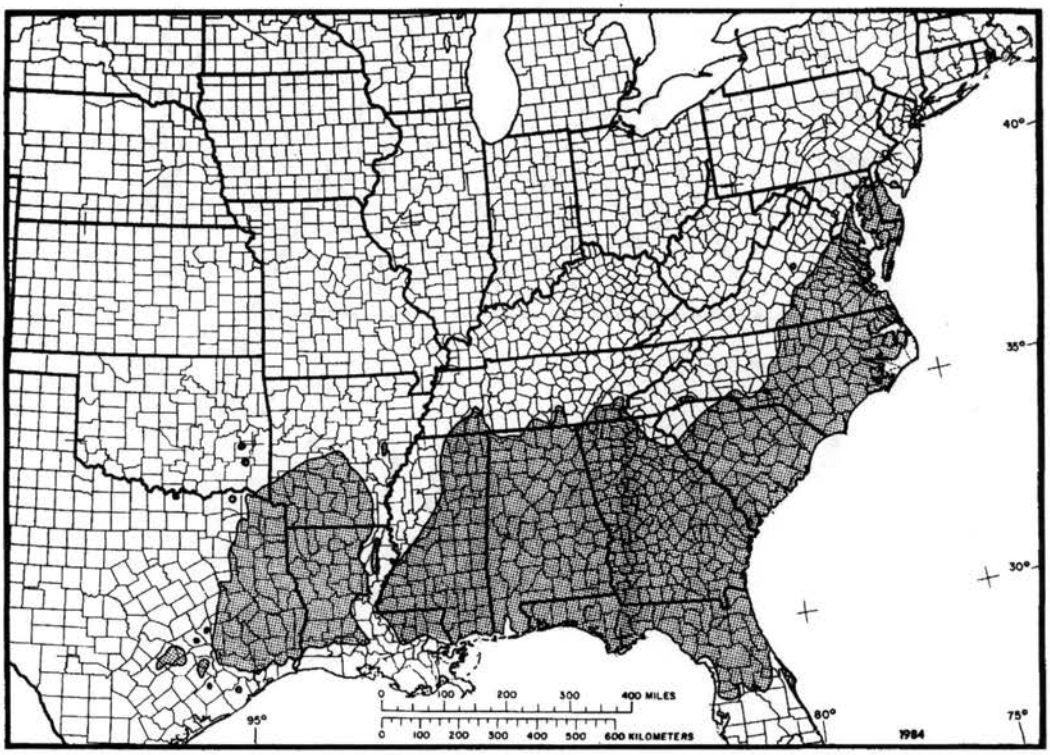

Fig. 5. Distribution of loblolly pine in the continental US (Baker and Langdon 1990).

1971). The main factor limiting extension of the species northward is the low winter temperature while lack of adequate precipitation limits western extension in Oklahoma and Texas (Fowells 1965). Loblolly pine performs best on Ultisols with small occurrences on Entisols, Spodosols, and Alfisols. The best growth is on moderately acid soils with imperfect to poor surface drainage, a thick medium-textured surface layer, and a fine-textured subsoil (Fowells 1965). It does not grow on Mollisols, calcareous river bottoms and terraces characterized by high base saturation and high pH, and Alfisols of the Coastal Prairie of Louisiana and Texas (McKee 1981).

Loblolly pine is found in pure stands and in mixtures with other pines and hardwoods, and in association with a great variety of lesser vegetation. Within the natural ranges the most important associates are longleaf pine (*Pinus palustris* Mill.), shortleaf pine (*P. echinata* Mill.), Virginia pine (*P. virginiana* Mill.), southern red oak (*Quercus falcata* Michx.), white oak (*Q. alba* L.), post oak (*Q. stellata* Wangenh.), blackjack oak (*Q. marilandica* Muenchh), sassafras (*Sassafras albidum* (Nutt) Nees), and persimmon (*Diospyros virginiana* L.) (Eyre 1980).

Moisture is the major critical factor in seedling establishment. Soil compaction and puddling also reduce root growth, seedling survival, and shoot growth (Foil and Ralston 1967, Fowells 1965, Grano 1971, and Langdon 1979). Competition affects growth in varying degrees depending on the site, amount and

size of competing vegetation, and age of seedlings (Fitzgerald *et al.* 1973). Silvicultural practices such as prescribed burning, use of herbicides, and mechanical treatments reduce growth and development of hardwood understories and favor growth of loblolly pine (Langdon 1981).

Forested areas of Oklahoma cover about 20 % of the state's total land area. Nearly half of this area (2.2 million ha) is commercial forests capable of producing forest products. Forests offer a multitude of benefits, directly or indirectly. In order to have sustainable resources from forests, they have to be managed properly (Lewis and Goodier 1990). Forest and conservation tree nurseries produce seedlings required for reforestation and afforestation. In 1998, 1.6 billion tree seedlings were produced in the United States and out of this 1.3 billion was from the South accounting for about 80 % of the total (Moulton and Hernandez 1998).

Research: Abundant information is available on the above-ground parts of loblolly pine seedlings. Many researchers have worked on the effectiveness of needles and their role in photosynthesis and transpiration. The aerial part is easy to work with and research resulted in a wealth of information for practical application. Water and minerals absorbed from soil are transported to the shoot for producing photosynthates. The shoot absorbs sunlight and produces carbohydrates for supplying to the growing parts. As seedlings in the nursery increase in size towards the end of the season there is an increasing demand for

water and nutrients. This is met by producing more lateral roots containing white zone and mycorrhizhal white zone.

Information about roots is limited compared to that of above-ground parts. Unlike aerial parts roots are fragile and there is a paucity of methods in exploring them. As roots are buried in the soil and extend into deeper profiles during seedling growth, extraction of all fine roots is difficult. Although many methods have been suggested in studying roots (Sutton and Tinus 1983), there is no single method to follow the growth of all fine roots in the soil itself. Moreover, many microorganisms, especially mycorrhizae exist in the rhizosphere that may interact with root functioning. Recently more attention has been directed towards working with roots and root systems. Advances in modern research methods have made this task easier. There are different methods for studying roots, the most common being extracting soil cores containing roots and separating them from the soil with minimum disturbance to roots.

The loblolly pine root system is characterized by having a tap root, horizontal laterals, sinker roots and fine roots (Carlson *et al.* 1988 and Harrington *et al.* 1989). The tap root grows into the soil and provides anchorage and support for the shoot and lateral roots. The tap root ceases growth in favor of an extensive lateral root system. About 20 to 30 first-order lateral roots (FOLR) grow horizontally in the soil and most of them exist in the upper layers. The FOLR spread out in the subsurface layer surrounding the base of seedlings. The

sinker roots arise from FOLR and grow downward. Fine roots arise from all of these long-lived roots that are mostly involved in water and nutrient absorption. The roots grow throughout the year, but most root growth occurs in April and May, and in late summer and early fall (Fowells 1965, Miller and Woods 1965, and Wahlenberg 1960). The variation in structure of any individual root is a consequence of its growth and maturation pattern (Esau 1977). The root is formed by an apical meristem and consequently growth occurs at the tip. Thus, a gradient in age exists along the length of root, with the youngest portion at the apex and the oldest towards the base.

The anatomy of a loblolly pine root is not uniform along its length due to changes in structure that occur with age and environmental conditions. According to McCrady and Comerford (1998) and Wilcox (1964), the morphology of pine roots is complex and heterorhizic, and individual roots vary anatomically along their lengths. During primary stages of growth, striking anatomical changes occur in the cortical and stelar regions. The changes in the endodermis are the most important and have a decisive role in regulating movement of water and nutrient ions. Therefore, the changes in the anatomy of seedling roots have a significant role in regulating the entry of water and ions into the stelar tissues for upward transport to the shoot.

Justification

There are several key components involved in seedling production; among them bed preparation, seed collection, nurture of seedlings, lifting and grading, storage and safe transport are important. As planting sites in Oklahoma are generally drought prone, seedlings must be physiologically adapted for survival in adverse conditions. The species, family, and stock type must be matched with conditions of planting sites. Therefore, preparation for producing high quality seedlings begins from collection of the best seed source, seed handling, and nurture of seedlings until lifted. Seedlings receive many cultural practices including irrigation, fertilization, pest and disease control, undercutting, lateral pruning, and top pruning. Seedlings are lifted with bare roots and are graded based on seedling morphology. When planted in the field they must be capable of survival in droughty conditions and competition with undesirable vegetation. Therefore, seedlings must be of superior quality to withstand adversities at planting sites. Seedling quality can be manipulated mainly through genetic selection and cultural practices.

Genetic improvement: One of the important parameters of reforestation success is selection of a seed source. The choice of species and family depends on the purpose of planting and site conditions. Oklahoma sites are generally drought prone and are at the western edge of the natural range of loblolly pine. Despite plentiful rainfall, high temperatures and low humidity cause water stress

in seedlings that have been recently planted. This interferes with establishment of transplanted seedlings.

Seedlings with long tap roots absorb moisture from deeper layers and those exhibiting a faster growth rate overtop competing vegetation. These traits are advantageous for transplanted seedlings especially on drought prone areas. Hallgren *et al.* (1993) reported that capacity to grow new roots rapidly after planting is a major factor determining planting success. According to Wells (1969), Wells and Wakely (1966), and Woesner (1972a, b) loblolly pine showed large genetic variation in drought resistance. Drought resistant types had deep, wide ranging root systems, xeromorphic needles (van Buijtenen *et al.* 1976) and reduced water use under drought (Bilan *et al.* 1977). Therefore, genetically improved progenies of such families may be ideal for planting in Oklahoma sites. Accordingly, families with a shallow root system will be the best choice for the eastern states and other areas with plentiful moisture. The number of FOLR is important in promoting faster growth in transplanted seedlings. According to Hallgren *et al.* (1993) root traits such as number of FOLR and root growth potential may be better indicators of seedling capacity for survival and performance than shoot traits. The number of FOLR is strongly controlled by genetic factors and root growth potential is susceptible to variations in environment.

Although a multitude of information is available on the physiological ecology of the shoot including photosynthesis and transpiration, much less has been done to examine interaction of roots with environment. In order to determine the ideal seedlings for planting on diverse sites, more research must be conducted involving root anatomy and morphology and combining the same with structure and function of roots. Families suited to the site will survive better especially in adverse conditions and compete with undesirable vegetation.

Seedling culture: Seedlings for conservation planting can be improved by incorporating changes in routine cultural practices in the nursery. The most common practices are irrigation, fertilization, pest and disease control, undercutting, lateral pruning, and top pruning. Loblolly pine seedlings grow continuously during the season until lifted in winter. The cultural treatments employed in the spring promote shoot growth at the expense of roots (Iivonen *et al.* 2001 and Sloan 1994). Roots are involved in absorption of water and nutrients to meet the demand of young seedlings. While treatments such as irrigation and fertilization encourage shoot growth, pest and disease control activities maintain seedlings with good health. According to Chauhan and Mishra (1996) treatments in the fall including undercutting and lateral pruning promote production of abundant lateral roots. Ectomycorrhizal fungi also play a significant role in enhancing growth of seedlings especially under nutrient-limiting environments (Eissenstat and van Rees 1994, Nylund and Wallander 1989, Sylvia 1990, Wallander 1995, and Wallander and Nylund 1991, 1992) and their

abundance increases towards the end of the season (Harvey *et al.* 1987 and Söderström and Read 1987). All these cultural practices result in vigorously growing seedlings ready for lifting in winter. Hallgren and Tauer (1989) and Hallgren *et al.* (1993) reported that harvesting seedlings in mid-winter makes them most resistant to transplant stress. According to Lavendar (1964), Lavendar and Wareing (1972), and Wakeley (1954), changes in environmental conditions such as temperature and photoperiod can alter seedling tolerance to stresses.

The symbiotic role of ectomycorrhizae in enhancing growth of pine seedlings is well documented (Brundrett 1991, Harley and Smith 1983, Kendrick 1992, and Sylvia 1990). Increase in the population of mycorrhizae and fall growth of seedlings have been studied by many researchers and a positive relation was noticed in most cases (Dixon *et al.* 1980, Muhsin and Zwiazek 2002, and Safir *et al.* 1972). Although mycorrhizae appear in late spring or summer, a significant increase in their abundance was noticed in nutrient-limiting environments (Sylvia 1990 and Wallander 1995). Abundant laterals in the subsurface layer of soil and plenty of mycorrhizae (Harvey *et al.* 1987 and Söderström and Read 1987) will increase the survival of transplanted seedlings. Laterals in the surface layers are beneficial because they grow exploiting the limited summer showers on transplanted sites (Carlson *et al.* 1988).

As cultural treatments are important in nursery management, the rate and timing of implementing these treatments have a significant role in producing vigorous seedlings. Planting success can be ensured if planted with healthy seedlings having plentiful laterals in the subsurface layer and abundant mycorrhizae. Therefore, research must be conducted to evaluate the significance of existing methods and suggest any amendments, if warranted.

Objectives: There were two main objectives for the present research: (i) to determine how morphological and anatomical traits of loblolly pine seedling roots are affected by environmental conditions, and (ii) to determine the absorptive capacity of loblolly pine seedlings in the nursery throughout the year and if there is a seasonal pattern in absorptive capacity comparable with transpiration demand. The two objectives were dealt with in detail in two separate chapters.

Chapter II contains details of root morphology and anatomical changes in the FOLR of loblolly pine seedlings in three different environmental conditions: mist chamber, peat-vermiculite, and loamy sand. The anatomical changes in the cortex and stele, and details of xylem maturation affected by different environments were examined. Chapter III contains details of anatomical changes in the roots of loblolly pine seedlings during the first year in the nursery. It also contains information on root parameters at different depths in the soil and how these parameters match the transpiration capacity of the shoot. The changes in the population of mycorrhizae were also examined.

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CHAPTER - II

ENVIRONMENTAL EFFECTS ON THE ANATOMY OF FIRST-ORDER LATERAL ROOTS (FOLR) OF LOBLOLLY PINE SEEDLINGS

ABSTRACT

Roots of loblolly pine seedlings contain three characteristic root zones [white, condensed tannin (CT) and cork], previously described by McKenzie and Peterson (1995a, b) in jack pine. Live cortex cells and passage cells in the white zone and passage cells in the CT zone are considered to be involved in the absorption of water and nutrients. The main goal of the present research was to determine environmental effects on anatomy of first-order lateral roots (FOLR) of loblolly pine seedlings. Morphological and anatomical measurements were conducted on the FOLR from a variety of growth conditions (mist chamber, peat-vermiculite, and loamy sand). Fluorescent staining methods of Brundrett *et al.* (1991) were adopted to determine anatomical changes, and xylem maturity was tested using Cellufluor (Calcofluor White M2R). The rooting environment was considered to be most favorable in the mist chamber due to least physical resistance and constant water availability. Rooting environment was least favorable in the loamy sand and intermediate in the peat-vermiculite. Root zones in the FOLR showed the typical anatomical traits including maximum number of

cortex cells and endodermal passage cells associated with uptake capacity greatest near the tip and decreasing with distance from the tip. The mist chamber provided the most favorable moisture conditions in the root environment. Roots in this environment had more tissue with a high capacity for water and nutrient uptake. These roots had the most white zone and passage cells. Roots in environments with less favorable moisture conditions had less absorptive tissue. The white and CT zones became shorter, the number of passage cells declined near the tip, and the live cortex was eliminated closer to the tip. These anatomical changes became barriers to water exchange suggesting that the priority for roots exposed to more desiccating conditions was to reduce loss of water from the root to the soil. Production of second-order lateral roots closer to the tip may have increased moisture absorption area, thus compensating for losses of the white and CT zones on the FOLR. This growth may have increased root absorption capacity in unexplored soil while root absorption in already explored and moisture depleted soil decreased. Further study is necessary to learn whether the earlier production of laterals in stressful conditions replaces the absorptive tissues lost in the main root. Some root traits appeared to be insensitive to the environment including the distance from the tip of the first conducting xylem, the number of endodermal cells, and the number of xylem poles.

INTRODUCTION

Loblolly pine survives in a wide range of environments in its natural range (Baker and Langdon 1990). The variations in environmental conditions occur even within a microsite during different periods of the growing season. When seedlings experience variations in environmental conditions they adapt to the new conditions. The seedlings grow faster when conditions are favorable and they become dormant when conditions become unfavorable. Actively growing seedlings produce abundant roots for absorption of water and nutrients. When seedlings are dormant or face adverse conditions roots undergo morphological and anatomical changes. The roots perform various mechanical and physiological functions and also act as signals of rhizospheric conditions. When roots encounter changes in the rooting environment they can signal the changes to the shoot. There is evidence that roots communicate drying soil to the shoot where stomates close as a consequence to conserve water (Kramer and Boyer 1995).

Root morphology

The root system of loblolly pine is characterized by the presence of a tap root supporting horizontal laterals, sinker roots and fine roots (Carlson *et al.* 1988 and Harrington *et al.* 1989). The tap root ceases growth after several years when it reaches an impervious layer or the water table (Ashe 1915, Harlow *et al.* 1978, and Wahlenberg 1960). The tree develops an extensive lateral root system that is active in absorption of water and nutrients. The distal end of a root

contains three zones: white zone, CT zone, and cork zone (McKenzie and Peterson 1995a, b and Peterson *et al.* 1999). During development roots undergo changes in morphology and anatomy along their length. According to Esau (1977) the variation in structure of any individual root is a consequence of its growth and maturation pattern. The root is formed by an apical meristem and as a result growth and elongation occur at the tip. Thus, a gradient in age exists along the length of the root, with youngest portion at the apex and oldest towards the base.

The difference in the length of root zones is a reflection of conditions in the rooting environment (Weaver 1919, 1920, Weaver and Clements 1929, and Wraith and Wright 1998). When variations occur roots undergo anatomical changes and these changes are followed by changes in external morphology also. These morphological changes affect total length of different root zones. Therefore, difference in the length of a particular root zone is the result of variations in the rooting environment. When conditions become favorable seedlings produce abundant white roots and during adverse conditions they become brown due to deposition of tannin. The tannin zone is less capable of absorbing water and nutrients due to a reduction in absorbing surface compared to white roots (Taylor and Peterson 2000).

The diameter depends on the type of root, its maturity or age and the environment (Enstone *et al.* 2001 and McCrady and Comerford 1998). But

changes in a single root along its length occur irrespective of environmental conditions. The growing tips of roots taper distally and they are protected by root caps. Immediately behind the tip, roots are larger in diameter and are mostly white. Proximal to the white zone roots become brown due to death and collapse of cortical cells in the CT zone (Enstone *et al.* 2001). The roots generally have a narrower diameter in the tannin zone. Basal to the tannin portion roots become darker in color and diameter begins to increase gradually due to secondary growth (McKenzie and Peterson 1995a, b). The change in diameter is affected by changes in conditions of the rooting environment by the movement of tannin and cork regions closer to tip (Wilcox 1962, 1964). The roots become increasingly swollen at their tips when conditions in the rhizosphere become adverse.

The changes in the anatomy and morphology of roots are reflected in the emergence of higher order laterals. The roots growing in favorable conditions contain longer white portions and roots facing adverse conditions develop browning closer to the tip (Enstone *et al.* 2001 and Wilcox 1968). The development of higher order laterals also follows a similar pattern. Therefore, emergence of laterals is delayed in roots growing in ideal conditions.

Root anatomy

Anatomical changes occur along the length of roots due to changes in environmental conditions and age. McCrady and Comerford (1998) and Wilcox

(1964) reported that morphology of pine roots is complex and an individual root varies anatomically along its length. The three characteristic zones (white, CT, and cork) proposed by McKenzie and Peterson (1995a, b) in pouch-grown jack pine was found to be suitable for loblolly pine also (Peterson *et al.* 1999). Each zone has its own characteristic anatomical feature resulting from the developmental stage of root and growth condition of the rooting environment. The anatomical changes appear to have a significant role in the absorption of water and nutrients (McKenzie and Peterson 1995a, b). The maturation of zones is compressed towards the root tip in slow growing roots that encounter adverse conditions. Therefore, roots undergo anatomical changes followed by changes in morphology to adjust to the new conditions in the environment.

The white zone in roots varies depending on the conditions in the environment (Taylor and Peterson 2000 and Wraith and Wright 1998). When conditions become favorable roots produce a longer white zone and during adverse conditions the length is significantly reduced. Therefore, the length of white zone is indicative of the suitability of the rooting environment. The death of cortex cells in the tannin zone reduces absorptive areas in roots. As roots undergo changes in anatomy due to changes in rooting environment the tissue affected most is cortex. The presence of cortex and number of cells in the cortex are important in deciding absorptive capacity of roots. The presence of live cortex increases absorptive surface of white roots more than that of brown or dark roots. The roots with a longer white zone contain more cortex cells

increasing the absorbing area. Therefore, presence and number of cortex cells in roots are influenced by environmental conditions.

The deposition of suberin lamellae (suberization) is the process in which endodermal cells are impregnated with suberin lamellae on the inner tangential walls. The suberization acts as an apoplastic barrier in the radial pathway of water and nutrients. Therefore, presence of unsuberized passage cells in the CT zone is significant in determining the amount of water and nutrients that can enter the stele. The cork zone is characterized by the presence of secondary growth. Although vascular cambium arises earlier in the tannin zone, cork cambium appears at the end of the CT zone marking the beginning of cork zone. According to Esau (1977) cork cells in roots typically arise in the outer stelar cells and thus they are produced interior to the endodermis. Therefore, due to the combined action of vascular cambium and cork cambium secondary growth causes an increase in root diameter.

The endodermis passes through three stages of development and a mature endodermis develops Casparian bands on the radial and transverse walls (Clarkson and Robards 1975 and van Fleet 1961). The Casparian bands usually appear within a few millimeters from the tip in the white portion. Proximal to the origin of Casparian bands suberin lamellae are deposited on the inner tangential walls. The deposition proceeds faster in the white zone and continues more gradually in the CT zone. This continues until all cells of the endodermis are

suberized marking the beginning of the cork zone. The presence of Casparian bands and suberin lamellae prevent apoplastic movement of water and ions (Clarkson and Robards 1975, Esau 1977, and Peterson 1988). The presence of unsuberized (passage) cells in the endodermis is significant in allowing water and nutrients to enter the stele. The presence of cortex cells and passage cells offers a large area for absorption in the white zone. Due to death of cortex cells in the CT zone only passage cells act as portals for the entry of water and ions. Therefore, suberization has a significant role in determining the amount of soil solution that enters the transpirational stream. The roots of seedlings respond differently to suberization in varying conditions of the environment. As suberization is the process in regulating movement of water and nutrients, deposition of suberin begins closer to the tip in adverse conditions. When conditions are favorable roots delay deposition of suberin which may allow greater water uptake.

Maturity of xylem

The extreme tips of roots containing immature tracheary elements are hydraulically isolated (Frensch and Steudle 1989) and the development of Casparian bands in this region prevents entry of water and ions into the stele through the apoplast (Enstone and Peterson 1992). Therefore, roots become conductive several millimeters behind the tip. The water and nutrients entering the stele must be transported axially to the shoot in the xylem.

The xylem must attain a certain level of maturity in order to be conductive. The xylem is a multicellular tissue within the stele appearing in the form of a crescent with 2 to 5 poles alternating with phloem (Enstone *et al.* 2001). When water and nutrients are within the cells of pericycle, the xylem has direct access for conducting them to the shoot. The protoxylem elements at the tips of the crescent are in direct contact with the pericycle. The metaxylem elements in each xylem pole grow centripetally and merge at the center of the stele. The development of xylem commences close to the tip of the root. Enstone *et al.* (2001) reported that in the tap roots of pot-grown loblolly pine xylem was functional 5 mm from the apex while it was functional 9 mm from the apex in pouch-grown roots.

Objectives:

Tree roots are highly variable in root morphology and anatomy because they are long-lived and grow in a wide variety of environments. Trees have tap roots, lateral roots of several orders and mycorrhizae. Their roots grow in many types of soils and environments. The present study was undertaken to extend the knowledge of root anatomy to FOLR and to a variety of growth conditions. First-order lateral roots were examined in three different environments that represented a gradient of increasing resistance and fluctuation in moisture availability: mist chamber, peat-vermiculite, and loamy sand. These were chosen to cover a relatively wide spectrum of soil conditions to bracket the range of variation in environmental response of root morphology and anatomy. In

addition, these root environments allowed easy extraction of relatively undamaged roots for investigations.

The main objective of this research was to determine how the morphological and anatomical traits of loblolly pine roots were affected by the soil environment. Of particular interest were traits associated with water and nutrient uptake capacity. Specific objectives included learning how the environment affected the following: (i) morphology of FOLR, (ii) anatomy of the cortex cells of the different root zones, (iii) pattern of suberization of the endodermis, and (iv) maturity and conductivity of the xylem along the root. First-order lateral roots are the link between tap root and higher order laterals and therefore, answering these questions will provide more information on the morphology and anatomy of roots. The three media in the current research involved extremes of rooting environments; so the results will provide a better understanding of root anatomy that can be used for predicting the response of roots to growing conditions. As growth of seedlings is directly related to response of roots in the rhizosphere, a better understanding on the anatomical changes under different conditions will be useful in defining better models of water uptake.

MATERIALS AND METHODS

Growth conditions

Loblolly pine seedlings from an Oklahoma source were provided by the State Forest Regeneration Center, Washington, Oklahoma. They were one-year-old seedlings produced under normal operational procedures. The seedlings were stored in a cold room in water-proof bags for several days prior to planting.

The seedlings were planted into three different media: (i) mist chamber, (ii) peat-vermiculite and (iii) loamy sand. The mist chamber was of 120 x 60 x 90 cm size and the top was covered with plastic rods lined with rubber seals (Fig. 1). The seedlings were held by the rubber seals suspended at the root collar with roots inside the dark mist chamber and tops in a lighted room. The bottom of the mist chamber was filled with a nutrient solution (Hoagland solution modified for pine). The solution contained macronutrients $NH_4 NO_3$ (0.5 mM), $(NH_4)_2 SO_4$ (0.25 mM), $Ca CO_3$ (0.3 mM), $Ca (NO_3)_2.4H_2O$ (0.2 mM), $Mg SO_4.7H_2O$ (0.3 mM), $KH_2 PO_4$ (0.2 mM), and $K NO_3$ (0.3 mM) and micronutrients $H_3 BO_3$ (4.6 µM), $Mn Cl_2.4H_2O$ (9.1µM), $Zn SO_4.7H_2O$ (0.8 µM), $Cu SO_4.5H_2O$ (µM 0.3), $H_2 Mo O_4.H_2O$ (1.1 µM), and Fe - sequestrene 330 (17.9 µM). An immersion pump produced a continuous mist spray for 10 sec every 5 min and the plants received a 14 h photoperiod at 24 °C. Seedlings were also planted into 7 L pots

Fig. 1. Front view of the mist chamber with partially open top showing freely hanging roots.

containing either peat-vermiculite (3:1 by volume) or loamy sand (77 % sand, 19 % silt, and 5 % clay) containing 30.5 mg kg^{-1} nitrate nitrogen, 27.0 mg kg^{-1} plant available phosphorus, and 58.0 mg kg^{-1} plant available potassium. Both the peat-vermiculite and loamy sand pots were watered 2 to 3 times each week and fertilized every 2 weeks with Peatlite Special (20N-20P_2O_5-20K_2O). The pots were placed on benches in a greenhouse with evaporative cooling and heaters to maintain temperatures of 27 to 32 °C during the day and 15 to 20 °C at night. The roof of the greenhouse reduced solar radiation to approximately 50 % of full sunlight as measured with Li-COR radiometer.

The rooting environment was considered to be most favorable in the mist chamber because it provided the least physical resistance to growth and constantly bathed the roots in nutrient solution. The loamy sand had the greatest and the peat-vermiculite intermediate resistance to root growth. The pots were watered 2 to 3 times each week depending on the weather and allowed to drain to field capacity.

Roots were collected singly from the three media in a completely randomized manner. In each environment 10 to 25 roots were sampled. The distinction between the white and CT zones was determined by visual observation (Fig. 2). Root color was considered a reliable predictor of root developmental stage in loblolly pine seedlings by many researchers (Enstone *et al.* 2001 and McCrady and Comerford 1998). The point where browning began

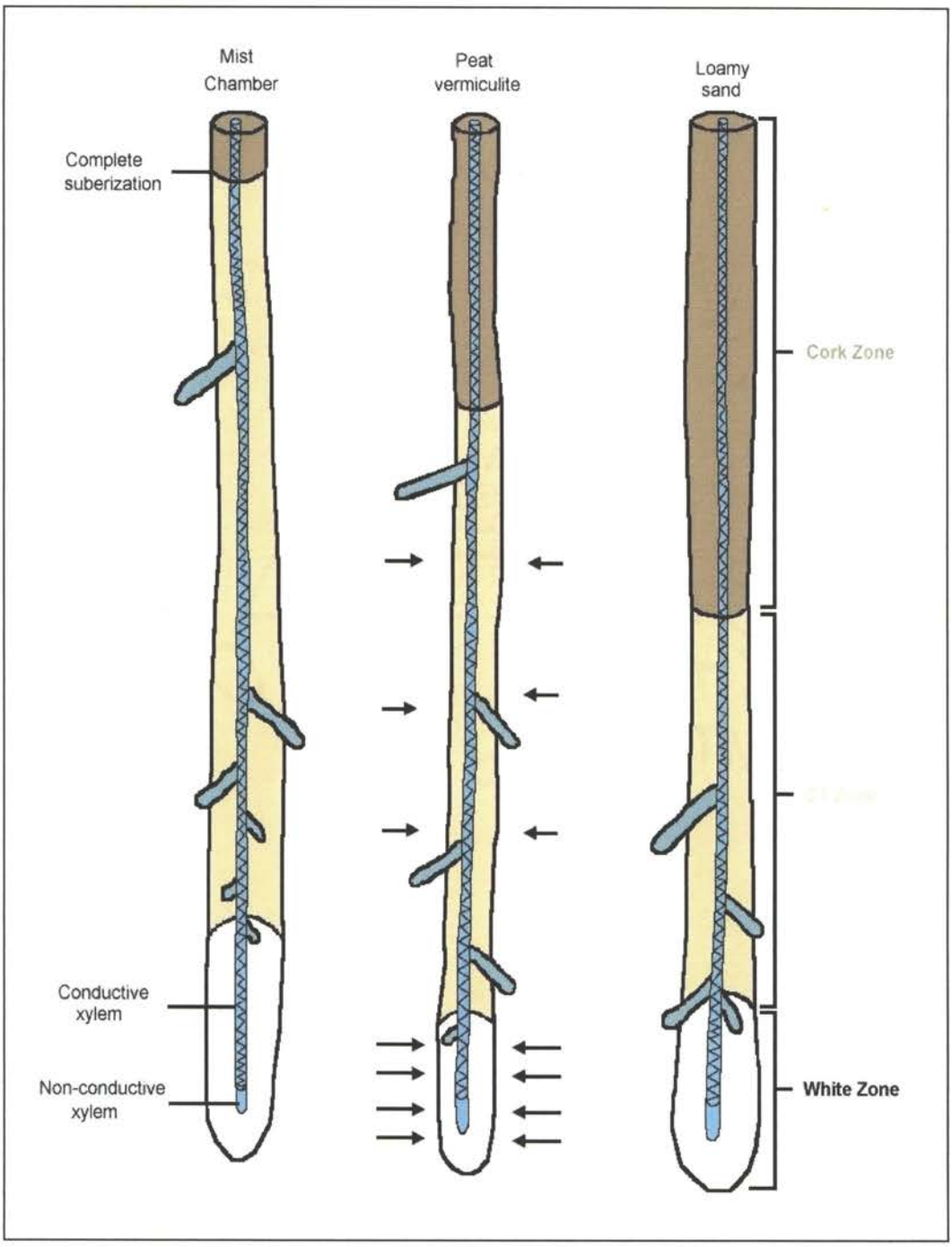

Fig. 2. Diagrammatic representation of roots in mist chamber, peat-vermiculite, and loamy sand. Arrows indicate hypothesized water flow into roots, intensity of flow shown by number of arrows. Emergence of second-order laterals was shown only in the white and CT zones.

was taken as the boundary between the white and CT zones. At the origin of the cork zone roots increased in diameter and exhibited a darker color. This is the point where suberization of all endodermal cells and production of a cork layer occurred. In the mist chamber color change was not very prominent and it was difficult to make a macroscopic decision. The lengths of the white and CT zones were measured using a ruler and the diameter of the white zone was measured using a caliper.

Sectioning and staining

The roots from the three environments were cut into 10 mm segments and placed in water in labeled containers. Each root provided 10 to 20 segments, root length permitting. The segments were placed between the folds of a sheet of parafilm (Fröhlich 1984) and sections were cut from the mid-point of each segment under a stereomicroscope. Two or three sections from each segment were stained with Fluorol Yellow 088 (0.005 % w/v) for 1 h following the protocol of Brundrett *et al.* (1991, Appendix A) and viewed under UV illumination. The best section from each segment was used for measurements.

Emergence of first second-order lateral

Most of the roots used for the experiment contained second-order laterals. Although lateral primordia were initiated in the white zone, they extended through the cortical tissue emerging near the boundary between the white and CT zones. Although initiation of primordia was not examined in the current study, in many

cross sections they were noticed originating from the outer stelar tissues of the white zone (Fig. 3). The laterals emerged in more or less an acropetal sequence, the youngest towards the distal ends of roots. The emergence of laterals was observed macroscopically and the distance between the root apex and the first-emerged lateral was measured using a ruler.

Number of cortex cells

The number of layers of cortex cells including endodermis was counted from the periphery. Two counts were made on opposite sides of each section and the average was taken. Although death and decay of cortex cells began in the CT zone many deformed cells were retained; these cells were counted. The death and decay affected first the outer cortex cells and proceeded with a centripetal progression.

Suberization of endodermis

The progression of suberization was determined along the white and CT zones until the beginning of a cork layer beneath endodermis. Cross sections from mid-point of 10 mm segments from the apex were stained with Fluorol Yellow 088 (0.005 % w/v) following the protocol of Brundrett *et al.* (1991, Appendix A) and viewed under UV illumination. The suberized cells fluoresced bright yellow and (unsuberized) passage cells were pale yellow. The number of suberized and unsuberized cells was counted in the sections from all segments

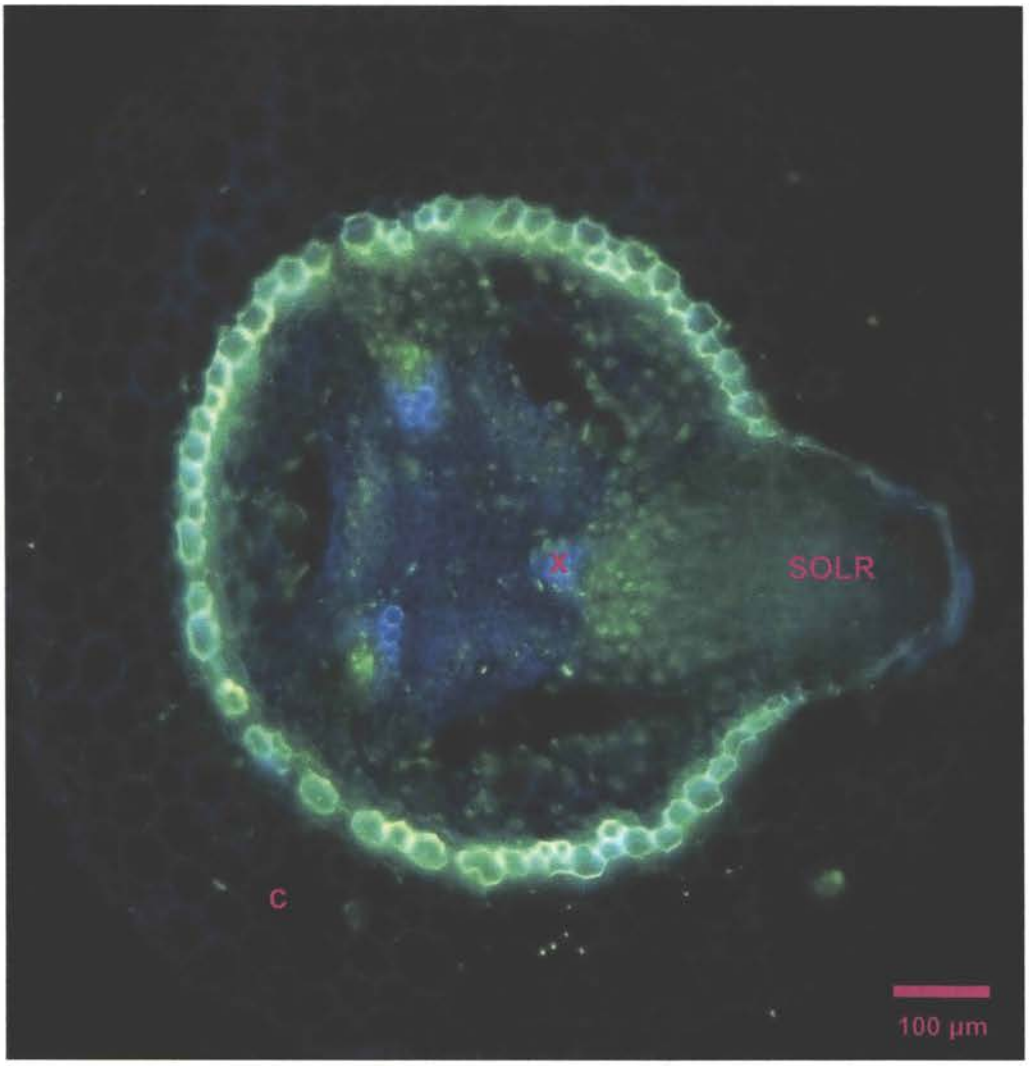

Fig. 3. Cross section at 40 mm from the apex of a mist chamber root stained with Fluorol Yellow 088 (100x). A second-order lateral root (SOLR) initiates at one of the three xylem poles (x) and penetrates through cortex cells (c).

until endodermis formed a complete ring of suberized cells. The number of xylem poles was also recorded.

Maturity of xylem

Maturity of xylem tracheids in the tips of white roots was tested using Cellufluor (Calcofluor White M2R) in a 0.01% w/v solution. It is an apoplastic fluorescent dye that binds to cellulose (Peterson and Steudle 1993). Tips of white roots about 50 mm long were immersed in tap water immediately after collection. The proximal ends of these roots were discarded by cutting under water and leaving the distal 35 mm. After gently blotting dry, three roots were laid flat on one end of a slide with a vaseline seal about 10 mm from the edge (Fig. 4). The end of the slide with roots was immersed vertically in a glass container containing Cellufluor allowing the distal exposed ends to dry in humid air. The vaseline prevented movement of stain along the root surface. As evaporation progressed it forced the upward movement of stain through the conductive portion of the xylem. After 4 h roots were crushed flat on a slide with a cover glass and examined under epifluorescence illumination. The tracheids that conducted stain appeared bright blue under UV light (Fig. 5). The stain moved to the root apex along the mature portion of the xylem. The distal end of staining was marked and the distance from the root apex to this point was measured using a ruler. The number of xylem strands in each sample was also counted.

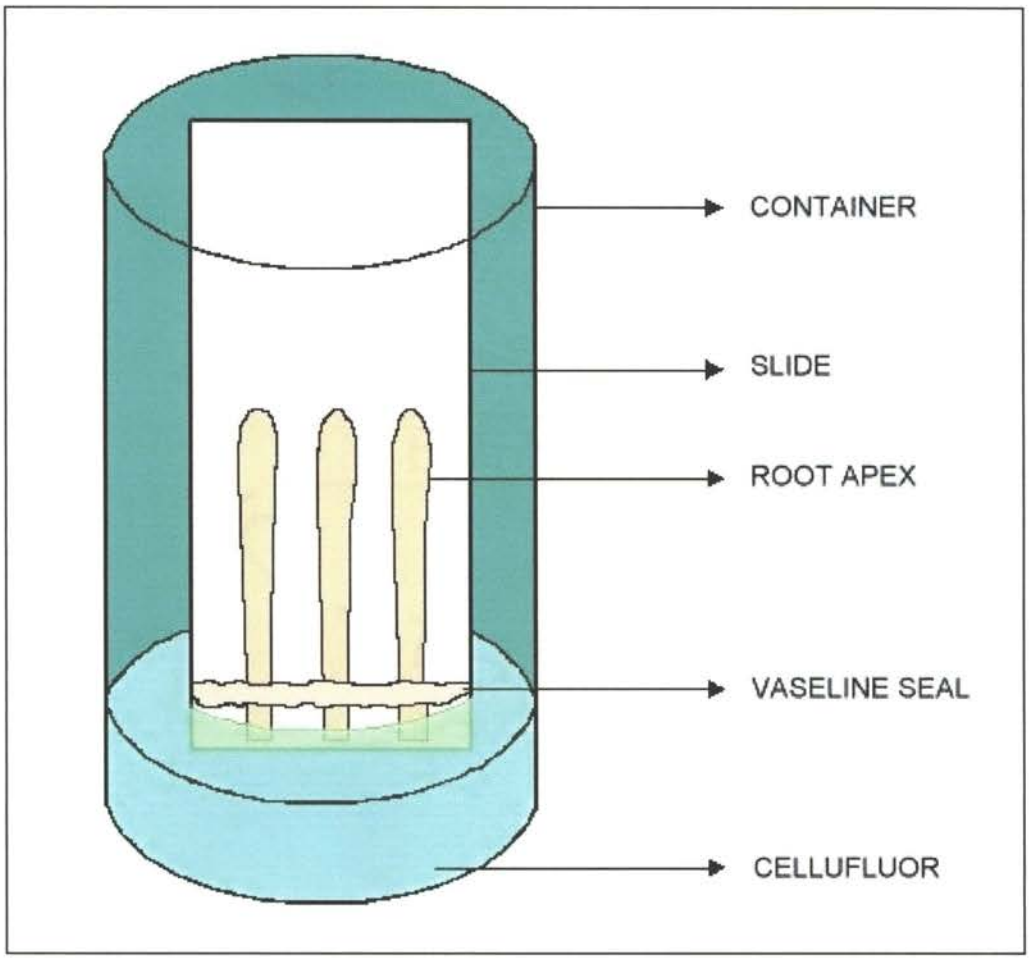

Fig. 4. Diagrammatic representation of staining white root tips using Cellufluor. The cut (basal) ends of roots were immersed in the stain.

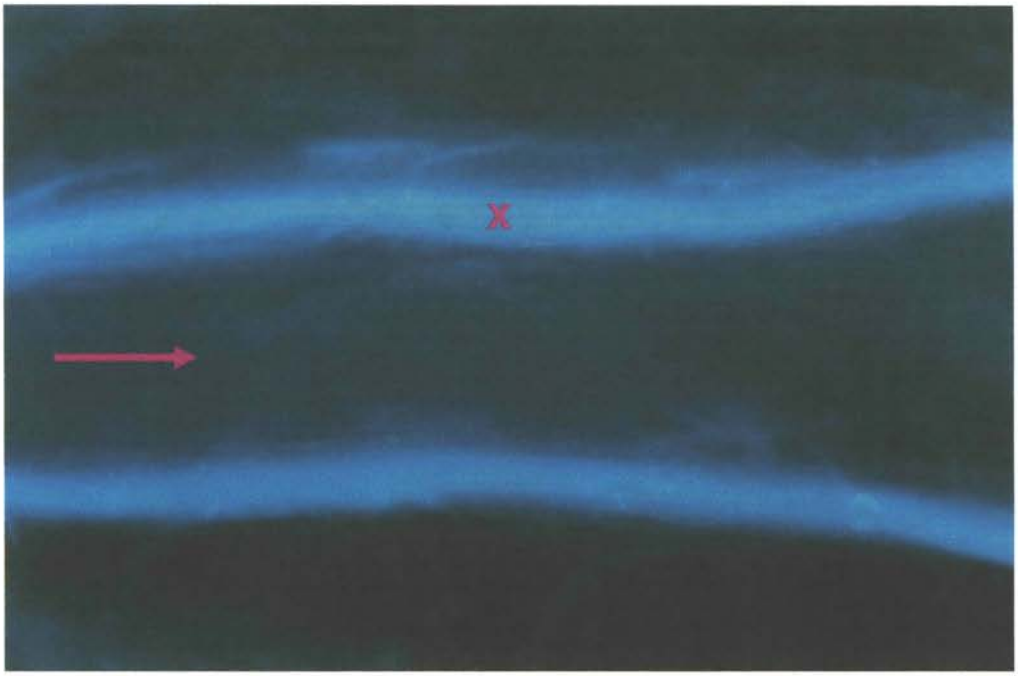

Fig. 5. Xylem (x) tracheids at the tip of a white root stained with Cellufluor (100x). The diarch xylem fluoresced bright blue under epifluorescent illumination. The arrow indicates the direction of stain movement from the base to the apex of the root.

Data analysis

Statistical analyses were performed using SAS and means and standard deviations were calculated (SAS 1999). Mean separations were carried out using LSD at $p \leq 0.05$

RESULTS AND DISCUSSION

Root Length

Enstone *et al.* (2001) reported that root color and diameter are two features that can be observed macroscopically without damage to the root. According to McCrady and Comerford (1998) color of primary roots of loblolly pine was a reliable predictor of developmental stage and it was related to vitality of cortical tissues. The FOLR of loblolly pine from the mist chamber, peat-vermiculite, and loamy sand exhibited significant differences in length of the different root zones.

White zone

Differences in the length of white zone existed in the FOLR from the three media. Mist chamber roots contained the longest white zone (48 mm) while in peat-vermiculite and loamy sand the white zone was shorter (Table 1, Fig. 6). In the mist chamber there was a continuous supply of nutrient-rich water in the form of mist spray at frequent intervals and roots always remained wet. Moreover, there was no physical resistance to the roots as they were hanging down during elongation. Due to the pendulous condition of roots gravitational forces may also have a significant role in influencing root elongation. During the time between two mist sprays, nutrient-rich solution drained along the root length and the meristematic tips always held nutrient ions required for growth. As the mist chamber was maintained at room temperature, no fluctuation in environmental

Table 1. Morphological characteristics of white and CT zones in the first-order lateral roots from mist chamber, peat-vermiculite, and loamy sand. Means within rows followed by different letters are different at $p \leq 0.05$, (sample size).

Root details	Mist chamber	Peat-vermiculite	Loamy sand
Distance of first conducting xylem from tip (mm)	6.63 (10)	8.91 (15)	8.53 (11)
Diameter of white zone (mm)	0.74 (10)	0.68 (20)	0.73 (12)
Length of white zone (mm)	47.93 [a] (10)	25.50 [b] (20)	29.33 [a, b] (12)
Length of CT zone (mm)	152.23 [a] (10)	24.67 [b] (20)	80.83 [c] (12)
Emergence of first 2° lateral (mm)	65.00 [a] (10)	55.74 [a] (15)	28.10 [b] (10)
Beginning of cork layer (mm)	200.00 [a] (10)	150.00 [b] (20)	110.00 [c] (12)

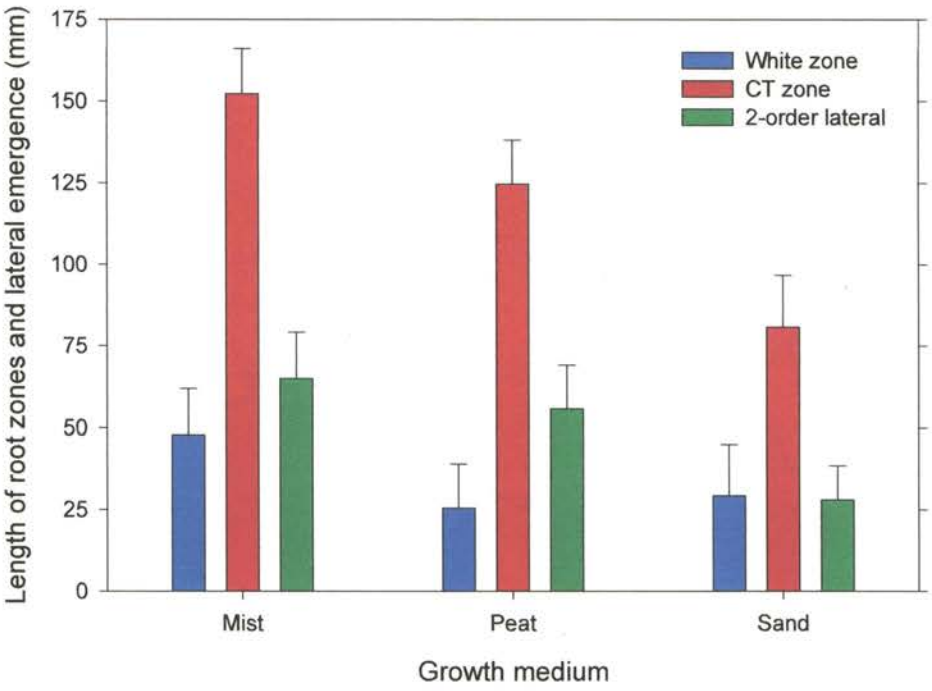

Fig. 6. Length of white and CT zones and distance between the root apex and the first-emerged second-order lateral in loblolly pine seedling roots from mist chamber, peat-vermiculite, and loamy sand (error bars = SE).

conditions occurred. This resulted in relatively constant photosynthetic activity required for preparing excess carbohydrates for translocation to roots resulting in enhanced root elongation. Therefore, these conditions favored faster growth of roots in the mist chamber and they contained more white zone compared to those in the other two media. Taylor and Peterson (2000) observed faster growth of roots in chamber-grown seedlings and this reflected the better conditions compared to those in the field. According to Wilcox (1962) faster growth of roots was associated with maturation of metaxylem and other structures farther from the tip. Thus faster growing roots would be expected to have long white zones. Enstone *et al.* (2001) observed that the white zone in the roots of pouch-grown loblolly pine was longer than that in the pot-grown roots. Although there was some difference in the growth conditions of mist chamber and pouch, the latter offered less impedance to root extension compared to pots. The longer white zone in mist chamber roots may permit more radial entry of water and ions due to an increased number of live cortex cells along the root. The roots in peat-vermiculite would have encountered periodic decreases in moisture between waterings. The resistance to root penetration would have been higher than in the mist chamber. It is possible that the periodic day conditions between waterings and greater resistance to penetration resulted in a shorter white zone than that in the mist chamber. The length of white zone in roots from loamy sand was nearly the same as in the peat-vermiculite. One distinguishing feature of loamy sand roots was the highly swollen nature of their tips compared to roots from other media. The swollen tips were not measured

separately because only a short segment of the tip was enlarged. This may be due to greater compaction of soil particles resulting in higher impedance during root elongation. According to Wilson *et al.* (1977) swollen root tips were characteristic of impeded roots. Therefore, root tips that encountered resistance produced more meristematic cells and became swollen at their tips.

Condensed tannin zone

The CT zone in the FOLR exhibited a gradual death and collapse of outer cortical cells. Roots behind the white zone appeared brown to the unaided eye. Richards and Considine (1981) reported that upon epidermal and cortical cell death, the release and oxidation of phenols normally enclosed within cell vacuoles result in a brown color on root surface. The roots from the mist chamber had the longest (152 mm) CT zone (Table 1, Fig. 6). The reason for the longer CT zone in the mist chamber may have been the favorable growth conditions. Roots encountered minimum resistance during root elongation and the intermittent mist spray containing nutrient-rich solution promoted faster root growth. Although the extension of white zone may have been faster, the development of cork zone could have been delayed resulting in a longer CT zone. Cell death and sloughing of cortex cells are characteristics of the CT zone. Both processes may have been delayed in the mist chamber due to favorable growth conditions. The favorable conditions may have promoted rapid growth and slow conversion to CT and cork zones. The result would have been longer white and CT zones. The longer CT zone may permit more radial entry of water

through an increased number of passage cells. Although the length of white zone in loamy sand roots was longer than that in peat-vermiculite, the length of CT zone was the minimum. The greater compaction in loamy sand may have influenced the development of cork zone closer to the tip resulting in minimum CT zone length.

The browning of white roots progressed much faster where the compaction in the rooting medium was greater. Therefore, the conditions in a rooting environment have a decisive role in determining the nature of root zones. Roots grew faster and contained more white and CT zones when resistance was a minimum as in the mist chamber. This offered more absorptive surface for the radial entry of water and ions into roots. In peat-vermiculite and loamy sand growth conditions were less favorable and resulted in shorter white and CT zones.

Root diameter

No significant difference occurred in the average diameter at the white zone of FOLR from the mist chamber, peat-vermiculite, and loamy sand. The diameter varied between 0.6 and 0.8 mm (Table 1) and it was almost uniform except at the extreme tip where the diameter was larger. The diameter in all three media declined gradually along the CT zone (macroscopic observation, not measured). In the mist chamber and peat-vermiculite diameter at the white zone had less taper towards the CT zone while in loamy sand the extreme tip of roots

was highly swollen and then declined abruptly. The gradual decrease in diameter of the CT zone coincided with death of cortical cells. This continued along the CT zone until reaching the cork zone where complete suberization of endodermis occurred internally. Then the diameter of roots increased along the cork zone (macroscopic observation, not measured) due to the combined effect of vascular and cork cambia.

McCrady and Comerford (1998) reported that majority of fine roots in loblolly pine were in the diameter range of 0.4 to 0.6 mm and white roots emerging from the tips of brown roots often had diameters exceeding those of parent roots. In the current study only FOLR were examined and they had a diameter of 0.6 to 0.8 mm. Enstone *et al.* (2001) reported that the diameter of tap roots in loblolly pine seedlings remained constant (about 0.6 mm) between 20 and 40 mm behind the tip and declined to the narrowest point (0.5 mm) at about 110 mm in pouch-grown roots. They also observed that changes in root diameter were correlated with internal anatomical changes and root shrinkage in the CT zone was due to death of cortex cells. Although diameter was not measured at the CT zone in the present study, diameter appeared to decrease. In the mist chamber, roots were hanging down without any resistance during elongation and they received mist spray at frequent intervals. Therefore, mist chamber roots had longer white and CT zones with almost uniform diameter and minimum taper. Wilcox (1962) reported that faster growing roots would be expected to have long white zones. According to Taylor and Peterson (2000) faster growth of

roots reflected better conditions available for root growth compared to those in the field. The swollen tips of white roots in loamy sand may be due to greater compaction of soil particles. Compared to the high porosity in peat-vermiculite, greater compaction in the loamy sand created greater resistance to growth. Wilson *et al.* (1977) reported that impeded roots contained swollen tips.

Emergence of 2° laterals

Emergence of second-order laterals was noticed near the transition from the white to the CT zone, although in many cross sections initiation of lateral primordia was noticed in the white zone (Fig. 3). Enstone *et al.* (2001) reported that primordia of FOLR or branch roots in the tap roots of loblolly pine originated invariably in the white zone and emerged more or less in acropetal sequence.

Second-order laterals emerged much closer to the root tip in loamy sand soil than in the others (Fig. 2 and 6). In the mist chamber lateral roots emerged farthest (65 mm) from the tip compared to those from peat-vermiculite and loamy sand (Table 1). In the mist chamber and peat-vermiculite, laterals emerged at the distal end of the CT zone, while in loamy sand they appeared very close to the proximal end of the white zone. Therefore, the rooting environment has a significant role in determining the initiation and emergence of laterals in loblolly pine seedlings. When conditions were favorable, roots grew faster and as a result, initiation and emergence of laterals were delayed. In the mist chamber root zones were longer and laterals emerged farthest back in the CT zone. In

peat-vermiculite laterals emerged in the CT zone much closer to the root apex while in loamy sand they emerged at the proximal end of the white zone. The adverse conditions in the loamy sand were responsible for the production of laterals closest to the tip. Enstone *et al.* (2001) observed the initiation of FOLR primordia at 7 mm and emergence at 46 mm from the tip in the tap roots of pouch-grown loblolly pine where the conditions were more favorable. In pot-grown roots harvested at different dates the initiation of primordia was closer to the tip at variable distances. According to Wilcox (1968) FOLR emerged near the tip due to suppression of an apical dominance in non-growing tap roots.

Roots respond faster to changes in the rooting environment depending on the severity of conditions. Roots in the mist chamber contained a longer white zone and the development of the CT zone was delayed due to minimum resistance in the rooting environment. This may have resulted in the delayed appearance of second-order laterals. In loamy sand resistance to root extension was greatest. This may have led to the emergence of second-order laterals closest to the apex. Peat-vermiculite roots were intermediate in the production of second-order laterals. Therefore, when minimum stress occurred in the rhizosphere the development of CT zone was delayed resulting in longer white and CT zones, and when conditions became unfavorable they adapted by the progression of 'browning' and production of second-order laterals closer to the tip. As roots required energy to grow through resistant soil they were in need of more water and nutrients for producing photosynthates. By the development of

CT zone closer to the tip, roots lost part of the absorbing surface and new higher order laterals were produced that may have functioned to replace the lost absorbing surface. Therefore, the absorbing surface in one FOLR was replaced by many higher order laterals for absorbing sufficient amount of water and nutrients. These young roots may be capable of exploring other areas in the soil where there is less resistance and more moisture. Thus seedlings may be capable of producing more photosynthates for translocation to roots for providing energy for the production of new laterals. This may be considered as an adaptation by seedlings to overcome the adverse conditions in the rooting environment. As a result, roots may become more fibrous and capable of exploring far and wide for water and nutrients in an adverse condition.

Number of cortex cells

The total number of cortex cells in a radial file was greatest (10) in the peat-vermiculite and 30 percent lower in both the mist chamber and loamy sand (Table 2, Fig. 7). This number included both live and dead cells and the endodermis. Near the tip all cells were alive and they gradually died as the root aged. The peat-vermiculite roots showed no decline in cortex cells in the white zone and a decline of 20 percent in the CT zone. Following the initial decline it appeared that the number of cells fluctuated from 6 to 8 up to the cork zone. The number of cortex cells declined by 25 percent in the white zone of the mist chamber roots and then fluctuated from 3 to 6 through the CT zone. The loamy sand roots lost nearly 50 percent of the cortex cells in the white zone and

Table 2. Anatomy of white and CT zones in the first-order lateral roots from mist chamber, peat-vermiculite, and loamy sand. Means within rows followed by different letters are different at $p \leq 0.05$, (sample size).

Cell dimensions	Mist chamber	Peat-vermiculite	Loamy sand
White zone			
Cortex cell layers (#)	7 [b] (9)	10 [a] (9)	7 [b] (11)
Passage cells (#)	33 [a] (13)	11 [b] (22)	5 [c] (13)
Endodermal cells (#)	69 (13)	62 (22)	65 (13)
Mean passage cells (%)	48	18	8
CT zone			
Cortex cell layers (#)	4 [b] (9)	8 [a] (9)	1 [c] (11)
Passage cells (#)	19 [a] (13)	5 [b] (22)	2 [c] (13)
Endodermal cells (#)	70 (13)	62 (22)	66 (13)
Mean passage cells (%)	28	8	3

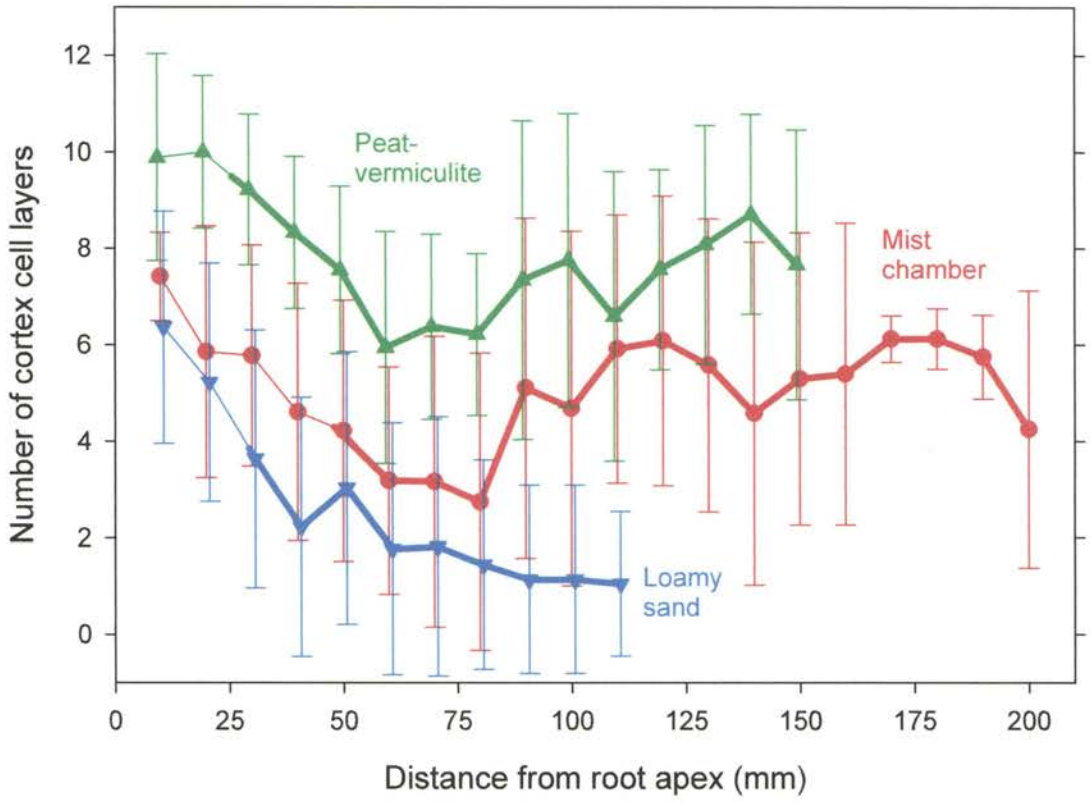

Fig. 7. Number of layers of cortex cells in the roots from mist chamber, peat-vermiculite, and loamy sand. Thin lines indicate white zone and thick lines indicate CT zone, n = 9 - 22.

continued to lose cortex cells in the CT zone until the average number of cells was slightly greater than one. This means only the endodermis was still intact.

These results show that in favorable conditions most of the cortex cells (more than 75 percent) will remain attached to the root even when dead. The number of dead and collapsed cells increased through the CT zone up to the cork zone. On the other hand, under abrasive conditions in a compact soil the cortex cells are sloughed rather early leaving only the endodermis and fragments of cortex cells in the CT zone. As a result, cortex cells declined continuously until reaching the cork zone. In the mist chamber there was no abrasion between outer cortex cells and rooting medium and hence they remained intact despite loss of turgidity. In contrast, peat-vermiculite offered moderate abrasion to the growing roots.

When the endodermis was mature it developed Casparian bands on the radial and transverse or end walls. The development of Casparian bands eliminated the apoplastic pathway for water exchange except for breaks or bypasses. In the mist chamber roots the endodermis was not fully developed until 20 mm from the apex (Fig. 8). The number of endodermal cells remained the same along the white and CT zones until crushed by the basipetal cork zone (data not shown). No significant difference existed in the total number of endodermal cells in the roots among the three media and an average of 65 cells constituted the endodermis (Table 2). Enstone *et al.* (2001) did not observe any

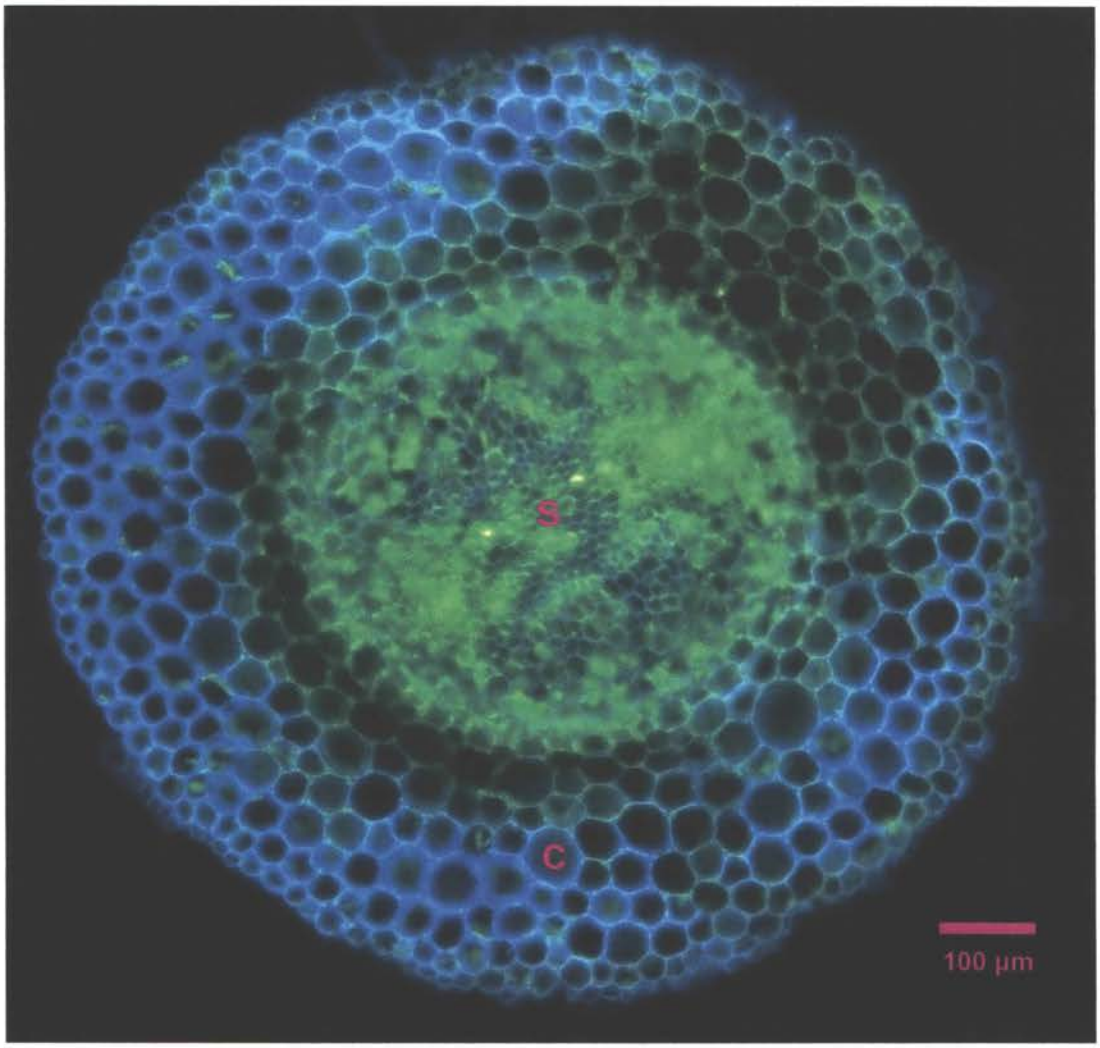

Fig. 8. Cross section at 10 mm from the apex of a mist chamber root stained with Fluorol Yellow 088 (100x). The multicellular cortex (c) encloses the central stele (s); the endodermis is not well developed at this point.

increase in the number of endodermal cells along the CT zone, but when secondary growth began the number increased due to anticlinal division and their tangential walls were stretched. By the time the endodermis was crushed, a layer of cork formed internal to it maintaining the function of apoplastic barrier.

There was a significant difference in the number of passage cells in the endodermis of roots from the three media. The number of passage cells was highest in the mist chamber roots and least in loamy sand roots (Table 2). Enstone *et al.* (2001) observed a higher number of passage cells in pot-grown roots than in pouch-grown roots. Although the number of endodermal cells remained the same in the white and CT zones, a significant decline occurred in the number of passage cells along the CT zone in all the three media. The mist chamber roots had longer white and CT zones and the smallest relative decrease in the number of passage cells in the CT zone. Peat-vermiculite roots recorded a large percentage decline in passage cells in the CT zone and the decline was greatest in loamy sand roots (80 %). The conditions in the three media determined the number of passage cells. Due to uniform growth conditions and availability of surplus nutrient solution, roots in the mist chamber contained longer white and CT zones and retained more passage cells. According to Wilcox (1962) the presence of a long white zone is characteristic of fast growing roots. In loamy sand roots deposition of suberin lamellae progressed much faster resulting in a faster decrease in the number of passage cells, presumably to prevent loss of water. In peat-vermiculite the conditions were more moderate

and roots had a longer CT zone compared to loamy sand roots and the decrease in the number of passage cells was also less.

Suberization of endodermis

The deposition of suberin lamellae on the inner tangential walls of the endodermal cells began in the white zone. The deposition was first initiated in the cells opposite the phloem and continued along the CT zone until the cells opposite to xylem poles were also suberized. Due to suberization, the number of passage cells in the endodermis declined significantly in the white zone and more gradually in the CT zone. The presence of Casparian bands on the radial and transverse or end walls disrupted the apoplastic continuity between the outer cortical cells and inner stelar tissues. Therefore, the presence of unsuberized (passage) cells in the endodermis was highly important in determining the amount of water that could enter into the stele (Peterson and Enstone 1996). The unsuberized cells which were aligned with xylem poles for some distance in the CT zone permitted entry of water into the stele through the symplastic pathway. When all the cells were suberized, a layer of cork cambium developed along the periphery of the stele and this marked the beginning of the cork zone (Enstone *et al.* 2001). By the production of more cork cells the cork cambium pushed the endodermis centrifugally and crushed it. The cork layer acted as an apoplastic barrier taking up the role of endodermis in regulating the movement of water and ions.

Suberization of all endodermal cells in the roots from the three media exhibited significant differences marking the beginning of a cork layer (Table 1). The roots from loamy sand developed complete suberization closest to the apex (110 mm) while it was farther from the apex of roots in peat-vermiculite and mist chamber (Fig. 9). In mist chamber roots endodermis was not developed at 10 mm from the apex and suberization was not yet begun. Suberization was noticed first at 20 mm from the apex and about 44 % of endodermal cells were already suberized. The CT zone began at about 48 mm and about 72 % of cells were suberized at this point. While suberization progressed much faster in the white zone it was more gradual in the CT zone. This is similar to the observation made by (Enstone *et al.* 2001) in the tap roots of pouch-grown loblolly pine seedlings. They observed that suberin lamellae first appeared between 10 and 20 mm and by 30 mm, 77 % of cells were suberized. This remained fairly constant until 70 mm. In the roots from mist chamber only about 10 % of cells remained unsuberized at 170 mm from the apex. Suberization of all endodermal cells occurred at 200 mm (Fig. 9) forming a ring around the stele. Further development of roots resulted in the initiation of a cork layer outside the pericycle. Taylor and Peterson (2000) observed faster growth of roots in chamber-grown seedlings reflecting the better conditions compared to those in the field. According to Wilcox (1962) along with faster growth, maturation of metaxylem and other structures occurred farther from the root tip. In the roots from peat-vermiculite suberization of endodermal cells began at 10 mm from the apex and about 80 % of cells were suberized (Fig. 9). Therefore, the number of

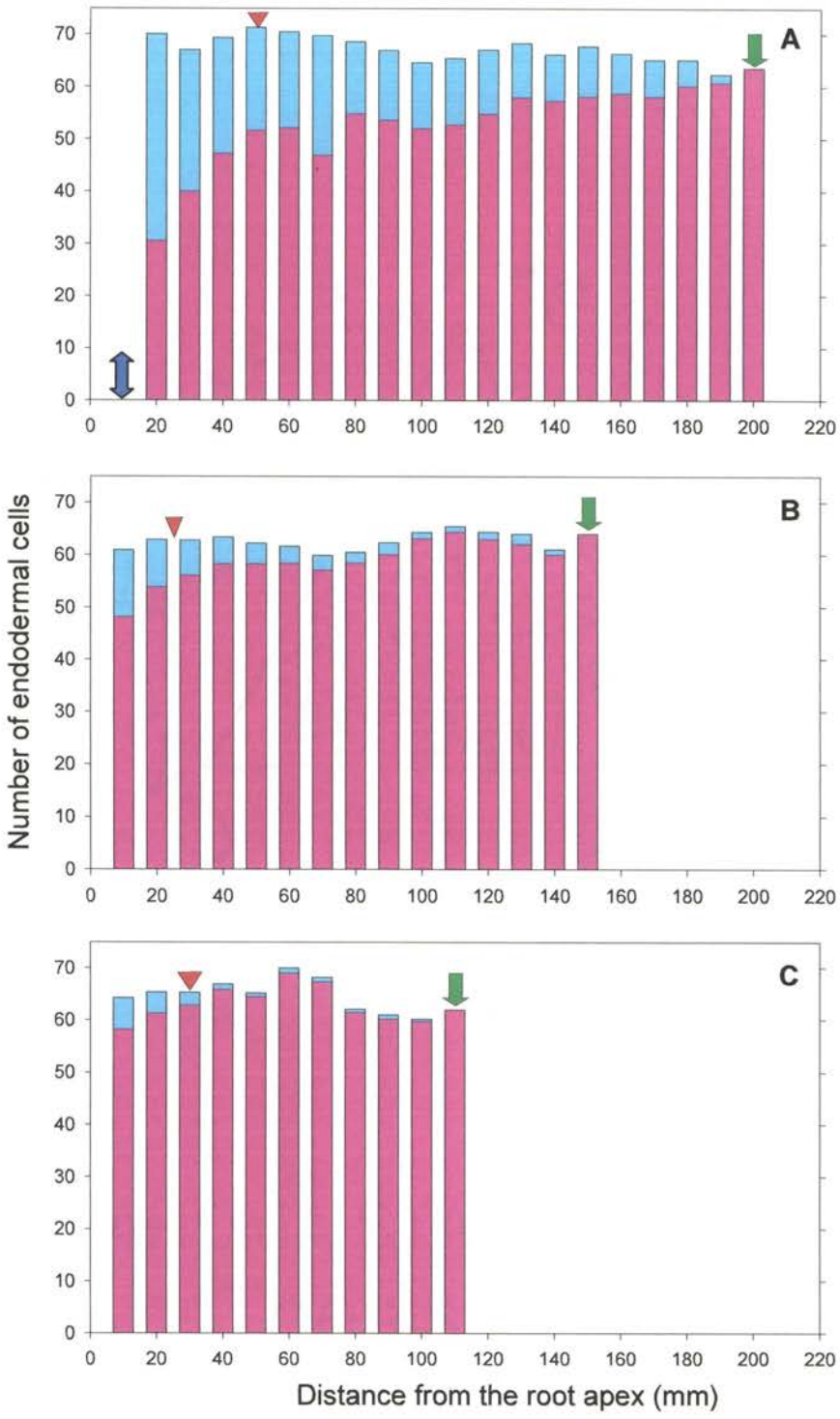

Fig. 9. Number of suberized ( ) and unsuberized ( ) cells in the endodermis in the first-order lateral roots of loblolly pine from mist chamber (A), peat-vermiculite (B), and loamy sand (C). (= no endodermal cells, = transition from white to CT zone, and = transition from CT to cork zone); n = 9 - 22.

passage cells was low in the white zone compared to that in the mist chamber roots. In the CT zone most cells (90 %) were suberized at 40 mm. All endodermal cells were deposited with suberin at 150 mm and the development of a cork layer initiated at this point crushing the endodermis centrifugally. In the roots from loamy sand suberization occurred closest to the tip compared to those from the other two media (Fig. 9). At 10 mm from the apex 90 % of cells were suberized. In the CT zone changes in the number of suberized cells was fairly gradual. A completely suberized endodermal ring was noticed at 110 mm (Fig. 10). Further development of roots resulted in the appearance of a cork layer outside the pericycle and the crescents of the xylem merged at the center (Fig. 11). Similar observations were made by McKenzie and Peterson (1995a) in pouch-grown jack pine and Enstone *et al.* (2001) in pouch-grown loblolly pine. The reason for the deposition of suberin closest to the tip in loamy sand roots may be the low availability of water due to faster drainage and low water holding capacity. As a result the higher density of loamy sand offered greater resistance to growing root tips. The more rapid suberization would expose less root area to water loss back into the soil.

McCrady and Comerford (1998) reported that suberization is an internal cellular process and may result from several conditions. Development of suberin lamellae in all cells of the endodermis was responsible for the death of cortex cells in roots of woody species (Enstone e*t al.* 2001 and Leshem 1974). As a result plasmodesmata connecting the endodermis with cortex cells on the outside

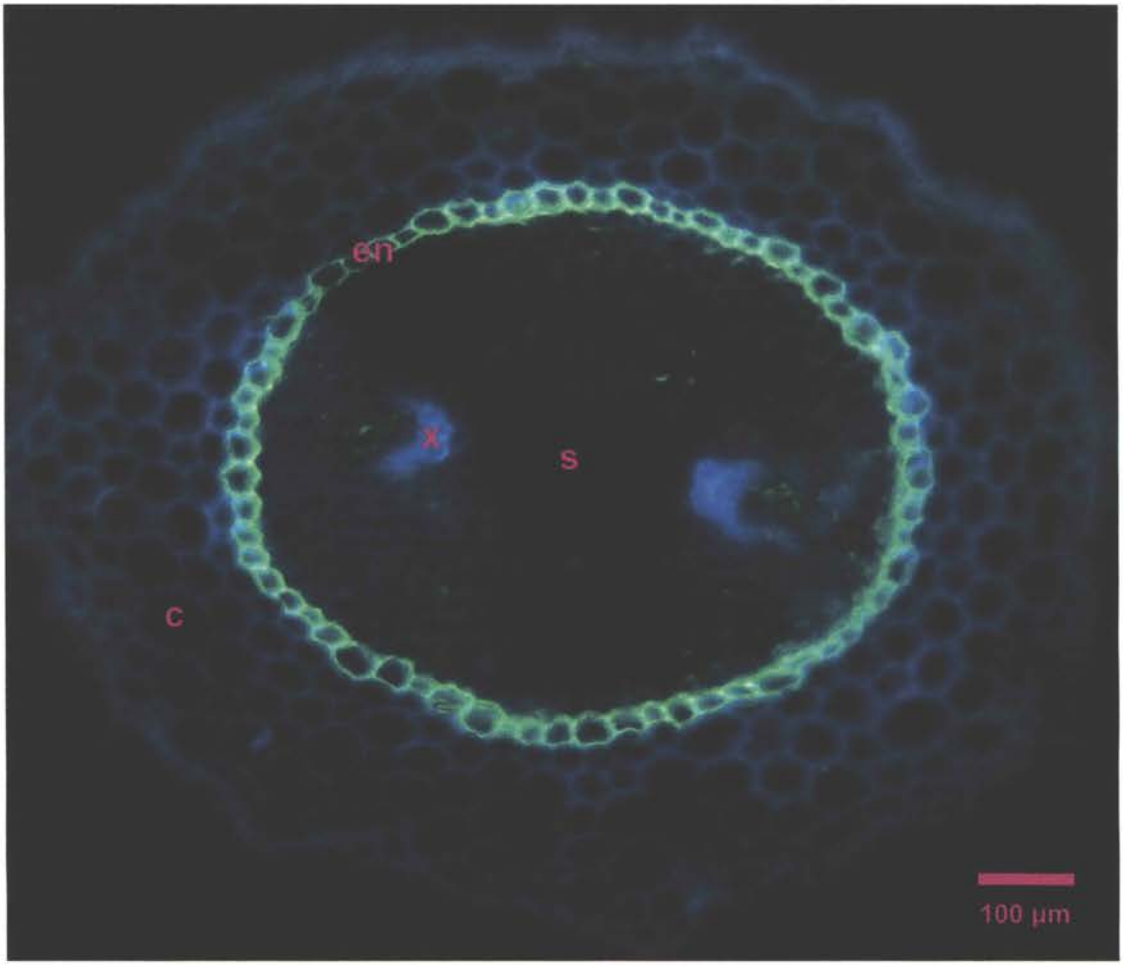

Fig. 10. Cross section at 110 mm from the apex of a loamy sand root stained with Fluorol Yellow 088 (100x). All cells of the endodermis (en) were suberized to form a ring around the stele (s) and the xylem (x) tracheids merge at the center during further growth. Although the cortex (c) was dead, three to four layers of cells were retained at this point.

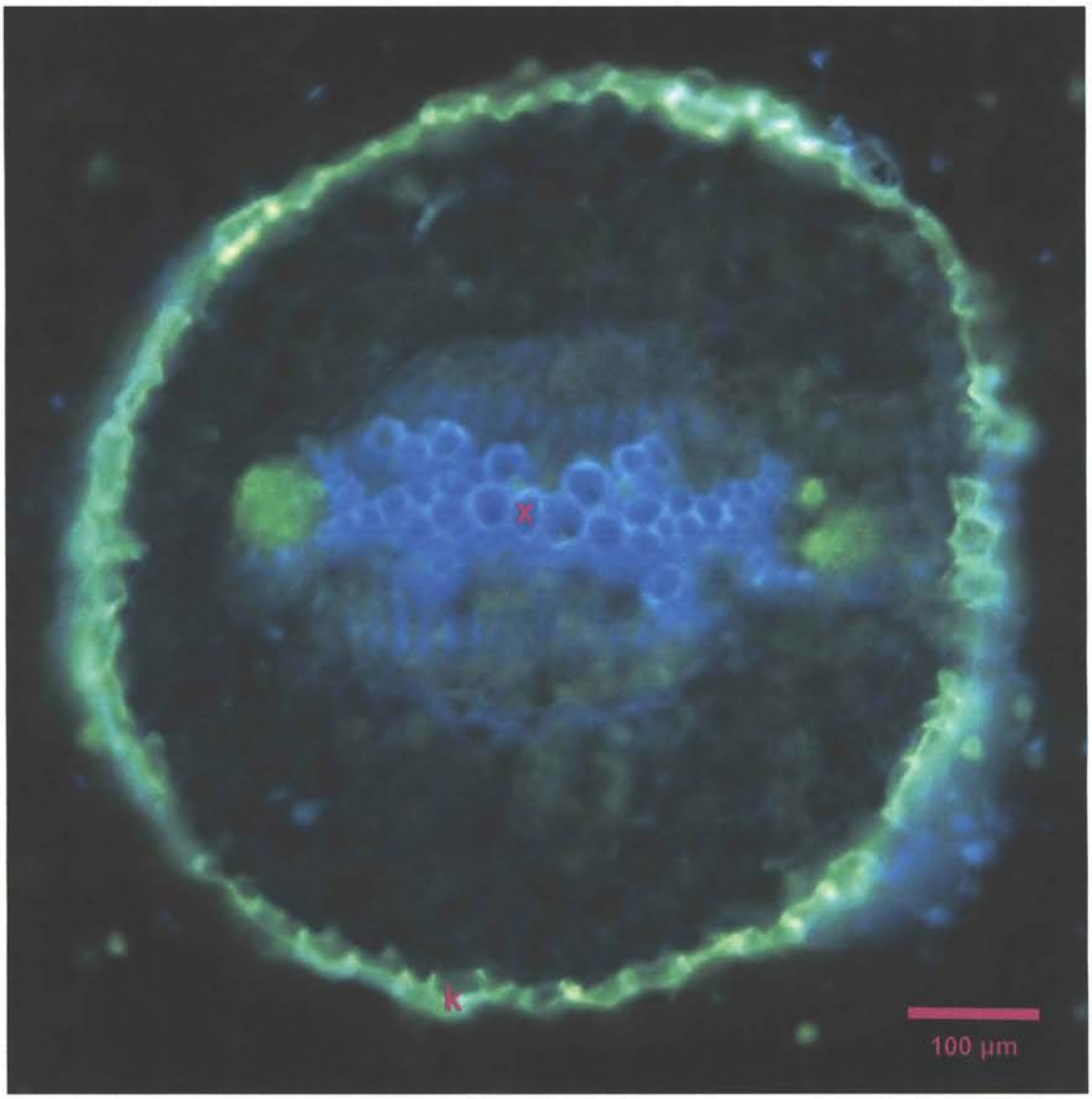

Fig. 11. Cross section at 130 mm from the apex of a loamy sand root stained with Fluorol Yellow 088 (100x). The two xylem (x) poles merged at the center of the stele. The endodermis was crushed by the centrifugal development of a cork layer (k) originated in the pericycle.

and pericycle on the inside are lost. Therefore, suberization of endodermis has a significant role in regulating the movement of water and ions into the stele. The more favorable conditions in the mist chamber delayed the deposition of suberin lamellae while the less favorable conditions in the other two media initiated the development early. This was very specific in loamy sand roots that resembled more or less a field condition. Therefore, when the conditions in the rooting environment become unfavorable, roots develop 'browning' closer to the tip marking the beginning of CT zone. As a result death of cortex cells progresses closer to the tip and the symplastic connection between cortex cells and stelar cells is lost. This may act as a protective measure to reduce the amount of water lost from the cells back into the drying medium. In peat-vermiculite and loamy sand there were periods of drier conditions due to faster drainage resulting in faster initiation of suberization. This might have resulted in the development of CT zone closer to the tip and death of cortex cells reducing the amount of symplastic flow and water loss. Thus, while passage cells permit the entry of a required amount of water and nutrients into the stele, suberization closer to the tip may prevent water loss back into the soil. Therefore, suberization of all cells closer to the tip may adapt seedlings to overcome adversities in the rooting environment ensuring better survival. This may be a favorable adaptation by seedlings growing in drought prone conditions where they can effectively utilize the limited availability of water and nutrients.

Maturity of xylem

The xylem was not conductive over the distal 7 to 8 mm of the root (Table 1) and environmental conditions did not have a discernable effect on the length of the hydraulically isolated tip. The youngest part of the root was developing Casparian bands in the endodermis and they were not mature to conduct water. Enstone *et al.* (2001) observed that in lateral roots of pouch-grown loblolly pine functional tracheids were present at 2 to 8 mm, while non-functional tracheids extended to within 2 to 4 mm of the tip. They also reported that walls of xylem tracheids distal to the zone where the stain moved had spiral thickening. This indicated that function followed wall thickening. Therefore, for conducting water at the tips of roots, xylem has to attain a certain level of maturity. Although tracheids began to mature distal to mature endodermis, they did not become functional until after Casparian bands had developed. Therefore, tracheids can transport water only when the walls thicken and Casparian bands form on the radial and transverse walls of the endodermis. As there was not much difference in the maturity of xylem in the roots among the three media, it is evident that tracheids in loblolly pine matured between 5 and 10 mm from the apex. As this occurred within the white zone of roots, they contribute significantly to increasing the absorbing surface of roots.

There was no significant difference in the number of xylem poles in the roots from the three media. An average of 2 xylem traces (diarch) was commonly observed, although sometimes triarch or even tetrarch roots were

noticed. Enstone *et al.* (2001) observed mostly triarch in pouch-grown and pot-grown loblolly pine. The pattern of xylem development was centripetal, as observed by Wilcox (1964) and reported later by Enstone *et al.* (2001). The crescent-shaped primary xylem contained protoxylem at the tips while metaxylem was at the apex. Further development occurred from the tips of metaxylem and the crescents were linked by bridges of tracheids. Xylem elements followed a centripetal pattern of development along the CT zone meeting at the center of the stele.

Therefore, the developmental stage of xylem determines whether it can conduct water and ions to the above-ground part. When new laterals are produced they cannot transport soil solution until they attain the required maturity. Hence, when lateral roots appear, they are not conductive immediately after emergence. But they attain maturity soon after emergence increasing the absorbing surface and conductive ability.

CONCLUSIONS

The following conclusions were made from the present research involving first-order lateral roots of loblolly pine seedlings under different environmental conditions:

- The white and CT zones were longest in the most favorable conditions and both zones became shorter as soil conditions became less favorable.

- Under favorable conditions production of higher order laterals was delayed and when roots encountered resistance it was closer to the tip. This may be a mechanism to increase absorbing white zone to replace that lost to production of CT zone.

- The death and sloughing off cortex cells in the CT zone occurred closer to the tip when roots experienced resistance. This may cause less symplastic resistance resulting in increased conductivity of water.

- While the number of endodermal cells was not affected by rooting environments, their development and deposition of suberin lamellae were delayed in the highly humid mist chamber.

- The number of passage cells declined much faster in the white zone than in the CT zone and this was more specific under adverse conditions. This may have been an adaptation to prevent water loss.

- The extreme tips of growing roots were hydraulically isolated and roots became conductive a few millimeters behind the tip. Roots did not have conducting xylem until Casparian bands appeared and tracheids matured.

- Root modifications due to increasing environmental stress appeared designed to restrict water and nutrient exchange with the soil. A reduction in water loss to a dry soil would be beneficial. A reduction in water uptake capacity would be negative, but the plant may be capable of rapidly increasing the uptake capacity when the environment becomes more favorable.

IMPLICATIONS AND FUTURE RESEARCH

- Information on the anatomy and its correlation to root functioning is important in tree improvement programs because selection of families for efficient use of soil water requires characterizing root properties. The current study demonstrated the feasibility of measuring root traits associated with water and nutrient uptake capacity, such as length of white zone, number of passage cells, suberization of endodermis, number of cortical cell layers and sloughing off cortex cells. Further research to determine genetic variation and control of these traits is necessary before they can be used to select for improved root traits.

- The changes in root anatomy were studied using first-order laterals under controlled conditions. It would be worthwhile to extend these findings to plants in the field and also to higher order laterals and mycorrhizae.

- According to Cruz *et al.* (1992) drought stress hastens endodermal maturation. As efforts to extend the range of loblolly pine towards west of its natural range into more drought-prone areas are underway, the performance of this stimulus has to be examined under drought conditions involving different families.

- The importance of CT zone in the absorption of water and nutrients has been under debate. Roots from the mist chamber had the longest CT zone with more passage cells. This seems to show that roots in the least stressful environment have the greatest capacity for water exchange with the soil.

- Roots in loamy sand turned brown much closer to the tip and suberization progressed faster. Second-order laterals initiated and emerged closer to the tip. These new roots close to the tip may have permitted greater flow of water into roots. It could be hypothesized that roots become more fibrous under droughty conditions; further investigation is needed to determine whether this occurs under field conditions.

- It would be informative to determine to what extent the production of new laterals in a hard soil will make up for the loss of white zone.

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CHAPTER – III

DEVELOPMENT OF ABSORBING SURFACE AREA OF ROOTS OF LOBLOLLY PINE SEEDLINGS

ABSTRACT

Roots of loblolly pine seedlings are characterized by having three zones [white, condensed tannin (CT) and cork], similar to those in jack pine previously described by McKenzie and Peterson (1995a, b). Plants produce white roots to increase absorbing surface to meet the growing demand of water and nutrients. Mycorrhizae are also considered to enhance the absorptive surface of roots under nutrient-limiting conditions. The primary goal of this study was to elucidate changes in the root anatomy related to water and nutrient uptake capacity of pine roots over the first year of growth and how these changes were related to factors such as ontogeny, seedling allometry, and weather. As roots grow vigorously twice (spring and fall) in a growing season I wanted to learn whether there were changes in the development of uptake capacity associated with this growth periodicity. I studied the distribution of roots and mycorrhizae with soil depth because trees grow allometrically with uptake and demand capacity for water

well balanced with the environment. Seedlings were harvested approximately every month from the state nursery using PVC pipes and soil cores were washed with tap water to extract roots. Roots were separated into different zones and anatomical measurements were made following different staining protocols and imaging techniques; the bulk of roots were scanned using Delta-T Scan. Cortical plasmalemma surface area (CPSA) was calculated following the method of Taylor and Peterson (2000). Seedling biomass growth was steady and rapid from July until mid-November for stems and leaves and until mid-December for roots. Over 90 percent of the roots were in the upper 20 cm of soil due to irrigation not penetrating deeper. White root tips appeared to reach a fairly constant level by July with some increase in early winter. In contrast, mycorrhizal tips were produced rather late and their number exploded from several hundred to 60,000 m^{-2} from October to mid-December. Nearly 90 percent of the root length and surface area were in the CT zone while over 98 percent of the CPSA was in the white zone and mycorrhizae. The amount of CPSA per unit of root length in the white zone increased 3-fold over the growing season due to increases in number and size of cortex cells. The ratio of CPSA to leaf area was at its peak in the hot summer months when evaporative demand was highest and declined in the winter when demand for water declined. Further research should be done to elucidate the degree of environmental and genetic control of root anatomy.

INTRODUCTION

Root morphology

Root color was used as the criterion to classify roots in the past; brown roots were 'suberized' and white roots were 'unsuberized' (Chung and Kramer 1975, Kramer and Bullock 1966, Sands *et al.* 1982, and van Rees and Comerford 1990). The three characteristic zones (white, CT, and cork) proposed by McKenzie and Peterson (1995a, b) in pouch-grown jack pine were also observed in loblolly pine (Peterson *et al.* 1999). Each of these zones has its own characteristic anatomical features that have significant roles in the absorption of water and nutrients (McKenzie and Peterson 1995a, b).

Root anatomy

The absence of a typical epidermis in the roots of loblolly pine (Mirov 1967) means the cortex is in direct contact with the soil solution. In the white zone the multilayered cortex is limited internally by a single layered endodermis that encloses a central stele. The endodermis comprises suberized and unsuberized (passage) cells. The number of passage cells is greater in the white zone compared to any other root zone. The Casparian band and suberization of endodermal cells act as apoplastic barriers to the movement of water and ions into the stele (Clarkson and Robards 1975, Esau 1977, and Peterson 1988). The cortex in the CT zone is dead and therefore, the outer tangential surfaces of passage cells are in direct contact with soil solution. As a result, the presence

and the number of passage cells in the endodermis are very critical in deciding the absorbing surface in the CT zone. This is particularly true when white roots undergo metacutization, a process by which the endodermis extends to the tip enclosing the apical meristem during adverse environmental conditions (Wilcox 1954, 1968). The cork zone, basipetal to the CT zone is characterized by secondary growth initiated by divisions of vascular and cork cambia. Enstone *et al.* (2001) reported that vascular cambium was active before the initiation of cork cambium. Therefore, separation of CT zone from cork zone is more realistic if done based on cork cambium development. In roots cork cambium typically arises in the outer stelar cells and as a result, cork cells are produced interior to the endodermis (Esau 1977). When cork cells form a complete ring at the periphery of the stele and continue growth, the endodermis is crushed. Therefore, the main function of cork layer is to act as an apoplastic barrier taking up the role of endodermis.

Significance of endodermis

The endodermis is a single layered parenchymatous tissue surrounding the stele. It passes through three stages of development (Clarkson and Robards 1975 and van Fleet 1961). During the initial stage (State I) mature cells are formed when they develop a Casparian band on the radial and transverse or end walls. During the second stage (State II) deposition of thin sheets of suberin lamellae on the inner tangential walls, and during the final stage (State III) deposition of a secondary cellulosic wall internal to suberin occurs. Although

Casparian bands and suberin lamellae combined with secondary walls prevent ion movement through the apoplast, there is a symplastic continuity. This is achieved through plasmodesmata connecting the endodermis with cortex on the outside and pericycle on the inside, that remain intact during deposition of suberin lamellae (Clarkson *et al.* 1971 and Robards *et al.* 1973).

Importance of passage cells

During the development of endodermis, cells that remain in State I (they develop a Casparian band on the radial and transverse or end walls) are called passage cells. They are aligned radially with protoxylem poles to facilitate transport of water and ions into the stele. Although passage cells also develop a Casparian band like other cells of the endodermis (Grymaszewska and Golinowski 1987 and Scott and Peterson 1979), the development is delayed (Peterson and Enstone 1996). Therefore, passage cells have a significant role in transport of ions into the stele, and in turn to the transpirational stream, thus offering areas of low resistance (Peterson and Enstone 1996). When cortical cells in the CT zone die, the endodermis becomes the outermost living layer of the root. Then plasmalemmae lining the outer tangential walls of passage cells are the only sites where soil solution contacts symplast (Peterson and Enstone 1996). Taylor and Peterson (2000) studied the relationship between cortical plasmalemma surface area (CPSA) and mycorrhizal association in roots of jack pine, and found that the white zone contained most CPSA in chamber-grown roots (85 %) while mycorrhizae contained the most CPSA in field-grown roots (80

%). In the CT zone CPSA was found only in the outer tangential surfaces of passage cells in the endodermis and the cork zone contributed no CPSA.

Role of ectomycorrhizae

Pine roots are known for having a symbiotic relationship with ectomycorrhizal fungi. Ectomycorrhizae have a significant role in extending the capacity of roots to take up water (Mudge *et al.* 1987 and Parke *et al.* 1983) and nutrients (Vogt *et al.* 1991). The role of ectomycorrhizae in the absorption of water and nutrients has been described variously. Although some researchers reported a negative or no effect of ectomycorrhizae on pine growth (Coleman *et al.* 1987, 1990, Sands *et al.* 1982, and Sands and Theodorou 1978), in most cases a positive relation was observed between the abundance of mycorrhizae and seedling growth (Harley and Smith 1983, Kendrick 1992, Muhsin and Zwiazek 2002, and Sylvia 1990).

When seedlings produce white roots spores of mycorrhizae in the soil invade them and colonize the cortical cells (Castellano and Molina 1989). The fungal mycelium multiplies within intercellular spaces and forms a Hartig net in the cortex and a mantle or sheath surrounding the root surface (Brundrett *et al.* 1996 and Massicotte *et al.* 1987). The mantle produces extramatrical hyphae that extend into the soil. The mantle combined with extramatrical hyphae increases the absorptive surface of roots (Sylvia 1990). Due to its very narrow dimension the extramatrical hyphae explore soil crevices far and wide that

otherwise are not accessible for roots or root hairs. The water and nutrients absorbed by extramatrical hyphae and mantle are exchanged to cortical cells at the Hartig net (Brundrett *et al.* 1996 and Sylvia 1990). Fungi benefit from photosynthates translocated from the parent shoot. Thus there is a symbiotic relationship between the roots of parent plant and ectomycorrhizal fungi (Harley and Smith 1983).

Cortical Plasmalemma Surface Area

The term "cortical plasmalemma surface area" was coined by Taylor and Peterson (2000) and it represents the absorbing surface lining the living cells of the cortex. The newly produced roots are white and they become brown or dark during maturation. As the whole root system of a seedling contains roots of different types, the amount of white zone is very important in determining the absorptive capacity of roots. According to Kamula *et al.* (1994) soil solution must be in contact with plasmalemmae of living cells to help roots in the absorption of water and nutrients from the soil.

Taylor and Peterson (2000) reported that capacity of different zones of a root to absorb nutrients from soil solution is influenced by the amount of viable plasmalemma surface area. The plasmalemma surface area has access to soil solution through the apoplastic continuum. In roots, apoplast is limited by hydrophobic wall areas such as Casparian bands and suberin lamellae. The Casparian bands appear in the white zone followed by the deposition of suberin

lamellae. The deposition of suberin begins in the white zone proximal to the Casparian band and continues in the CT zone until reaching the cork zone. The apoplastic movement of water and ions into the stele is reduced significantly when Casparian bands (Baker 1971, Peterson *et al.* 1993 and Robards and Robb 1974) and suberin lamellae (Botha and Evert 1986, Evert *et al.* 1985, and Wenzel and McCully 1991) appear. At the proximal end of the CT zone the endodermis forms a complete suberized ring marking the beginning of the cork zone. Therefore, root tissues such as the endodermis and periderm (cork) act as barriers to the radial movement of soil solution. Taylor and Peterson (2000) reported that roots of seedlings exhibit periodic growth with alternating periods of elongation and dormancy. This might have a strong influence on the anatomy of different root zones. Therefore, the amount of plasmalemma surface contributed by living tissues is a major factor determining the ion uptake capacity of roots. According to Wilcox (1962) plant roots in temperate zones grow in spring and fall and cease growth in summer and winter. The growing roots undergo anatomical changes followed by changes in morphology to adjust to changing conditions of environment. During ideal environmental conditions roots contain a large amount of plasmalemma surface for absorbing more water and nutrients in order to meet the demand of the growing shoot.

Radial pathways of soil solution

Three distinct transport routes (apoplastic, symplastic, and transcellular) facilitate the radial transport of water and ions into the roots (Steudle 1994). The

apoplastic transport occurs through the walls of living cells, skirting the protoplasts. The symplastic transport occurs through cytoplasm of individual cells and plasmodesmata linking adjacent cells. The transcellular movement occurs when the substance passes through the plasmalemmae of cell walls, cytoplasm, and tonoplast lining the vacuoles, i.e. repeatedly through membranes. As a result, the soil solution crosses two cell membranes per cell layer. It must also include at least part of the apoplast as the substance moves from one cell to the next. Due to the difficulty in distinguishing between symplastic and transcellular movements, they are grouped together as cell-to-cell pathway. Several lines of evidence support the cell-to-cell pathway as the major route for the transport of water through unmodified parenchyma cells (Canny and Huang 1994 and Peterson *et al.* 1993). The specific cells through which materials move in the radial path are cortical parenchyma, endodermis, pericycle and stelar parenchyma (Peterson *et al.* 1999). Ions initially diffuse into roots through the walls of outermost cortex cells and then may be actively transported across the plasmalemmae. Ions are assumed to diffuse from the cytoplasm of one cell to the next through plasmodesmatal connections until reaching pericycle or stelar parenchyma. Once in the walls of pericycle and stelar parenchyma, ions diffuse along their concentration gradient to the lumens of tracheary elements. Most movement of water in the roots is driven by gradients in water potential (Peterson *et al.* 1999).

Ion and water absorption

As the extreme tip of the root is hydraulically isolated (Melchior and Steudle 1993), water enters most rapidly in a zone approximately 2 to 10 cm behind the tip depending on the species and season. Several studies have directed to the importance of new root growth in water and nutrient uptake. In loblolly pine, root hydraulic conductivity was closely related to the number of new roots (Carlson 1986). Sands *et al.* (1982) found conductivity in the unsuberized roots of loblolly pine to be 2.6 times greater than that in the suberized roots.

In order to achieve ion uptake, movement through the symplast is indispensable especially in the endodermis. The white zone, with its living cortex, has a large plasmalemma surface containing space for many protein carriers, and thus has a greater potential for ion uptake. As the deposition of suberin lamellae in the cell walls of the endodermis does not sever the plasmodesmatal connections between neighboring cells, the symplastic diffusion of ions into pericycle is not impeded (Peterson *et al.* 1999). In the CT zone, death of cortical cells drastically reduces membrane surface available for ion uptake. Therefore, presence of endodermal passage cells is critical for ion uptake in this zone of the root. During early secondary growth, cork cells form a continuous layer beneath the endodermis. Then the sites for ion uptake into symplast are reduced to nil at the cork zone. In dormant roots the CT zone extends closer to the tip, but the endodermis still retains passage cells. Thus, even dormant roots have some

potential for ion absorption via combined passive transport to interior living cells where active transport occurs.

The effects of root development on water uptake are even more difficult to predict than that for ions. In the white zone when the endodermis has only Casparian bands but no suberin lamellae, the major resistance to water uptake occurs in the living parenchyma of the cortex (Peterson and Steudle 1993). Progressive development of suberin lamellae in more endodermal cells would increase the resistance to water flow in the transcellular pathway in the older regions of the white zone. In the CT zone the number of passage cells remains somewhat constant and progressive death of cortex and abrasion of outer layers may decrease resistance to the inward flow of water. But deposition of condensed tannin in the cell walls may have an effect in reducing the entry of water. In the cork zone, cell maturation increases resistance in both apoplastic and cell-to-cell pathways, and water movement is drastically reduced. In dormant roots the passage cells of the CT zone offer a low resistance region for the entry of water into the stele (Peterson *et al.* 1999).

Background

The three root zones (white, CT and cork) in loblolly pine seedlings identified by Peterson *et al.* (1999) was based on the previous report of similar zones in jack pine by McKenzie and Peterson (1995a, b). The authors reported that white roots are anatomically suited for efficient ion uptake due to the

presence of a living cortex. In contrast, the CT zone is also capable of ion absorption due to the presence of endodermal passage cells in both living and dormant roots. Some researchers have studied the anatomical changes in loblolly pine roots. One study concerned only seedling tap roots (Enstone *et al.* 2001). Another dealt mostly with seedling traits in plantations (McCrady and Comerford 1998). There has been no study to quantify the absorbing surface in all root orders in relation to growth of loblolly pine seedlings. Taylor and Peterson (2000) determined the absorbing surface in jack pine roots in Ontario, Canada, where seedlings grew in relatively cool soils. It would be worthwhile to study CPSA in loblolly pine seedling roots in a warmer environment such as in Oklahoma.

Loblolly pine seedlings, the most widely planted species in Oklahoma, are raised in the state nursery for one year. As Oklahoma sites are more drought prone than eastern regions of its natural range, loblolly pine seedlings may experience water stress when planted in the field. Although there is plentiful rainfall, high temperatures and low humidity cause water stress especially in seedlings that have just been planted and are not established. A major factor determining planting success is the capacity to grow new roots rapidly after transplanting (Hallgren *et al.* 1993). According to Carlson *et al.* (1988) the capacity of roots to supply water on these drought prone sites is especially important in the survival of seedlings. Roots respond to the changes in environmental conditions with changes in internal anatomy. No previous attempt

was made to quantify these changes and correlate them with growth of seedlings. As environmental conditions in Oklahoma are warmer than those in the eastern states with a longer growing season, seedlings may grow more vigorously. Mycorrhizae were considered to play a potential role in enhancing the uptake of water and nutrients by roots (Bowen 1973 and Rousseau *et al.* 1994). As the presence of mycorrhizae tends to be enhanced by nutrient-limiting environments (Maronek *et al.* 1982 and Marx *et al.* 1982) the conditions in the nursery may discourage them. Fertilizers applied in the nursery must be depleted by plant use and/or leaching to create ideal environments for mycorrhizal colonization. When mycorrhizal populations increase they may enhance the absorptive surface of roots. Therefore, it is important to study the anatomy of different root types to determine their effects on growth of seedlings. Cultural treatments including irrigation, fertilization, undercutting, and lateral pruning in the nursery are aimed at producing high quality seedlings. These treatments modify the seedlings to be successful after planting on diverse sites. Root traits such as number of FOLR and root growth potential were considered better indicators of seedling survival and performance than shoot traits (Hallgren *et al.* 1993).

Objectives

The primary goal of this study was to elucidate changes in the water and nutrient uptake capacity of pine roots over the first year of growth in the nursery and how these changes were related to factors such as ontogeny, seedling

allometry, and weather. Root uptake capacity was determined by anatomical and morphological characteristics known or assumed to be associated with root uptake functions, such as number of root tips, root length, root surface area, and CPSA. This research was done to aid in the development of models of root uptake of water and nutrients, selection of traits for genetic improvement and assessing nursery seedling quality, and understanding tree seedling function.

The research was designed to seek answers to specific questions including how the uptake capacity was distributed among the different root zones (white, CT, and cork) and types (tap root, lateral roots, and mycorrhizae). Distribution of roots at various soil depths was studied to learn what soil layers were exploited by roots. Previous research showed that roots have two annual periods of rapid growth (spring and fall) in the nursery and field (Dougherty *et al.* 1994, Iivonen *et al.* 2001, and Sword 1998). The summer was regarded as too hot and the winter too cool for rapid growth; spring and fall were apparently ideal for root growth. Changes in the development of uptake capacity associated with this growth periodicity were investigated. As mycorrhizae are considered to have a significant role in enhancing the uptake capacity of roots (Mudge *et al.* 1987, Muhsin and Zwiazek 2002, Parke *et al.* 1983, and Vogt *et al.* 1991), changes in root abundance that may help in the absorption of water and nutrients were examined. It is usually assumed that trees grow allometrically with uptake and demand capacity for water well balanced with the environment. This study

sought to learn whether the changes in root uptake capacity tracked the demand capacity of the shoot.

MATERIALS AND METHODS

Study site

The seedlings were harvested from standard nursery beds at the Oklahoma State Forest Regeneration Center, Washington, 90 miles south of Stillwater, OK. The seedlings belonged to a single genetically improved open-pollinated loblolly pine family. The beds were east-west with a length of 210 m and a width of 1.5 m. Seeds were sown in 7 rows with a spacing of about 21 cm between rows (Fig. 1). Within each row seedlings were about 2 to 3 cm apart. Seedling density was 190 m^{-2}.

Sampling

Three replications, about 4.5 m long, were established in different beds about 50 m apart and marked with flags (Fig. 2). Seedlings were harvested every 30 to 40 d from May until mid December using 10 cm diameter PVC pipes. Four pipes were used for sampling from each bed and they were inserted up to a depth of 60 cm. Two pipes were used for sampling within seedling rows, and two between adjacent rows, eliminating the border rows (Fig. 3). The samples were selected randomly. The pipes from within rows contained about 4 to 5 seedlings each while the other set contained only roots without any shoot. In June there were no roots in pipes sampled between rows. After lifting, the bottom ends of the pipes were closed with PVC caps drilled with holes to prevent any soil and/or root loss during transit to Stillwater.

Fig. 1. Seedbeds in the nursery showing loblolly pine seedlings planted in seven rows.

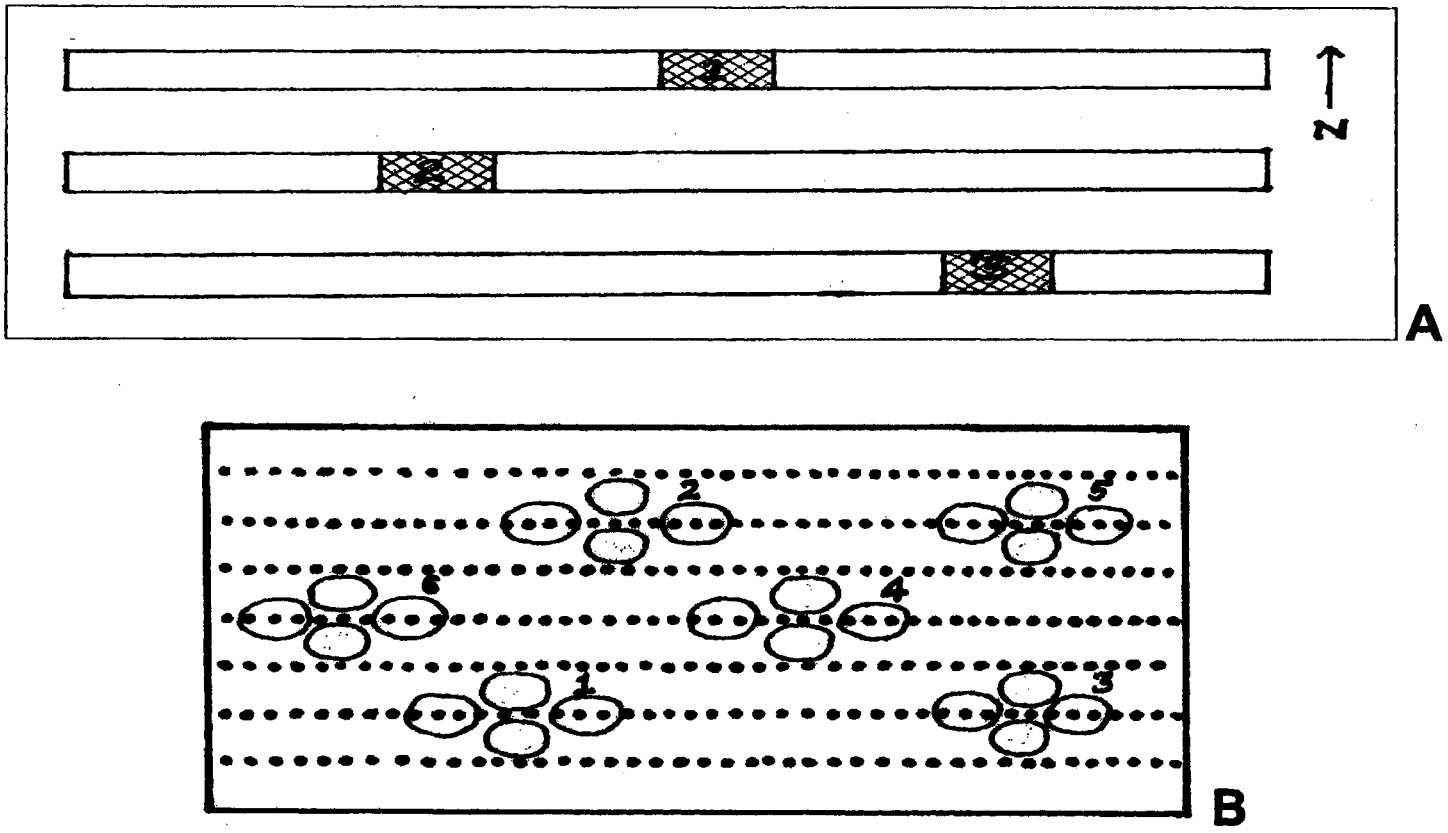

Fig. 2. Plot plan showing replications (A) and sampling (B). Two pipes were used to sample from within row and two between rows. Numbers on the right indicate harvest order.

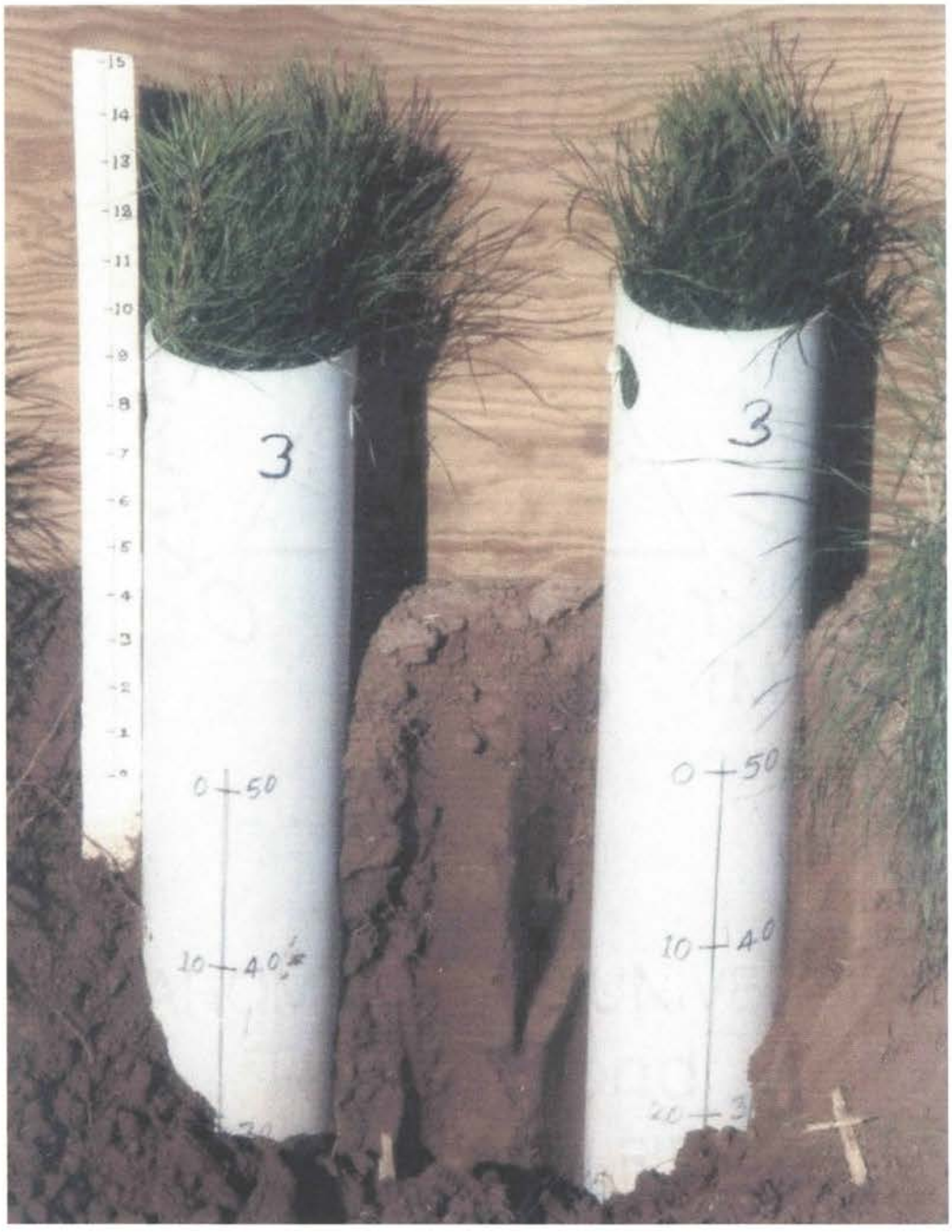

Fig. 3. Two pipes inserted up to 50 cm within seedling row.

Extraction of roots

Water was poured into the pipes from the top and the bottom half was soaked overnight in water to ease the extraction of soil. The cap was removed and another pipe cut lengthwise to make a channel was connected to the bottom end of pipe using three bolts and wing nuts (Fig. 4). Holding the sampled pipes in a slanting position, the bottom end of the split pipe was tapped on a hard surface. This helped the soil containing roots to slide out of the pipe. The soil core was cut into 10 cm segments using a sharp scalpel. The tap roots in the pipes from within rows were cut using a sharp scissors to separate the segments. The segments were washed gently with tap water to remove adhering soil particles and roots were collected. As seedling roots in the nursery were undercut at 20 cm and laterals were pruned in October, all roots below 20 cm were discarded in later harvests. Roots that were completely dead or exhibited signs of decay determined by visual observation were discarded. Roots were immersed in bottles containing clean water and stored at cold temperatures just above freezing until measurement. The seedling stems were cut at the soil surface and the shoot was collected separately.

Data collection

Shoot: The stem diameter in the hypocotyl region was measured using a caliper. The length of stem including the hypocotyl was measured using a ruler and the number of branches was counted. The surface area of 25 needles randomly selected from each pair of cores (only 10 needles in June) was determined using

Fig. 4. Soil core extracted from the PVC pipe showing 0 to 10 cm profile and seedlings.

Portable Leaf Area Meter - Li-COR 3000. The stem and needles were dried in a forced air oven at 70 °C until completely dry (minimum 48 h) and dry weights were recorded.

Roots: Roots were separated into three categories: tap roots, lateral roots, and mycorrhizal tips. It was not possible to separate laterals by order, as they were severed from the seedlings in the nursery by the harvesting technique. Only the samples taken within a row had tap roots, and there were no mycorrhizal tips until August. The white zones from tap and lateral roots were separated from the proximal part after visual inspection. The entire mycorrhizal tip was considered to be white zone. The proximal parts of the roots were analyzed to determine CT and cork zones. Roots were cut into 10 mm segments and stained with Fluorol Yellow 088 (Brundrett *et al.* 1991) to determine where all cells of the endodermis were suberized. This information was used to separate the CT zone from the cork zone from visual observation. Cross sections were taken from the white zone of the tap roots, lateral roots and mycorrhizal tips for determination of cell dimensions and number of passage cells. Length and diameter of white, CT, and cork zones of tap and lateral roots were determined by scanning the roots and analyzing the images using Delta-T SCAN software (Dynamax Inc., Houston, Texas). White roots were stained with methyl violet (3% aqueous solution) to provide a dark surface for scanning. After scanning roots were dried and weighed.

Mycorrhizal tips were handled differently from the other root types. There were too many tips to measure each separately. A sub-sample of 25 mycorrhizal tips was taken from each core and measured for diameter and length. These tips were dried and weighed. The remaining mycorrhizal tips were also dried and weighed. These data were used to calculate the total values for mycorrhizal tips.

Other root parameters

Root length density/intensity, L_v (m m^{-3}): This is the length of root per unit volume of soil.

Specific root length, SRL (m g^{-1}): Specific root length is the length of root associated with a unit of dry weight. The specific root length is relevant to the root system allocation strategy. It represents the relative soil exploitation potential.

Root area index, RAI; Leaf area index, LAI (m^2 m^{-2})*:* The surface area of roots was calculated from the diameter and length of roots scanned using Delta-T SCAN. Surface area is highly important because of the direct contact of roots with soil particles. The ratio of root surface area to unit ground area is RAI. LAI was calculated in similar fashion.

Calculation of cortical plasmalemma surface area

Cortical plasmalemma surface area (CPSA) is the membrane surface of cortex cells in the white zone and mycorrhizal white zone and also the outer tangential surface of passage cells in the endodermis of white zone, CT zone, and mycorrhizae. Cortical plasmalemma surface area was calculated following the method of Taylor and Peterson (2000). Three cross sections each from the mid-points of the white zone, CT zone, and mycorrhizae from each of the within row pipes were used to count the number and diameter of cortex cells and number and tangential width of passage cells. Similarly, three root segments each from the mid-points of white zone, CT zone, and mycorrhizae were used to determine the length of cortex and passage cells. The number of cortex cells was determined from images of cross sections captured by imaging software (Optronics MagnaFire, Goleta, California) attached to a Nikon E 600 microscope and projected on paper. The image was split into eight sectors and all cells in four sectors opposite and alternating with each other outside the endodermis were counted (Fig. 5). The total number of cortex cells was calculated from this. The diameter of cortex cells and outer tangential width of passage cells were measured using Image-Pro Plus (Version 4.1 - Media Cybernetics, Silver Spring, Maryland). The length of cortex cells and passage cells were measured from tissues cleared with 10 % bleach and stained with Fluorol Yellow 088 (Fig. 6). As there was no dimorphism, the passage cells were differentiated from suberized endodermal cells by their thin walls. Total CPSA included membrane area in the tangential, radial, and transverse surfaces of cortex cells, and outer tangential

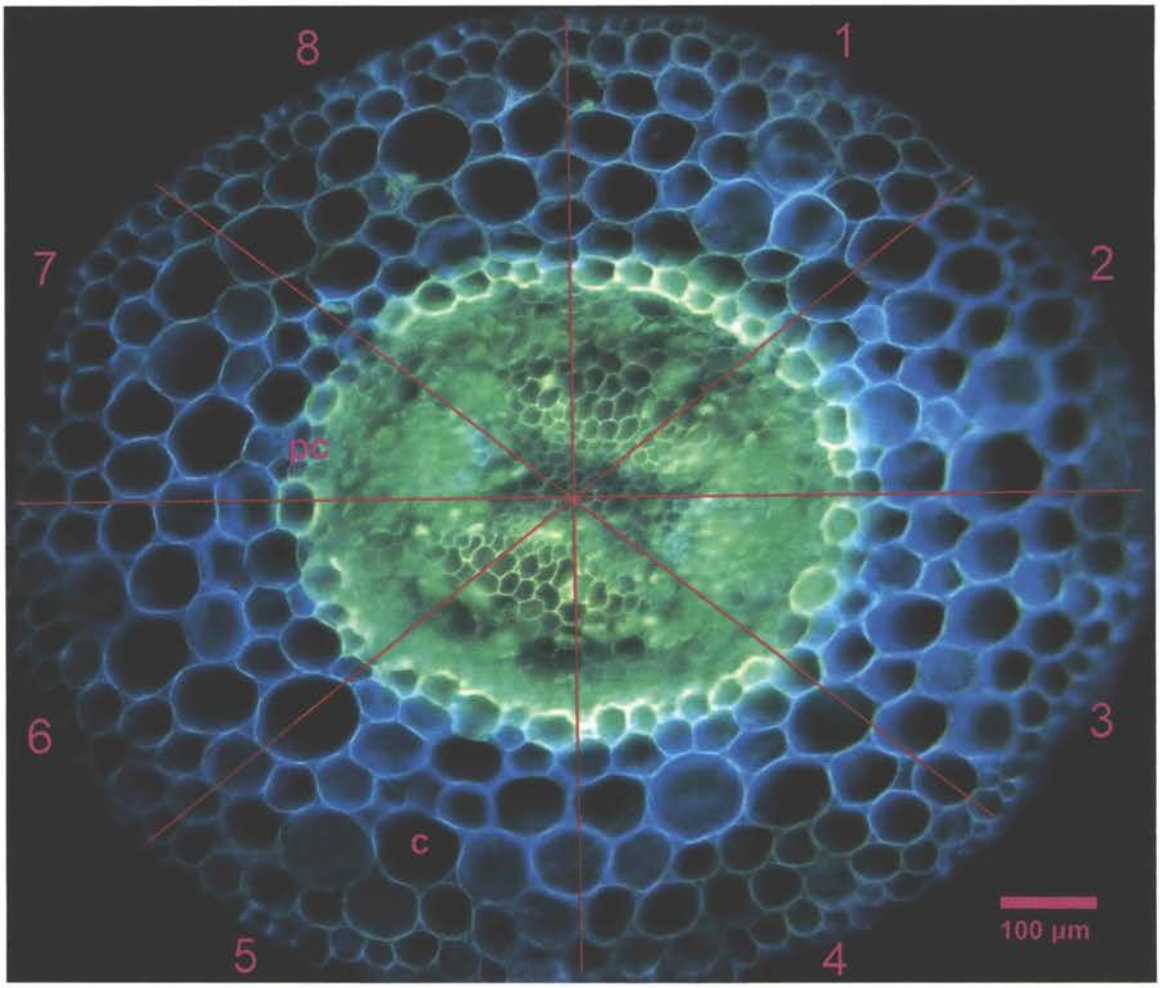

Fig. 5. Image of the cross section of a root (100x) split into eight sectors for counting cortex cells (c) and measuring the tangential width of passage cells (pc). All cortex cells in four sectors opposite and alternating with each other were counted.

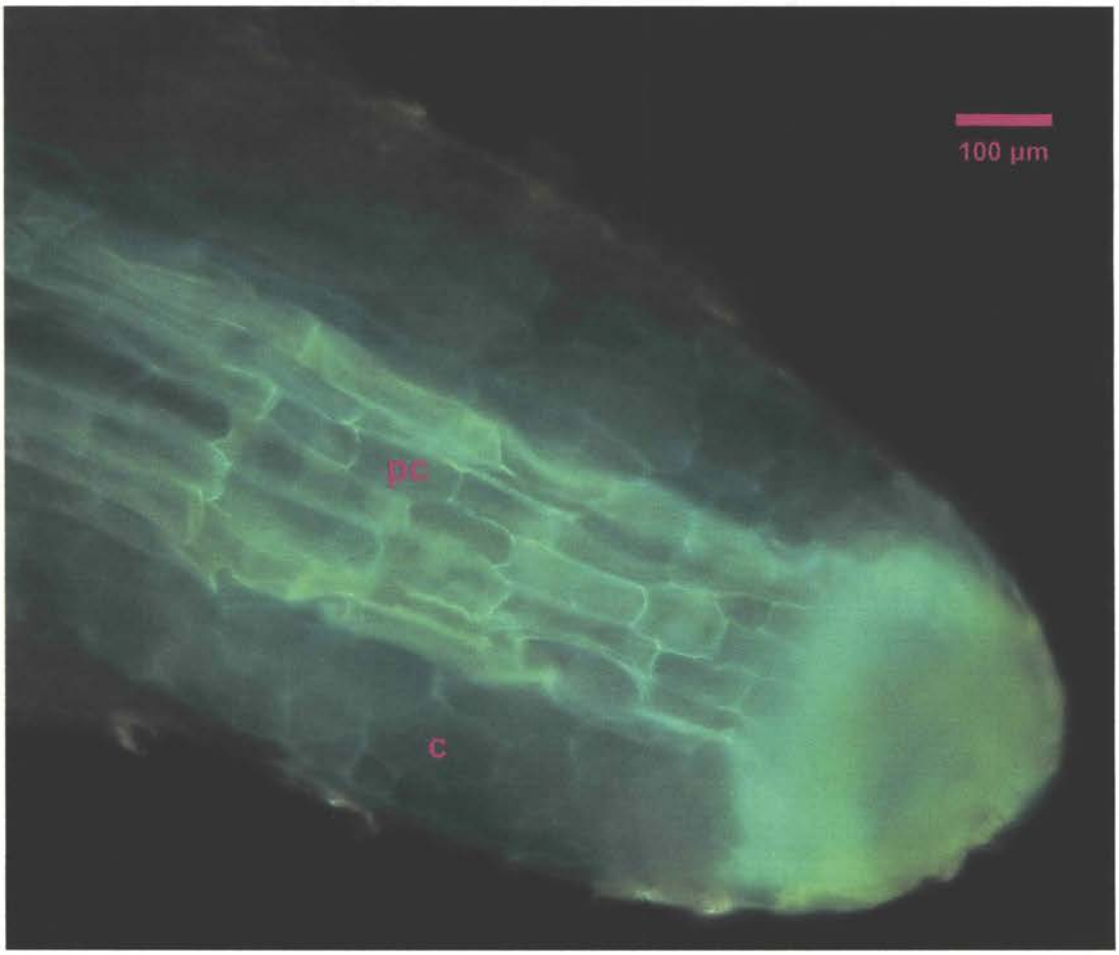

Fig. 6. Tip of a root cleared using 10 % bleach and stained using Fluorol Yellow 088 (100x). The length of cortex (c) and endodermal (en) cells were measured. The passage cells (pc) with thin walls were differentiated from suberized cells.

surface of passage cells in the endodermis per unit length of root. The presence of mycorrhizal hyphae (Fig. 7) was detected by clearing and staining root segments using Chlorazol Black E (Brundrett & Kendrick 1988 and Brundrett *et al.* 1984, Appendix B).

Cortex cells

The radial and tangential plasmalemma surface area of cortical cells in 1 mm of root, S_1 (mm^2) was calculated assuming the cells as cylinders and were 1 mm long, ignoring the transverse walls in between two cells (Eq. 1).

$$S_1 = n(h.2\,\pi\, r_c), \qquad (1)$$

where h = 1 mm, r_c = average radius of cortex cells in cross section (mm), and n = the number of cortex cells in cross section.

The transverse surface area of two membranes in 1 mm of root, S_2 (mm^2) was calculated using equation 2.

$$S_2 = n(\pi r^2_c).2h.h_c^{-1}, \qquad (2)$$

where hc = length of cortical cells (mm) and 2 representing the two membranes on both ends of the cell.

Passage cells

The CPSA of passage cells in 1 mm of root, P (mm^2) was determined by equation 3.

$$P = n_p.w_p.h, \qquad (3)$$

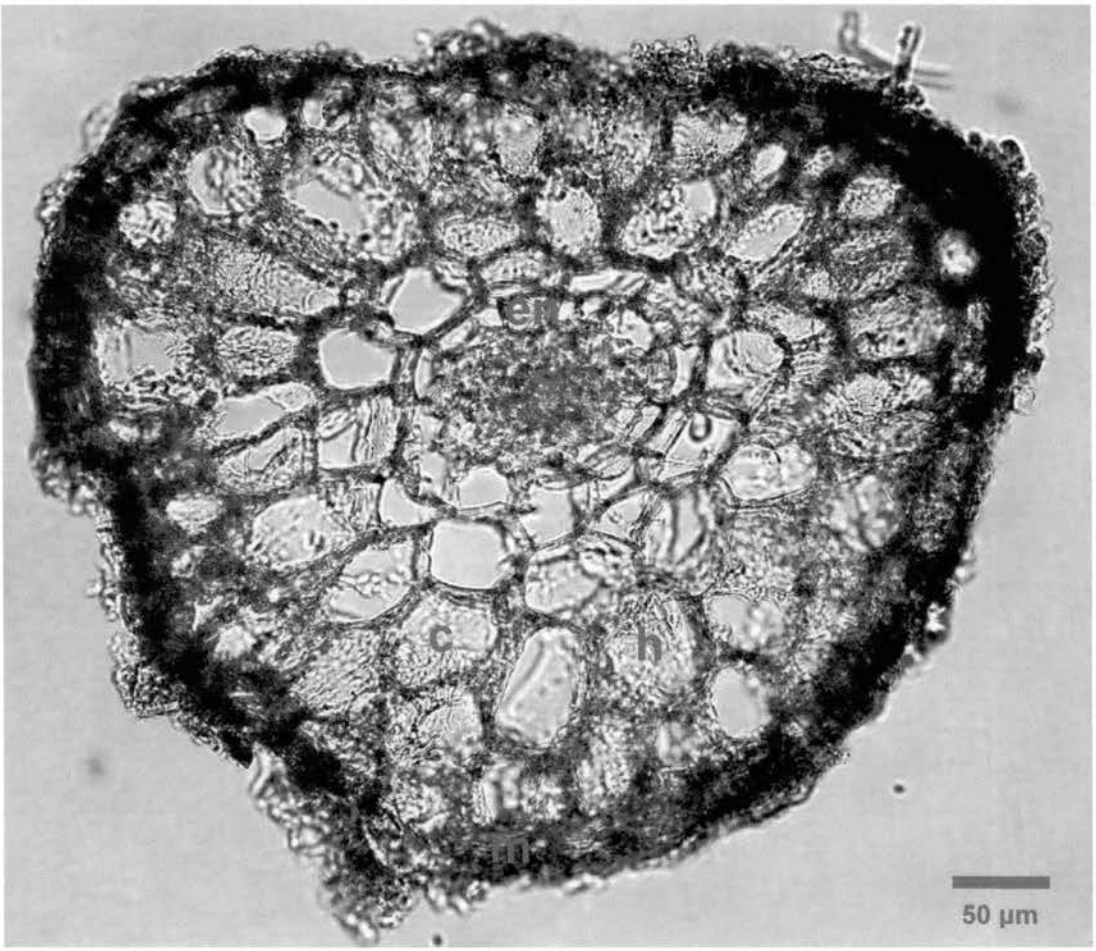

Fig. 7. Cross section through the white zone of a mycorrhizal root stained with Chlorazol Black E (200x). Mycorrhizal hyphae (h) colonize the intercellular spaces among cortex cells (c) to form a Hartig net and do not cross the endodermis (en) to reach the stele (s). The root surface was ensheathed by a mantle (m) and extramatrical hyphae protrude from the root surface to penetrate the soil.

where *np* = number of passage cells in cross section, *wp* = width of outer tangential face, and *h* = 1 mm.

Analysis of data

Most results were calculated on a land area basis such as root area per m^{-2}. Means for each replicate were based on 2 sub-samples from within the row and 2 such sub-samples from between the rows. There were three replicates. Statistical analyses were performed using SAS and means and standard deviations were calculated at $p \leq 0.05$ (SAS 1999).

Environmental parameters

Mean monthly values of air and soil temperatures from May to December 2001 for McClain County, OK were collected from the Oklahoma Climatological Survey (Mesonet). Normal temperature (30-year average) was collected from the nearest Co-operative Observation Network Station, National Weather Service, Purcell, OK (NOAA).

RESULTS AND DISCUSSION

Dry weight

Dry weight of the roots, stems, and needles increased slowly in June and by rapidly increased from July through November. Growth of the stems and needles declined abruptly after November in contrast to root growth that continued until mid-December (Fig. 8). The prolongation of growth into November and December may have been caused by unusually high temperatures (5 °C above normal) during both months (Fig. 9).

Although root biomass provided much less information than root length or specific root length, it has been the parameter most often used to quantify roots (Eissenstat 1992 and Fitter and Hay 1987). In June tap roots reached a depth of 30 cm and by July a few had grown to 50 cm. As soil below 25 to 30 cm was dry in July and August despite routine watering, further root penetration by tap roots did not occur until after August when irrigation water once again penetrated deeper soil layers apparently due to cooler temperatures and less evaporation. In mid-summer abundant lateral roots were produced in the shallow surface layers where they could absorb irrigation water. Mycorrhizal roots were first observed in August and they showed huge increases towards the end of the growing season. In September and October when environmental conditions became favorable, tap roots resumed growth and most grew to 40 to 50 cm.

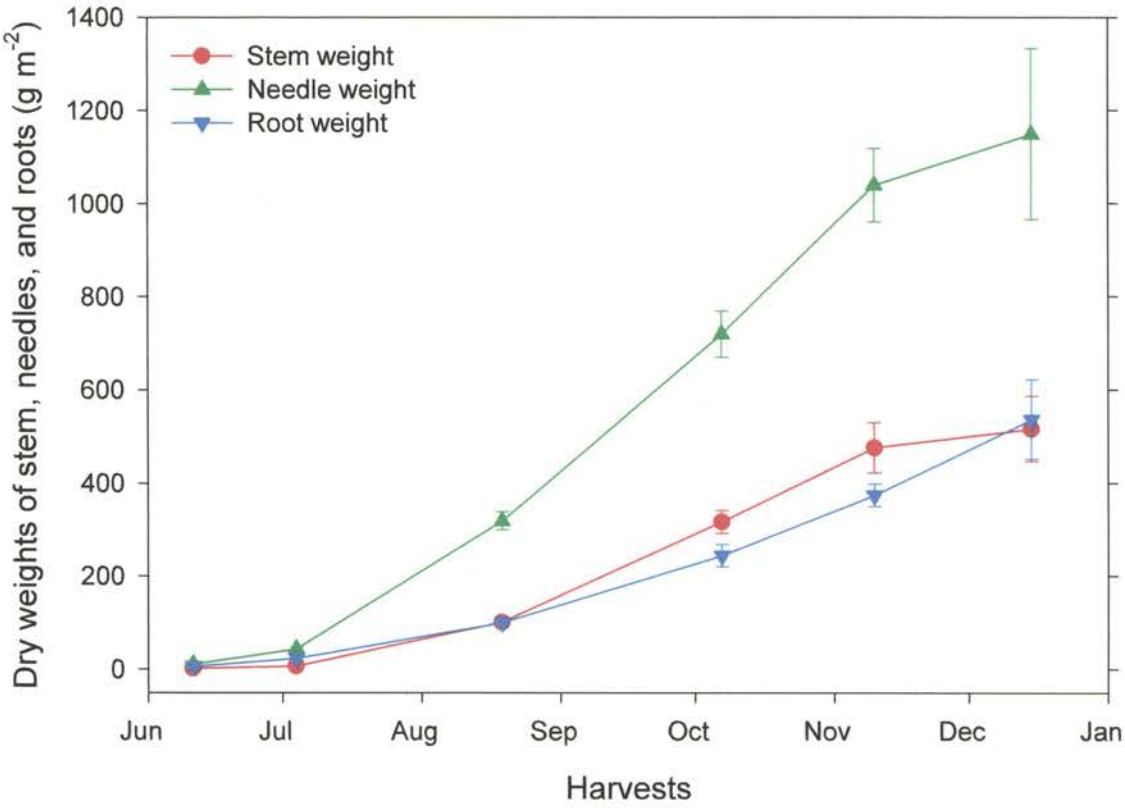

Fig. 8. Seasonal increase in dry weights of stem (includes lateral branches), needles, and roots of first year nursery-grown loblolly pine seedlings, n = 3.

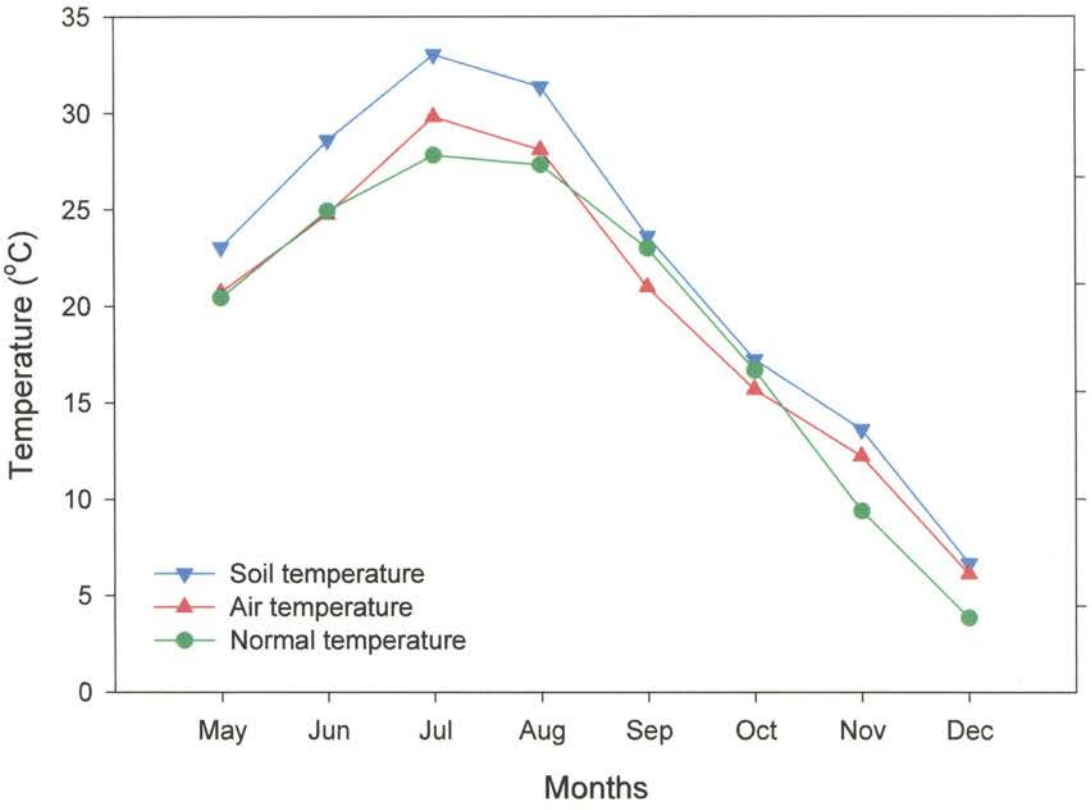

Fig. 9. Mean monthly soil and air temperatures at Washington, OK (Mesonet), and normal temperature (30-year average) at Purcell, OK (NOAA).

The undercutting of the nursery beds in October eliminated the roots below 20 cm. The effect was tempered by the fact that there was never more than 20 % of the root dry weight below 20 cm (Fig. 10). As the current research did not include a non-undercut treatment, it is not possible to determine the effect of undercutting on root growth. The plot of the data showed that despite undercutting and lateral pruning, the dry weight of roots continued to increase and nearly doubled from October to December. The November and December harvests showed a proliferation of white roots and mycorrhizae indicating that undercutting and lateral pruning may have stimulated new root production. Chauhan and Mishra (1996) reported that the number of lateral roots increased in seedlings subjected to undercutting. They also stated that one of the most sought after effects of root undercutting was to increase lateral root dry weight due to production of abundant vigorous roots of small diameter instead of a large tap root. Iivonen *et al.* (2001) reported that root growth was most intense at the end of growing season when shoot growth slowed down. Sung *et al.* (1993) reported that root growth in loblolly pine seedlings was slow in July when the major carbon sinks were stems and needles. Towards late November and early December loblolly pine roots became the main sucrose sink until the following spring. Miller and Timmer (1997) reported that biomass increment was much greater during the hardening phase characterizing a marked shift in carbon allocation to roots. Bed preparation practices, addition of mulch and fertilization in the nursery may have provided ideal growth conditions for the accumulation of roots in the surface layers.

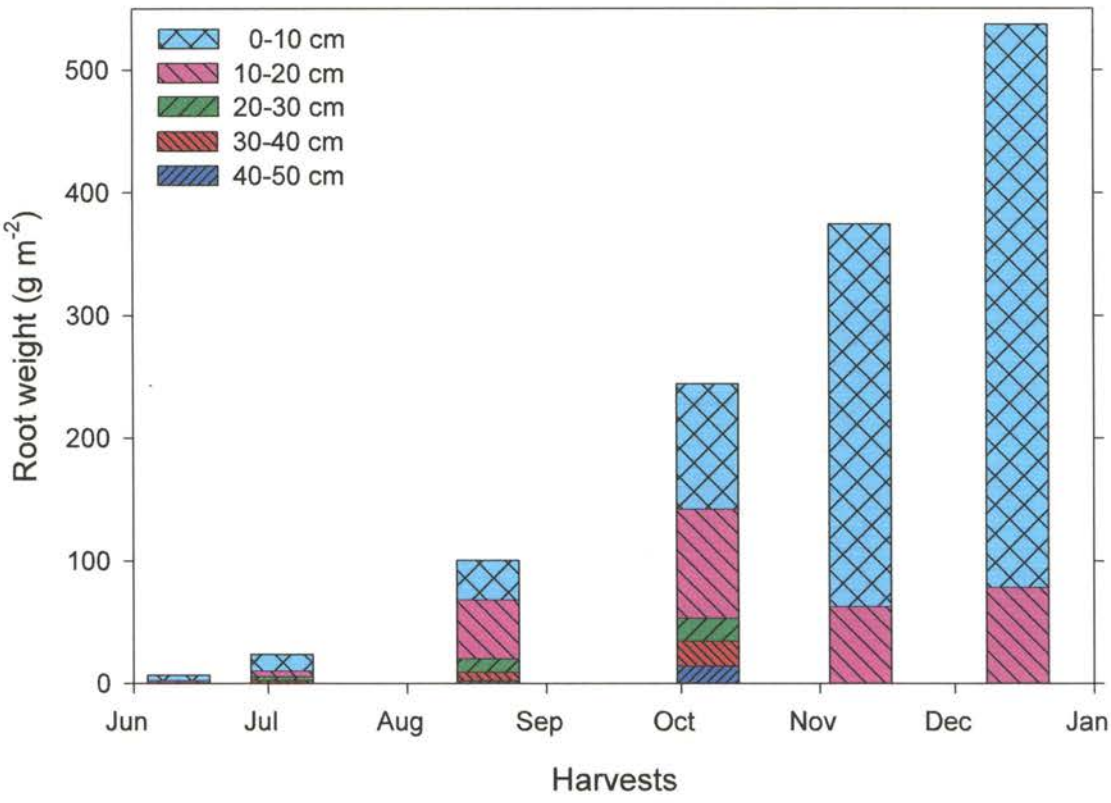

Fig. 10. Seasonal increase in dry weight of roots of first year nursery-grown loblolly pine seedlings at different soil depths, n = 3.

Height and diameter

Seedling height increased rapidly from June through August, then continued to increase slowly until growth stopped in mid-November (Fig. 11). Seedlings grew to a height of 251 mm and diameter of 4.7 mm with a needle surface area 5.4 m^2 m^{-2} in mid-December. Stem height and diameter are considered two major parameters to assess quality of nursery stock and used to cull and grade seedlings (Mullin and Christl 1981, 1982, Reese and Sadreika 1979, and von Althen 1969). Stem diameter has frequently been cited as the measure most strongly related to seedling performance in the field (Mexal and Landis 1990, Mullin and Christl 1981, 1982, Mullin and Svaton 1972, and Thompson 1985). The average diameter for this study was far greater than the minimum of 3 mm for a saleable seedling from the state nursery.

Root diameter

Root diameters increased steadily from June until the end of the season. The mean diameter of white and CT roots was near 0.7 mm and unchanged from June to December (Fig. 12). In June only the tap roots and a few FOLR were present. Later in the season seedlings produced higher order laterals resulting in multiple ramification. The white roots soon turned brown increasing the amount of CT zone. The CT zone contained roots of all orders. Therefore, the average diameter of roots did not vary much during the year. Mycorrhizae first appeared in August with a diameter of 0.35 mm and increased to 0.4 mm by December. The reason for the increase in diameter of mycorrhizae may be due to the

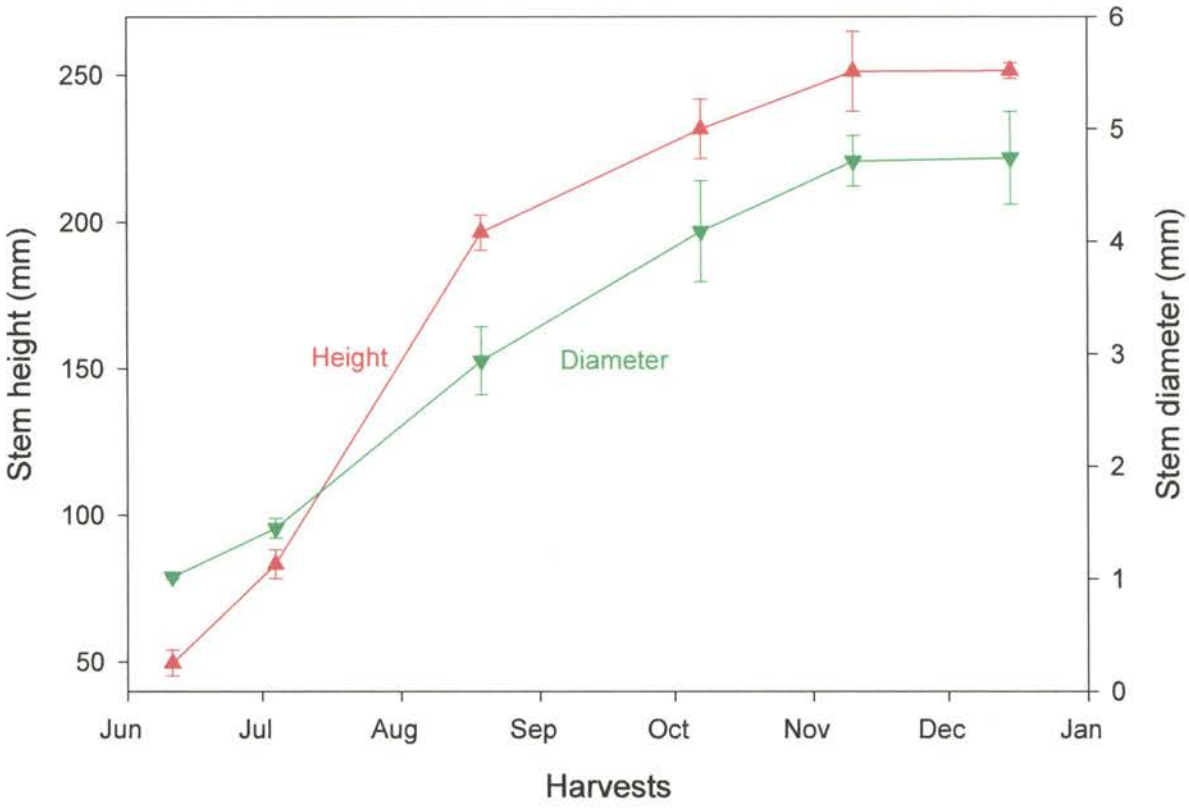

Fig. 11. Seasonal increase in stem height and stem diameter of first year nursery-grown loblolly pine seedlings, n = 3.

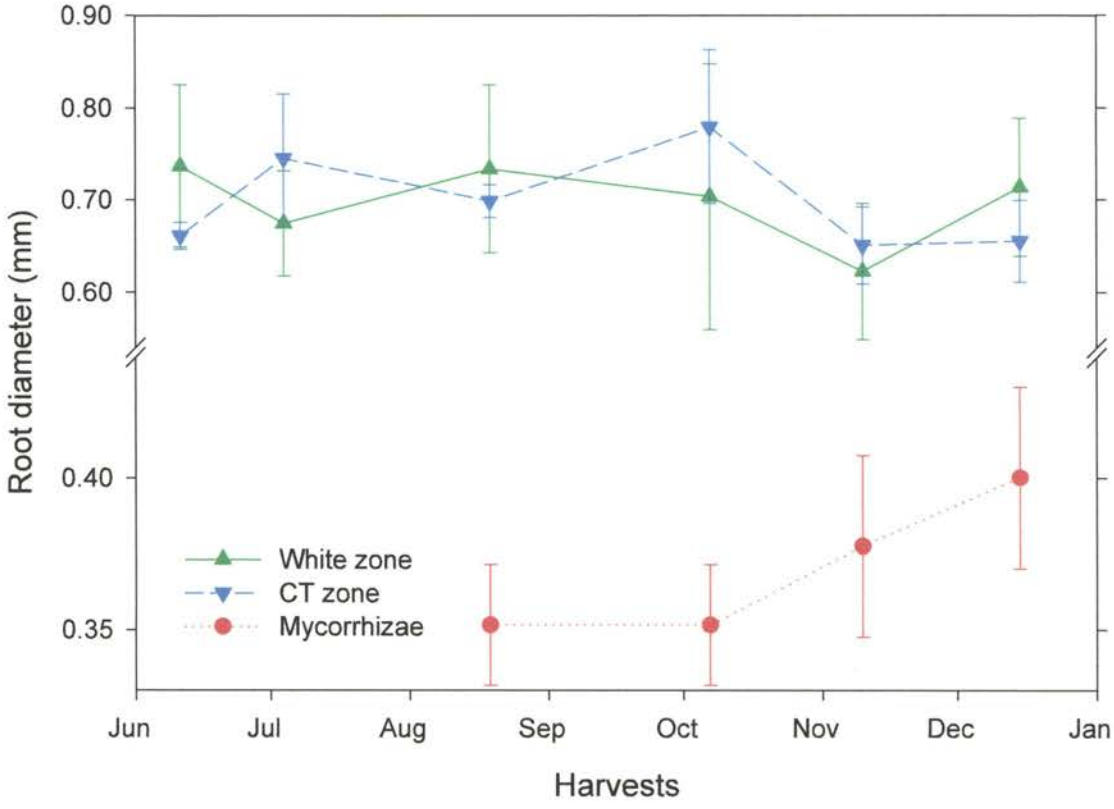

Fig. 12. Seasonal changes in root diameter of white zone, CT zone, and mycorrhizae of first year nursery-grown loblolly pine seedlings, n = 3.

accumulation of carbohydrates. Mycorrhizae increased enormously late in the year after undercutting and lateral pruning.

Production of white roots and mycorrhizal tips

The number of white roots increased to around 6000 m^{-2} in early July and did not change much until increasing slowly to 9000 m^{-2} in October and November and finally reaching 13,000 m^{-2} in December (Fig. 13). In contrast, mycorrhizal tips did not appear until a few were found in mid-August. Following gradual increases in mycorrhizal tips in September and October the number exploded in November and reached over 60,000 m^{-2} in December. The vast majority of root tips were in the top 20 cm of soil (Fig. 14). In July many root tips were deeper than 20 cm, but later in the summer irrigation was not adequate to penetrate deeper than 20 cm and the dry soil limited production of white roots below this level. After October the undercutting eliminated all the roots below 20 cm.

The possible reasons for late appearance of mycorrhizae in the field are manifold. The fungi in the soil may have declined due to cultural operations such as site preparation practices and fumigation the previous September. Miller *et al.* (1995) reported that tillage can dramatically affect function of mycorrhizae in an agricultural system and soil disturbance reduced effectiveness of mycorrhizal symbiosis. Once fungi come in contact with fine feeder roots they penetrate outer cortex and become established by forming a Hartig net in the intercellular

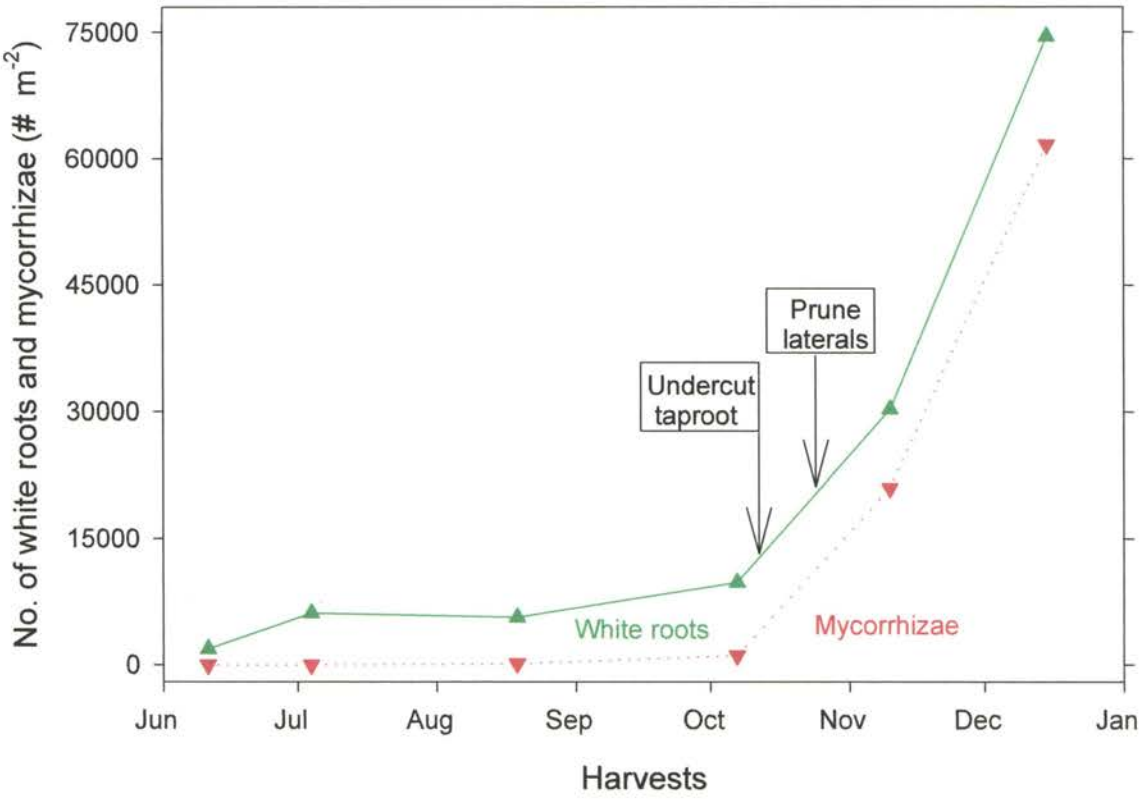

Fig. 13. Seasonal increase in cumulative number of white root tips and mycorrhizae of first year nursery-grown loblolly pine seedlings, n = 3.

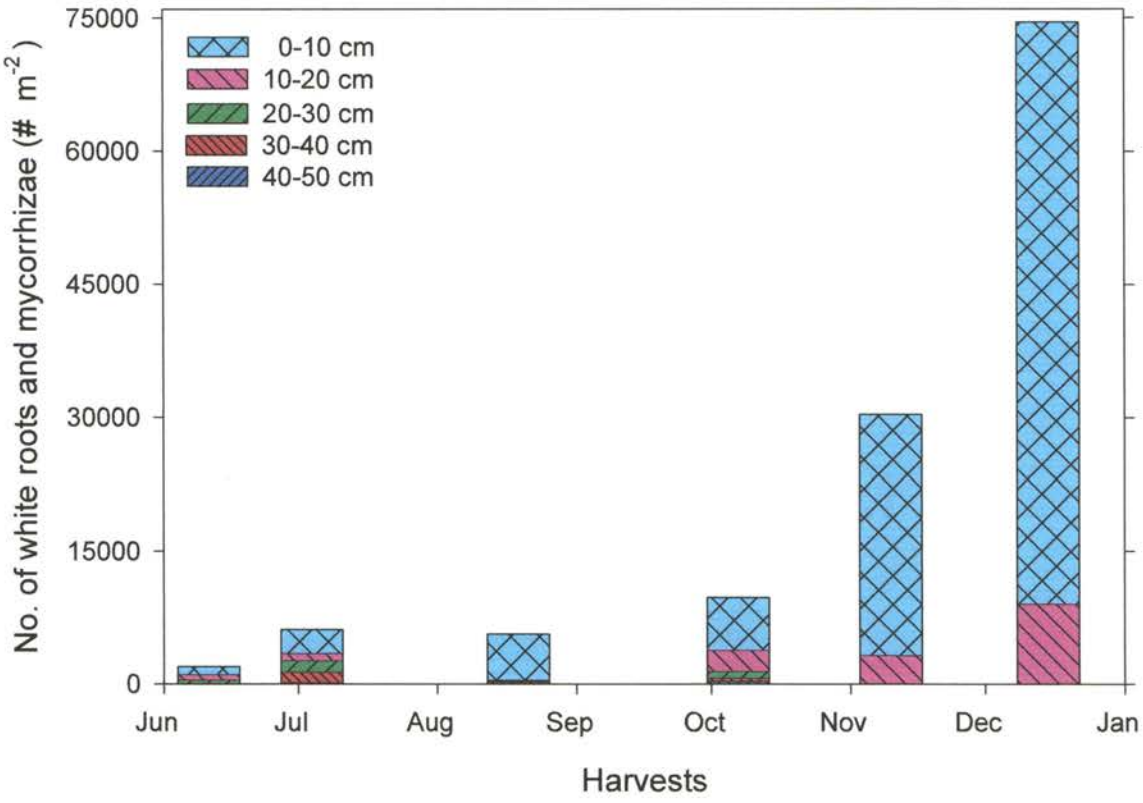

Fig. 14. Seasonal increase in number of white root tips and mycorrhizae at different soil depths of first year nursery-grown loblolly pine seedlings, n = 3.

spaces and a mantle or sheath around roots. For promoting vigorous growth of seedlings in the first growing season fertilization was done in the nursery until August only. This may be one of the reasons for late appearance of mycorrhizae. Abundant research conducted to study the relation between levels of fertilizer and formation of fungal infestation by *Pisolithus tinctorius* proved that high levels of soluble NPK fertilizers reduced formation of ectomycorrhizae (Danielson *et al.* 1984, Ekwebelam and Reid 1983, Maronek *et al.* 1982, Marx *et al.* 1982, Pope and Chaney 1984, Ruehle 1980, Ruehle and Wells 1984, and Rupp and Mudge 1985). After fertilization was ended in August nutrient level in the soil may have declined gradually due to absorption by seedlings as well as leaching into deeper layers. This change may have favored production of more mycorrhizal tips.

The increase in the number of mycorrhizal tips was significantly greater compared to the increase in the number of white roots. Undercutting tap roots below 20 cm and pruning laterals in October promoted production of abundant new white roots and mycorrhizal tips. The reason for the explosion in the number of mycorrhizae may be due to the presence of living tissue in the newly formed white roots. Brundrett and Kendrick (1988) reported that most mycorrhizae were formed by relatively fine higher order laterals. The responsiveness or capacity of plants to exploit small scale or short duration changes in water or nutrient availability by rapidly producing new roots was thought to be an important determinant of their success during competition for

soil resources (Fitter 1987, Grime *et al.* 1986, and St. John *et al.* 1983,). According to Castellano and Molina (1989) ectomycorrhizal fungi also produce growth regulators that stimulate feeder root elongation and branching, thus increasing total number of feeder roots produced. Such root branching also benefited absorption of nutrients by increasing root surface area. It is recommended that nurseries prepare seedlings to explore top layers of soil when transplanted to the field by encouraging the maximum number of fine roots and mycorrhizae in the upper layers (Harvey *et al.* 1987).

Dierauf *et al.* (1995a, b) reported that root pruning had a striking effect on root morphology and the more frequent the pruning the greater the effect. They observed that seedlings pruned four times had many more fine roots and mycorrhizae, and tended to have diffuse multiple tap roots. As each undercut was done at increasing depths two or more new 'sinker roots' often formed. Söderström and Read (1987) suggested that ectomycorrhizal mycelia depend on current photosynthates for respiration and growth and as a result both seedling roots and associated mycorrhizal fungi became major sinks in late fall and winter.

Harley and Smith (1983) and Hayman (1983) reported that a major function of mycorrhizal hyphae was to explore a great volume of soil to extract available forms of phosphorus. According to Harley (1989), the main role of mycorrhizae was to acquire nutrients by exploring soil volume with hyphae that are both more responsive and more extensive than roots themselves. As

fertilization in the nursery ended by August, the depletion of nutrients in the soil may have stimulated mycorrhizae to proliferate abundantly to exploit nutrient-limiting environments. It is also important to consider distribution and function of extramatrical hyphae of ectomycorrhizae. For mycorrhizae to be effective in nutrient uptake, hyphae must be distributed beyond the nutrient depletion zone that develops around roots. A nutrient depletion zone develops when nutrients are removed from soil solution more rapidly than they could be replaced by diffusion (Sylvia 1998). In the field, a positive correlation was reported between the length of fungal hyphae and phosphatase activity associated with ectomycorrhizal mantles (Häussling and Marschner 1989). According to Sylvia (1990) extramatrical hyphae of mycorrhizae could bind soil particles together and improve soil aggregation. Thus the increase in the vast distribution of mycorrhizae by huge increases in the number of mycorrhizal tips in the surface layers may have been beneficial in increasing the effectiveness of roots in absorption.

The high production of mycorrhizae in the fall may have an important beneficial effect on seedlings because absorption of water is hindered in the winter due to decreasing viscosity of water at low temperatures. Demand for water is lower in the winter than in the summer but the soil remains cold and there are warm periods when the atmospheric demand can be quite high. The relationship between water viscosity and root hydraulic conductivity was strong at low temperatures in mycorrhizal roots (Muhsin and Zwiazek 2002). Mycorrhizal

roots were capable of maintaining relatively higher hydraulic conductivity at low temperatures compared with nonmycorrhizal roots. Dixon *et al.* (1980) and Safir *et al.* (1972) reported that root hydraulic resistance increased with decreasing temperatures most rapidly in nonmycorrhizal plants. They also observed that ectomycorrhizae had a lower hydraulic resistance to water uptake than non-colonized roots. Therefore, the presence of abundant number of mycorrhizae with absorbing surface in the fall may help meet seedling demand for water.

Root Length, L_a

Root length is an important characteristic in determining the ability of roots to absorb water and nutrients. Loblolly pine roots steadily increased in length on a land area basis (L_a, m m^{-2}) in June and accelerated growth in July through September (Fig. 15). The undercutting halted increases in L_a in October and was followed by the greatest increase of the year in November and early December. Root growth in July and August may have been inhibited by the high temperatures of the warmest part of the year when irrigation did not penetrate below about 30 cm. Even before undercutting about 80 percent of L_a was less than 20 cm deep, perhaps because of drier soil at deeper levels (Fig. 16).

The major component of L_a was the CT zone (Fig. 15). Although the cork zone was a small component early in June, this component continued to increase steadily throughout the year. In contrast, the absolute amount of L_a attributable to white roots was constant throughout the year. Mycorrhizae were not produced until August and never were more than a very minor part of total L_a. Although

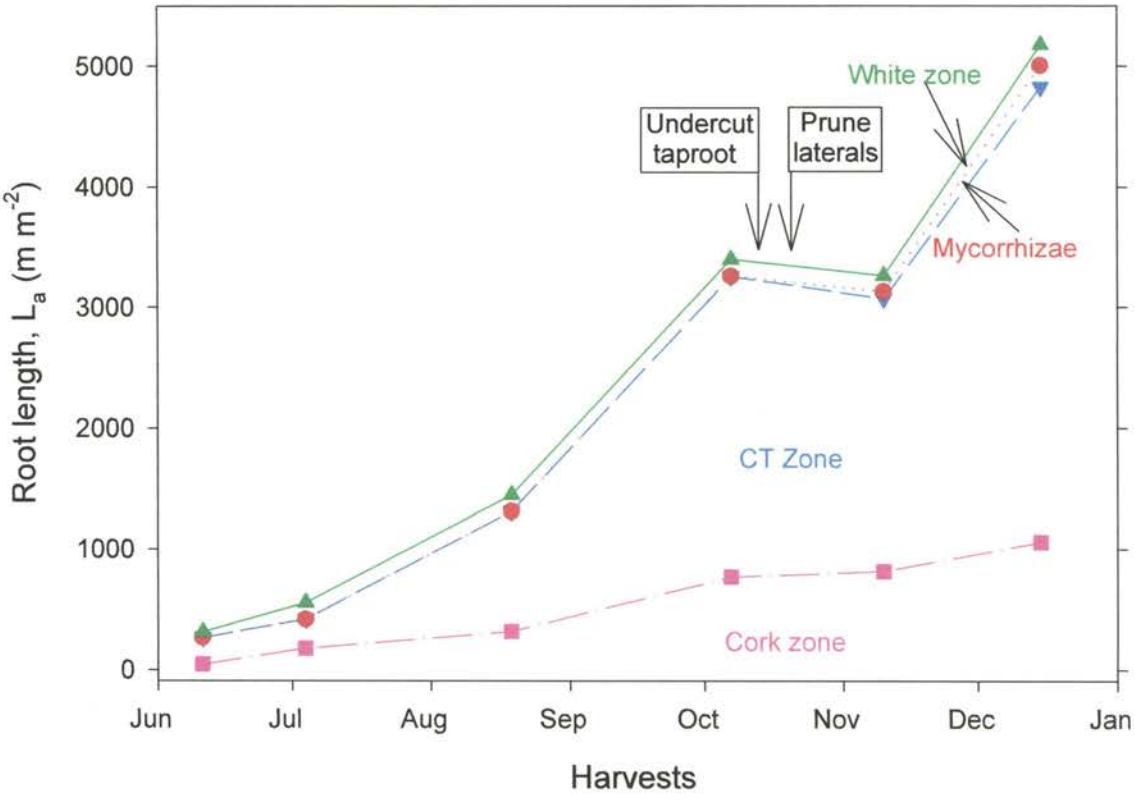

Fig. 15. Seasonal increase in cumulative length of white zone, CT zone, cork zone, and mycorrhizae of first year nursery-grown loblolly pine seedlings, n = 3.

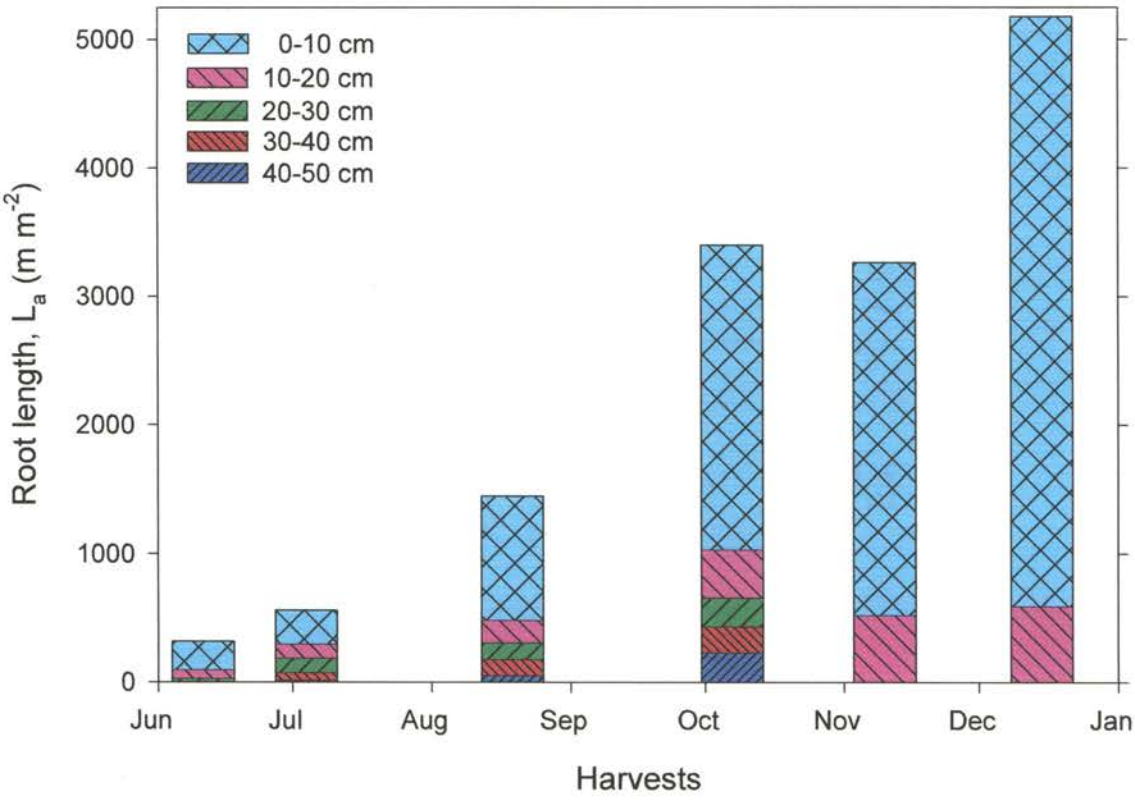

Fig. 16. Seasonal increase in length of white zone, CT zone, cork zone, and mycorrhizae at different soil depths of first year nursery-grown loblolly pine seedlings, n = 3.

white zone and mycorrhizae are considered the most important root zones involved in absorption of water and nutrients (Taylor and Peterson 2000), they did not make up much of the root length. This is because the newly produced white roots soon became CT zone and the basal region of CT zone became cork zone. This resulted in a decrease in the absorptive area and an increase in the length of CT and cork zones. Despite its increased length, the CT zone did not compensate for the large absorbing surface of white roots due to death of cortex cells. The seedlings produced more white roots as fast or faster at times as they changed to CT zone. This resulted in a significant increase in the length of roots containing CT and cork zones and a nearly constant amount of white zone.

Although mycorrhizae appeared in August there were only a few of them. But their number exploded towards the end of season when environmental conditions became favorable. The lateral roots infested by mycorrhizae lost the capacity for further elongation and their tips became forked containing white zone ensheathed by fungal mantle. The length of this white zone capable of absorption was very short compared to the proximal part and other root zones. As mycorrhizae needed living tissue to gain photosynthates (Söderström and Read 1987), they cannot survive in the dead cortex of CT zone and in the cork zone. Although huge increases in the number of mycorrhizal tips occurred, their total length was much less compared to other root types. Therefore, despite higher conductivity of the white and mycorrhizal zones due to live cortex cells, they contributed little to L_a compared to CT and cork zones.

The Regeneration Center seeks to produce healthy seedlings with abundant lateral roots and mycorrhizae at the end of the growing season and several cultural operations were implemented in the nursery to achieve this objective. Seedling roots were undercut below 20 cm in October resulting in a decline in root length in November. Ten days later laterals were pruned between adjacent rows resulting in further reduction in root length. The result of undercutting and pruning during environmental conditions ideal for root growth was that seedlings increased root length by nearly 60 % in a little more than a month. Miller and Timmer (1997) reported that root growth increased by 4 to 8 fold during hardening period, characterizing a marked shift in carbon allocation that occurred during dormancy. The rapid root growth in November and December increased the capacity for absorption of water and nutrients and storage of carbohydrates over the winter. Although air temperature was falling, soil temperature remained higher than air temperature until December (Fig. 9) and this favored continued growth of roots. Other studies have also shown the most intensive period of root growth was at the end of growing season after completing most shoot growth (Iivonen *et al.* 2001).

Root distribution/root length density, L_V

The capacity of roots to extract moisture from soil depends on the total amount of roots and root distribution with depth (Bowen 1985 and Levitt 1980). Recent studies suggested that total weight or root length were not always a good predictor of capacity to take up water. Previous research has shown differences

within species in drought resistance correlated with root density and distribution in wheat and loblolly pine (Hurd 1975 and van Buijtenen *et al.* 1976). Root distribution or root length density (L_v) at different soil depths may be important in determining the capacity for absorbing water and nutrients.

Root length density was always 5 to 10 times greater in the 0 to 10 cm level than at 10 to 20 cm (Fig. 17 and 18). The largest component of L_v at the end of the growing season was the CT zone (73 %), followed by the cork zone (21 %), while mycorrhizae and white zone contributed very little (3.5 % and 2.5 %, respectively). Roberts (1976) observed that 77 percent of root length was within 0 to 30 cm depth for loblolly pine, while it was 79 percent for Scots pine. Although roots penetrated into deeper layers, maximum root distribution was observed within surface layers. During summer roots were abundant in the upper layers for absorbing water and nutrients that were plentiful. Due to site preparation practices and addition of mulches in the nursery, upper layers contained loose soil and plenty of organic matter that encouraged production of maximum laterals. Fertilizers applied to seedlings also encouraged growth of roots. The white zone and mycorrhizae with their live cortex cells may have had higher conductivity than the CT zone, but its large length and wide distribution may make the CT zone very important in providing the shoot with soil solution. Moreover, when root growth declined in August especially at 10 to 20 cm due to extreme summer heat and evaporation, surface area generated by white roots was low. At this time, the CT zone with its wide distribution explored water and

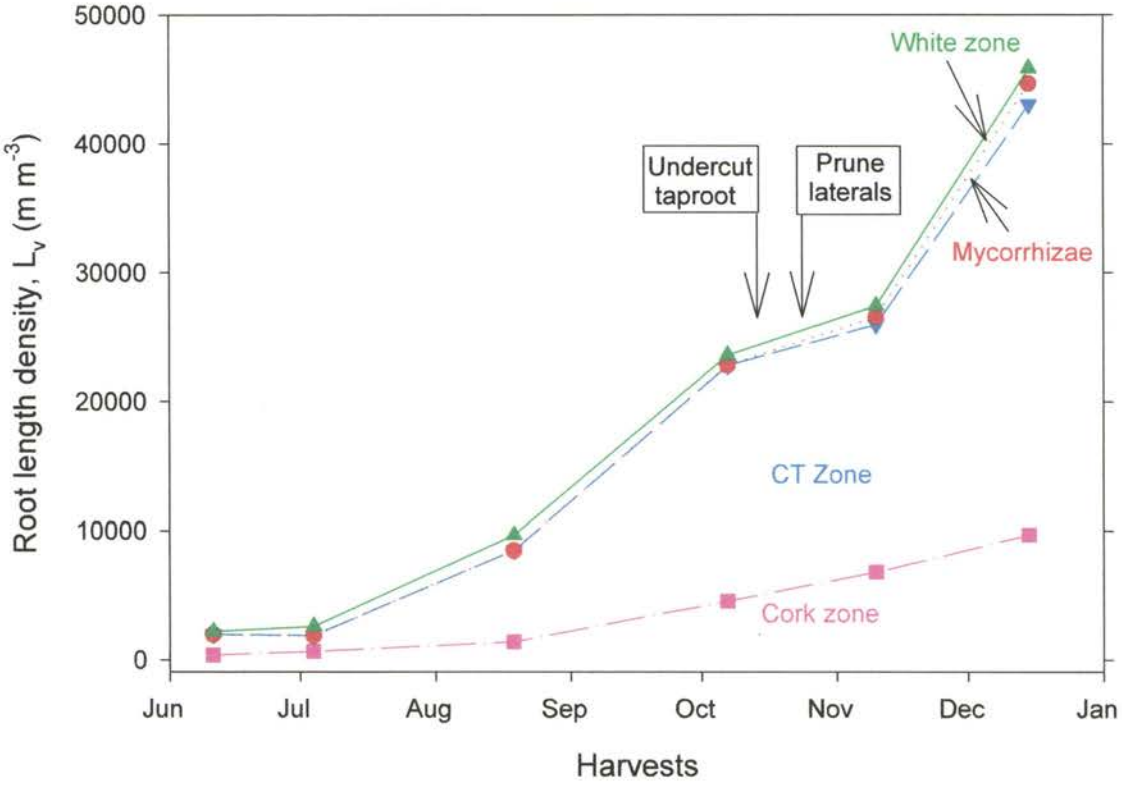

Fig. 17. Seasonal increase in cumulative root length density of white zone, CT zone, cork zone, and mycorrhizae of first year nursery-grown loblolly pine seedlings in 0 to 10 cm soil depth, n = 3.

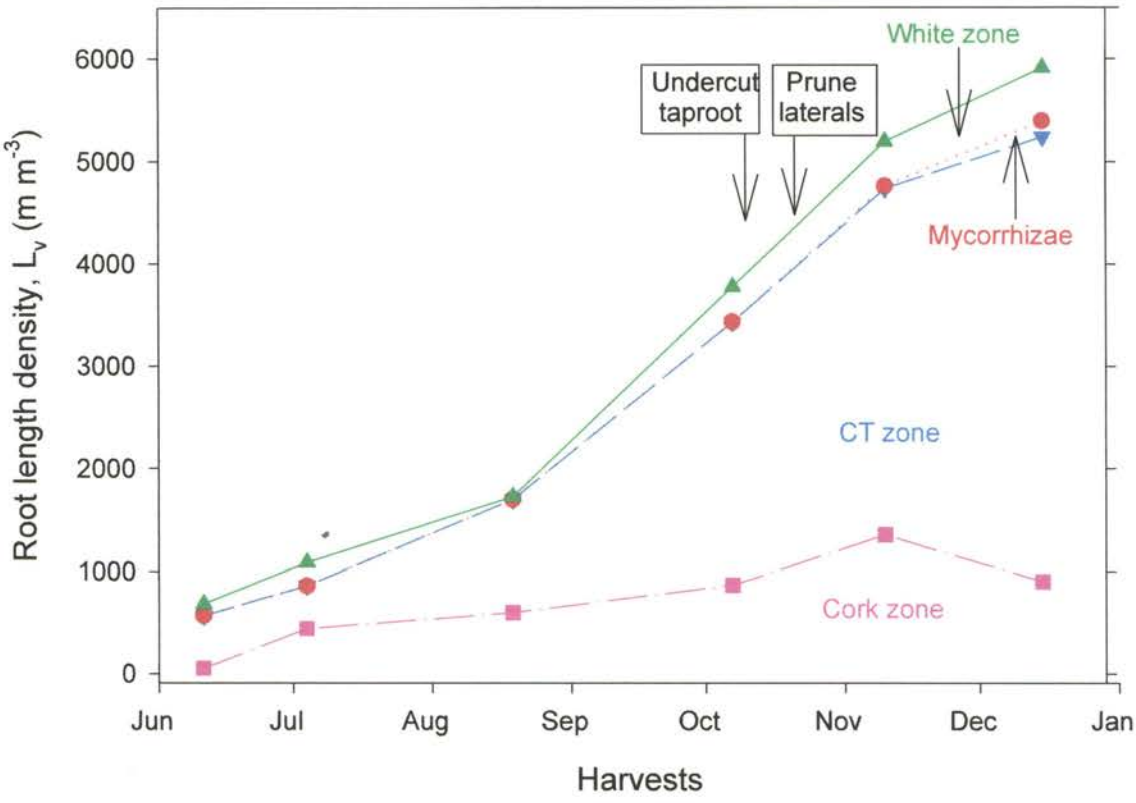

Fig. 18. Seasonal increase in cumulative root length density of white zone, CT zone, cork zone, and mycorrhizae of first year nursery-grown loblolly pine seedlings in 10 to 20 cm soil depth, n = 3.

nutrients from far and wide. Although the cork zone had no significant role in exploring the soil solution, it conducted soil solution axially and offered mechanical support to the shoot. Due to undercutting and lateral pruning many fine roots were truncated, but this did not affect length of cork zone due to its location in the upper soil layers.

Root length density in the subsurface layer (10 to 20 cm) was about one tenth that of the upper layer (Fig. 18). Root distribution in this layer followed a similar trend in the upper layer with the minor difference that white zone and mycorrhizae were a greater percentage of L_v. Klepper *et al.* (1973) found high near-surface root density in cotton (*Gossypium hirsutum* L.) and declining root density with depth, under well-watered conditions, but after a drying cycle maximal root density was in the deepest portion of the root zone. The undercutting and pruning treatments in October severed tap roots and many lateral roots and resulted in a proliferation of new white roots which was obvious in the increase in the white zone L_v at 10 to 20 cm, although the effect was obscured in the surface soil.

Specific root length

Specific root length, SRL (length per unit biomass, m g^{-1}) was considered very important in representing soil exploitation potential of roots. Atkinson (1990) reported that although SRL varies among species, a high SRL represented an effective use of resources to maximize soil contact. Eissenstat (1991) found a

positive correlation between SRL and root growth rate among eight different citrus rootstocks growing in moist, disturbed soil. He suggested that increased water uptake by roots with greater SRL indicated the ability of roots to better exploit enriched soil microsites.

Mycorrhizae appeared to be energetically more efficient in creating root length because of its high SRL than that of the white root zone which is considered the other major uptake zone of roots (Fig. 19). Early in the growing season SRL was at its highest for all root types and it declined thereafter, although SRL of the CT zone increased at the end of the year. The lower SRL at the end of the growing season could result from the accumulation of carbohydrates due to high photosynthesis during the mild weather and reduced growth rates. Although roots were in a period of rapid growth, leaf growth had slowed and stem growth had stopped. This means it is possible that more carbohydrates may have been transported to the root than could be consumed in growth and the excess was stored for later use.

Surface area

Eissenstat (1992) and Fitter and Hay (1987) reported that root surface area is one of the important parameters in explaining the significance of roots in their capacity to explore soil for water and nutrients. They also suggested that parameters used to predict nutrient absorption in increasing order of predictive

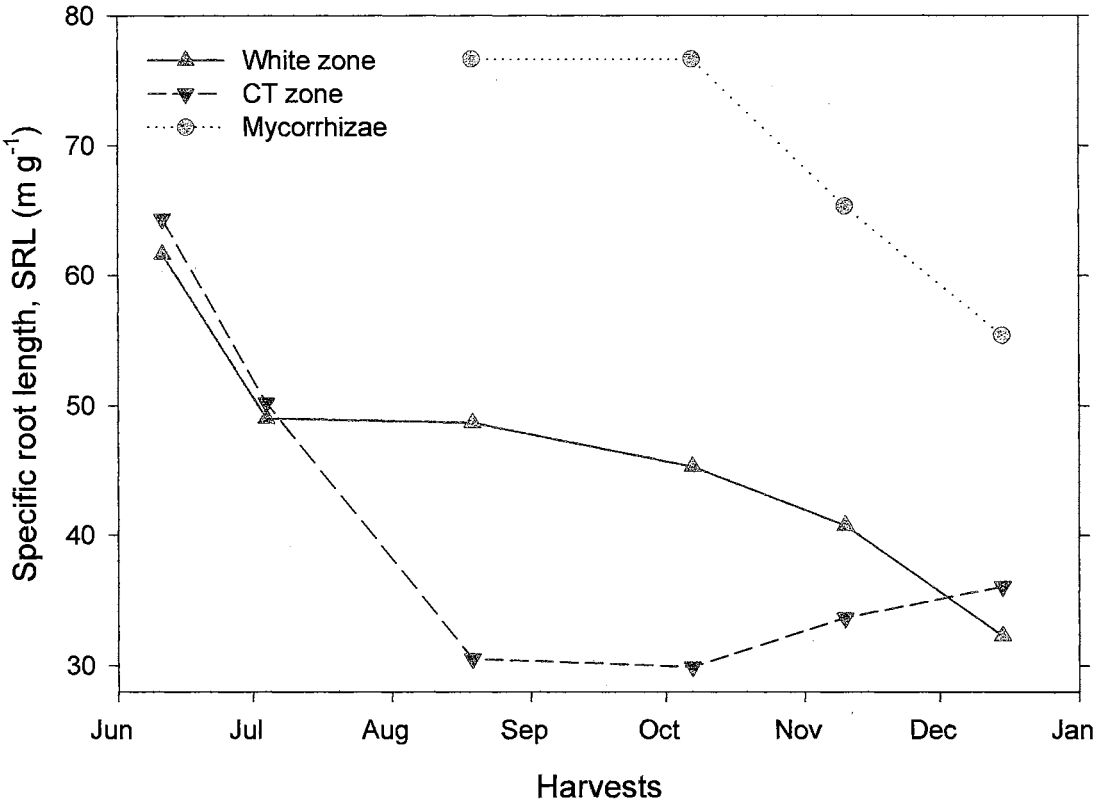

Fig. 19. Seasonal changes in specific root length (SRL) of white zone, CT zone, and mycorrhizae of first year nursery-grown loblolly pine seedlings, n = 3.

ability were: total root biomass < fine root biomass < root length < root surface area < rhizosphere volume.

The total surface area of all root types approached 8 $m^2 m^{-2}$ in December, the highest value for the year (Fig. 20 and 21). The major contributor to surface area was the CT zone followed by the cork zone, white roots and mycorrhizae. Compared to CT zone surface area of white roots was less, but they were highly efficient in absorbing soil solution because of the abundant live cortex cells and passage cells. Although the CT zone contained only passage cells for absorbing soil solution, the largest surface area generated was highly beneficial in exploring soil for water and nutrients. Russell (1977) and Marschner (1995) reported that when the level of an element is lacking in the soil it has to be explored from distant areas by increasing accessibility of roots. Therefore, the large surface area generated by the CT zone was highly beneficial to explore for soil solution from distant areas. When white roots turn brown they occupy the whole root distribution zone and this is particularly true for surface layers due to abundance of fine laterals. There was a gradual increase in the root surface area in the nursery from June until October. The major advantage of increased surface area is more specific when there is a need for exploring beyond the root zone. According to Bhat and Nye (1974), Russell (1977), and Marschner (1995), mineral nutrients such as phosphorus have very limited mobility in soils and mycorrhizae are considered efficient in absorbing such elements by increasing soil contact using extramatrical hyphae extending into crevices that are otherwise

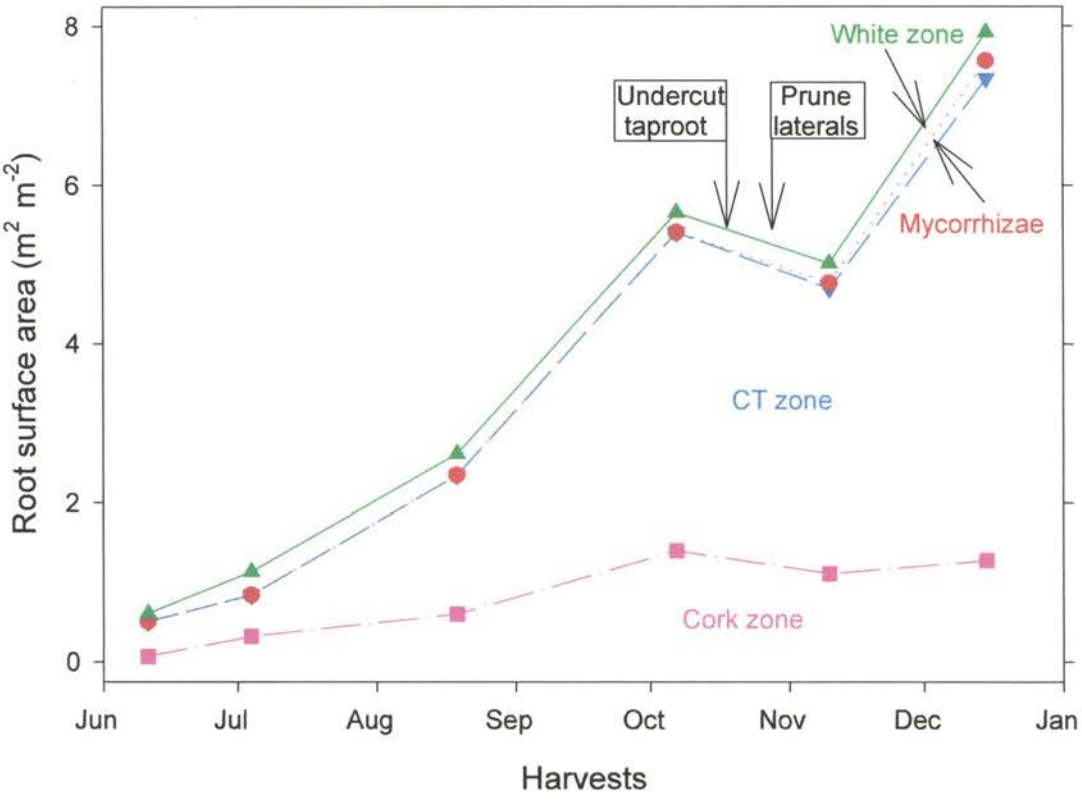

Fig. 20. Seasonal increase in cumulative surface area of white zone, CT zone, cork zone, and mycorrhizae of first year nursery-grown loblolly pine seedlings, n = 3.

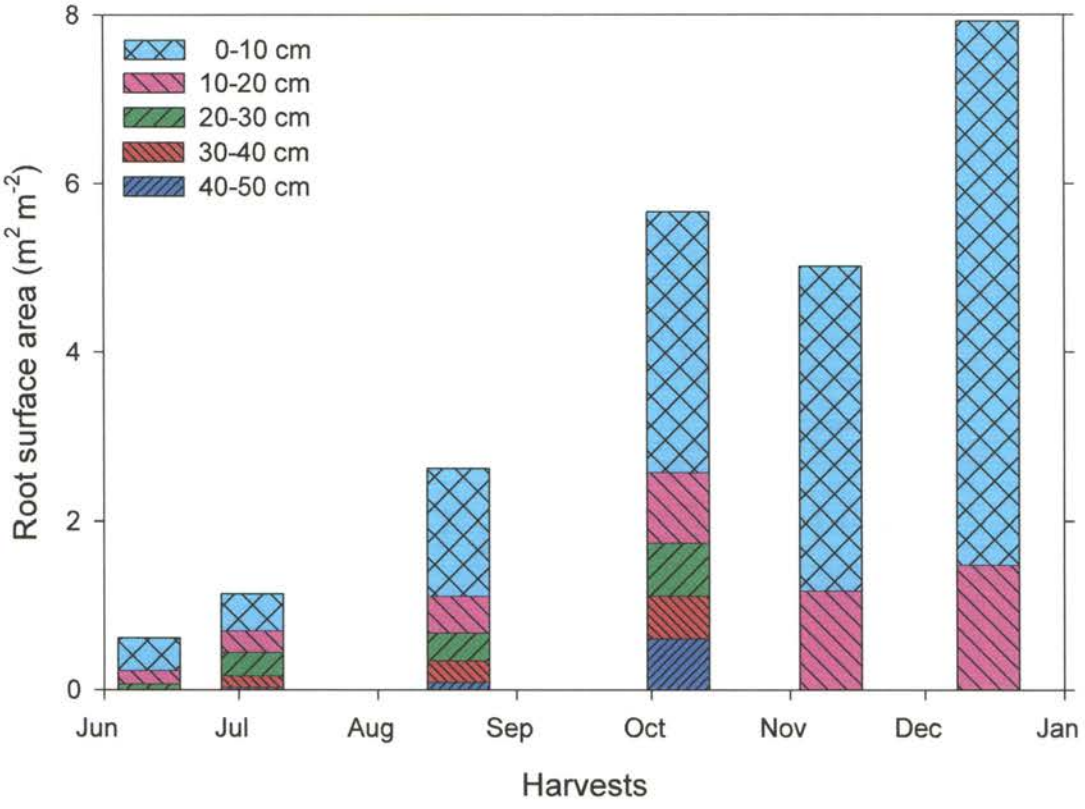

Fig. 21. Seasonal increase in surface area of white zone, CT zone, cork zone, and mycorrhizae at different soil depths of first year nursery-grown loblolly pine seedlings, n = 3.

inaccessible to roots themselves. According to Brundrett *et al.* (1996) plants must bypass the depletion zone for P by further root activity elsewhere in the soil to obtain more P. The outcome of this quest for P (and other relatively immobile soil resources) should largely be determined by surface area of a plant's root system. Marschner (1995) and Russell (1977) reported that demand for a particular mineral nutrient depends on plant internal requirements, while supply of that nutrient primarily depends on its availability and mobility in soils. Although the surface area generated by mycorrhizae was little compared to that of CT zone and white zone, they could explore more soil resources using extramatrical hyphae proliferating from the mantle around the root surface. Sylvia (1998) suggested that when a nutrient is deficient in soil solution, the critical root parameter controlling its uptake is surface area. Hyphae of mycorrhizal fungi have the potential to greatly increase absorbing surface of roots. Rousseau *et al.* (1994) found that while extramatrical mycelia (aggregates of hyphae) accounted for less than 20 % of total nutrient absorbing surface mass, they contributed nearly 80 % of absorbing surface area of pine seedlings. It is also important to consider the distribution and function of extramatrical hyphae. Ectomycorrhizal fungi produce growth regulators that stimulate branching of feeder roots, thus increasing total number of feeder roots produced. Such branching benefits in the absorption of nutrients by increasing surface area of roots (Castellano and Molina 1989). For mycorrhizae to be effective in nutrient uptake, hyphae must be distributed beyond the nutrient depletion zone that develops around roots when nutrients are removed from soil solution more rapidly than replaced by diffusion.

As a result of undercutting tap roots and pruning laterals in October, there was a decline in root length resulting in a decrease in surface area. Although shoot growth declined after November, roots continued growth producing more laterals and generating more absorbing area. With proliferation of long lateral root branches and ectomycorrhizae, absorbing surface area of the root system increases (Eissenstat and van Rees 1994 and Lynch 1995). Due to decline in shoot growth more photosynthates were translocated to roots resulting in the accumulation in roots, and in turn, production of more fine laterals. These laterals along with increased number of mycorrhizal tips containing white zone were able to absorb more soil solution. Chauhan and Mishra (1996) reported that removal of part of the root system has an almost immediate effect on root:shoot growth rate. Although shoot growth declined after root pruning, an increase occurred in the growth of roots resulting in an increase in surface area. This is due to the production of more laterals proliferated from truncated ends making the whole root system more fibrous to explore more soil solution because of increased surface area.

Quantifying cortical plasmalemma surface area

Cell dimensions

White zone: Roots of loblolly pine contained no clearly defined outer epidermis as it was sloughed off during root extension through soil (Mirov 1967). In the white zone cells involved in the absorption of water and nutrients are live cortex cells and passage cells in the endodermis surrounding the stele. The distal 15 to

25 mm of roots were white zone. The cross section of the cortex in the white zone contained an average of 102 cells in June (Table 1) and the number of cells steadily increased until October when it was over twice the June number. The number of cells stayed at this high level through December. Taylor and Peterson (2000) observed similar increases in jack pine roots between August and September. The increase in number could reflect ontogenetic changes in the first year of seedling growth from seed.

The average length and diameter of cortex cells appeared to change in opposite directions over the growing season (Table 1). The cells were long and thin (0.174 x 0.035 mm) in June and short and wide (0.126 x 0.045 mm) in December. Again, this may have simply been an ontogenetic change. In June the seedlings were at the beginning of a period of rapid growth and in December they were at the end of a period of active growth.

The number of cells in the endodermis increased from nearly 44 in June to 57 in December while the number of passage cells was at 8 in June and around 11 the rest of the year (Table 2). The reason for the changes in the number of endodermal cells may be due to ontogenetic changes. There did not appear to be much variation in passage cell dimension; their width ranged from 0.023 to 0.028 mm and length usually varied from 0.100 to 0.120 mm with one exceptional value of 0.160 mm in August. There was no difference in the length

Table 1. Seasonal changes in number, diameter, and length of cortex cells in the white zone and mycorrhizae from June until December. Mean and (standard error), n = 3.

Root type	Cortex cells		
	No.	Diam (mm)	Length (mm)
White zone			
Jun	102.2 (0.347)	0.035 (0.002)	0.174 (0.010)
Jul	143.1 (0.929)	0.041 (0.001)	0.166 (0.017)
Aug	167.7 (1.114)	0.038 (0.003)	0.169 (0.001)
Oct	238.7 (3.656)	0.038 (0.002)	0.174 (0.005)
Nov	218.2 (4.726)	0.043 (0.004)	0.144 (0.004)
Dec	211.7 (2.737)	0.045 (0.003)	0.126 (0.001)
Mycorrhizae			
Aug	50.5 (0.644)	0.032 (0.005)	0.057 (0.001)
Oct	59.6 (1.337)	0.042 (0.002)	0.066 (0.005)
Nov	65.0 (0.658)	0.044 (0.003)	0.070 (0.004)
Dec	76.1 (1.240)	0.058 (0.001)	0.074 (0.004)

Table 2. Seasonal changes in number of endodermal cells and number, tangential width, and length of passage cells in the white zone, CT zone, and mycorrhizae from June until December. Mean and (standard error), n = 3.

	Endodermal cells	Passage cells		
Harvest	No.	No.	Width (mm)	Length (mm)
White zone				
Jun	43.7 (0.551)	8.0 (1.079)	0.025 (0.0004)	0.116 (0.004)
Jul	48.7 (0.709)	13.3 (0.825)	0.026 (0.0005)	0.116 (0.005)
Aug	49.0 (0.709)	11.0 (1.248)	0.025 (0.002)	0.160 (0.012)
Oct	56.3 (1.665)	12.1 (1.082)	0.023 (0.002)	0.122 (0.003)
Nov	54.6 (1.652)	9.3 (0.502)	0.023 (0.001)	0.105 (0.004)
Dec	57.2 (1.960)	11.1 (1.634)	0.028 (0.001)	0.103 (0.003)
CT zone				
Jun	48.3 (0.586)	5.3 (0.297)	0.024 (0.001)	0.142 (0.011)
Jul	51.0 (1.464)	9.0 (0.529)	0.025 (0.001)	0.150 (0.010)
Aug	53.3 (0.173)	5.2 (0.385)	0.026 (0.002)	0.143 (0.003)
Oct	55.1 (1.856)	7.0 (0.191)	0.025 (0.001)	0.156 (0.008)
Nov	54.2 (1.253)	3.6 (0.064)	0.026 (0.001)	0.115 (0.005)
Dec	53.0 (0.961)	2.0 (0.230)	0.024 (0.0003)	0.121 (0.001)
Mycorrhizae				
Aug	11.3 (0.289)	2.4 (0.082)	0.026 (0.002)	0.087 (0.008)
Oct	12.4 (0.404)	2.6 (0.231)	0.028 (0.001)	0.093 (0.007)
Nov	12.7 (0.702)	3.0 (0.330)	0.030 (0.0003)	0.088 (0.012)
Dec	13.4 (0.850)	3.2 (0.294)	0.030 (0.002)	0.115 (0.010)

of suberized and unsuberized (passage) cells of endodermis which is consistent with the finding of Enstone *et al.* (2001).

Condensed tannin zone: The CT zone extending between the distal white zone and proximal cork zone contained about 73 % of total length of roots. Despite the extensive length, absence of live cortex in the CT zone makes it less efficient in providing absorbing surface. Although death of cortex is a programmed event in loblolly pine, Deacon (1987), Enstone *et al.* (2001), and Leshem (1974) reported that formation of suberin lamellae on the endodermal cells was responsible for the death of cortex in the CT zone. As a result, the tangential surface of passage cells had direct access to soil solution.

The number of cells in the CT zone endodermis varied only slightly around 50 cells and the number of passage cells decreased from a high of 5 to 9 cells in June and July to 2 in December (Table 2). The width of passage cells in the CT zone varied from 0.024 to 0.026 mm and length declined from high values of 0.142 to 0.150 mm in June and July to 0.121 mm in December.

Mycorrhizae: The cell dimensions of mycorrhizae were much smaller than those of white roots. There were fairly large increases in cell numbers and sizes from August to December. The number of cortex cells in cross section increased by over 50 % (Table 1). Cortex cells increased in diameter by over 80 % and in length by nearly 30 %. The number of endodermal cells changed slightly from 11

to 13 and there were 2 to 3 passage cells (Table 2). Passage cell width increased from 0.026 to 0.030 mm and their length increased from 0.087 to 0.115 mm.

Cortical plasmalemma surface area

The CPSA of the cortex had three components (Table 3). The contributions of these components in the white zone was around 90 % for the tangential and radial cell surfaces, 10 % for the transverse cell surfaces and 1 % for the outer tangential cell surfaces of passage cells. In mycorrhizae the contribution was around 75 % for the tangential and radial cell surfaces, 25 % for the transverse cell surfaces and 1% for the outer tangential cell surfaces of passage cells. The only CPSA in the CT zone came from the outer tangential surfaces of passage cells. CPSA per unit length increased three fold in the white zone from June to December and in mycorrhizae from August to December. In the white zone this was due mainly to the doubling in the number of cells. In mycorrhizae it was due to increases in cell number and size. CPSA per unit length in the CT zone was very small and it declined by over 80 % from a high in July to December due to decrease in the number of passage cells.

The total CPSA for the entire loblolly pine root system increased over 15 fold from June to December. The greatest part of the CPSA was always in the white zone and mycorrhizae contributed a significant part after August (Table 3, Fig. 22). Although the CT zone contribution was very small, the vast majority of

Table 3. Cortical plasmalemma surface area per unit length of root in the white zone, CT zone, and mycorrhizal white zone. S1 = tangential and radial surfaces of cortex cells, S2 = transverse surface of cortex cells, and P = outer tangential surface of passage cells; mean and (standard error), n = 3.

Harvest	S1	S2	P	Total CPSA
		($mm^2\ mm^{-1}$)		
White zone				
Jun	11.261	1.135	0.200	12.596 (0.967)
Jul	18.563	2.311	0.340	21.213 (0.627)
Aug	19.747	2.193	0.277	22.217 (1.956)
Oct	28.407	3.092	0.277	31.776 (1.534)
Nov	29.185	4.308	0.216	33.709 (3.914)
Dec	29.917	5.364	0.312	35.593 (2.854)
CT zone				
Jun	...	...	0.129	0.129 (0.009)
Jul	...	...	0.226	0.226 (0.006)
Aug	...	...	0.137	0.137 (0.008)
Oct	...	...	0.176	0.176 (0.004)
Nov	...	...	0.093	0.093 (0.004)
Dec	...	...	0.048	0.048 (0.005)
Mycorrhizae				
Aug	5.138	1.460	0.063	6.661 (1.138)
Oct	7.826	2.471	0.073	10.369 (0.912)
Nov	8.980	2.810	0.090	11.881 (0.825)
Dec	13.856	5.408	0.096	19.361 (0.454)

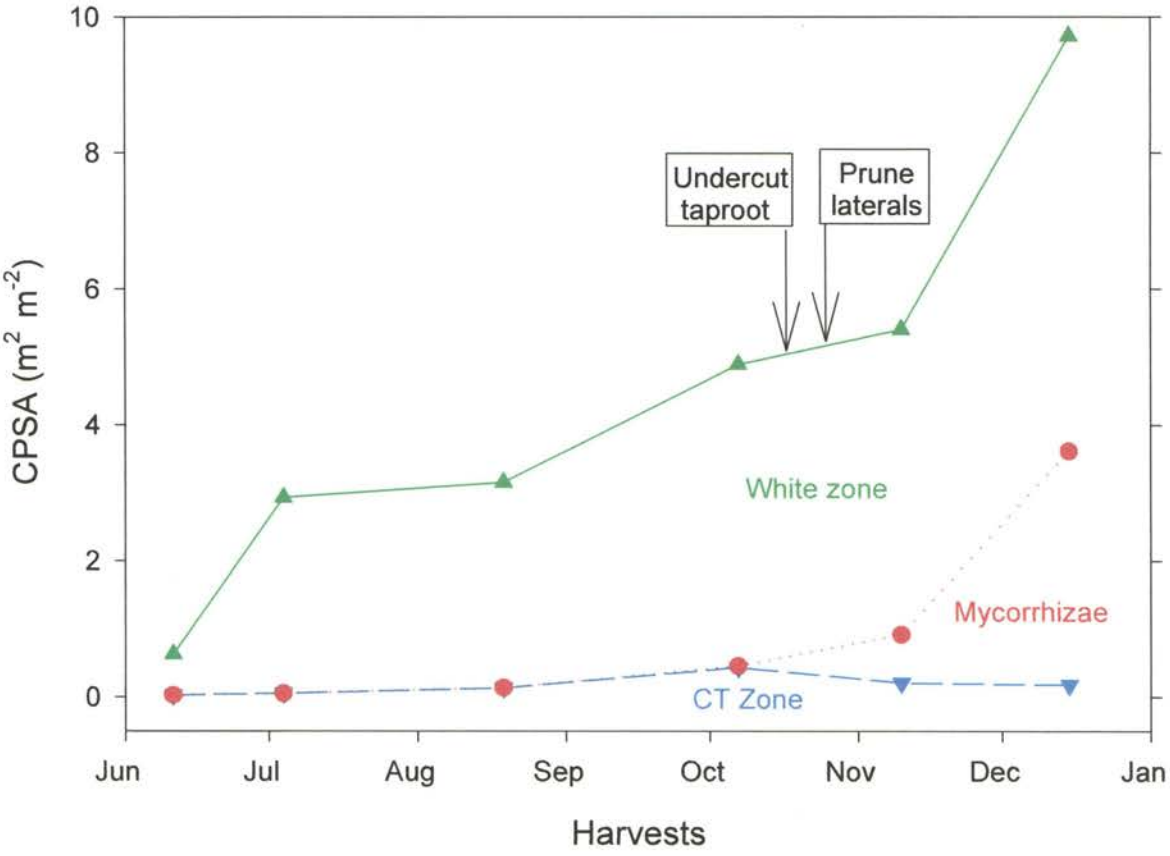

Fig. 22. Seasonal increase in cumulative CPSA in the white zone, CT zone, and mycorrhizae of first year nursery-grown loblolly pine seedlings, n = 3.

root length was CT zone which means these roots had access to the resources in a huge volume of soil. Enstone *et al.* (2001) reported that as most root length consisted of a CT zone, this zone should be capable of ion uptake and passage cells represented a low-resistance path for the inward flow of water. McCrady and Comerford (1998) observed that 84 % of primary brown roots had a cortex that was crushed or collapsed. Only the passage cells in these roots had access to soil solution.

Most CPSA was contributed by the white roots in the surface 10 cm (Fig. 23). This was particularly true in August when irrigation water was not supplied fast enough to moisten the deeper layers. In October more laterals penetrated into deeper layers and this contributed to a significant amount of CPSA in the 10 to 20 cm soil layer. After undercutting and lateral pruning in October, roots below 20 cm did not contribute any absorbing surface. As a result of root pruning abundant laterals containing white zone were produced within the upper 20 cm that significantly increased absorbing surface area. Therefore, the CPSA involved in the absorption of water and ions in November and December was from within this layer. The role of mycorrhizae was very important between November and December when an explosion in the number of mycorrhizae resulted.

Taylor and Peterson (2000) reported that ions must access membranes before they can be absorbed into the symplast; and therefore, CPSA contributed

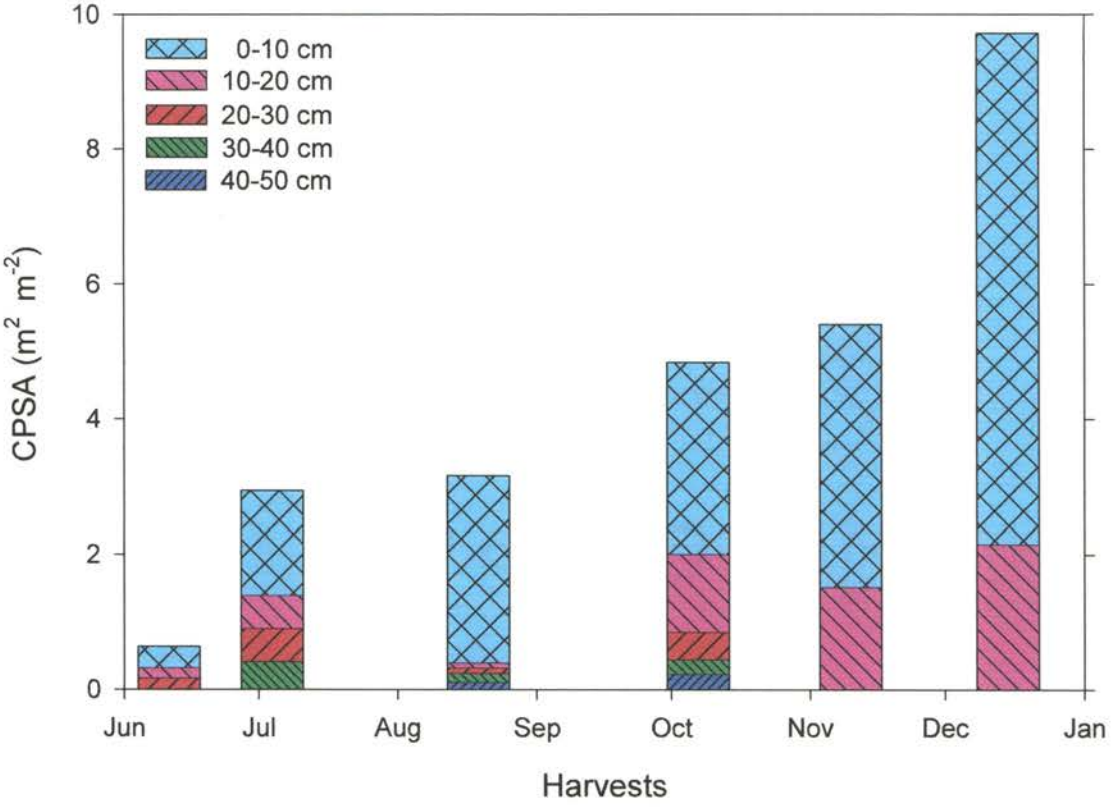

Fig. 23. Seasonal increase in CPSA of white zone, CT zone, and mycorrhizae at different soil depths of first year nursery-grown loblolly pine seedlings, n = 3.

to the capacity of roots for ion uptake. They also observed that the contribution of CPSA by white and CT zones changed little during the course of their study. Instead, the mycorrhizal zone was the dominant contributor both to overall CPSA and changes in CPSA. In the present study, most CPSA was found in the white roots followed by mycorrhizae and a very small contribution by the CT zone. One of the reasons for this difference may be the late appearance of mycorrhizae in August. Until the appearance of mycorrhizae all CPSA was in the white and CT zones. Pruning lateral roots in October resulted in a significant increase in the number and length of white roots and a decrease in CT zone bearing mycorrhizae. The amount of CPSA per unit of root length in loblolly pine was very similar to that of *Pinus banksiana* Lamb. (Taylor and Peterson 2000). No other comparisons can be made, as the amount of CPSA has not been published for other species.

It is tempting to speculate on efficient strategies for increasing CPSA and limiting negative effects and costs. It seemed that the best opportunity for increasing CPSA was the cortical cells of the white zone and mycorrhizae, as these cells varied a lot in number and dimensions. The absorbing area could be increased by increasing cortical cell number. This could have negative effects because an earlier comparison among five species showed a negative relation between cortex thickness and root conductivity (Rieger and Litvin 1999). An increase in CPSA could be achieved without increasing cortex thickness by

increasing the number and reducing the size of the cells at the same time. This strategy may have the added benefit of reduced cost for increased CPSA.

Cortical plasmalemma surface area per unit root length varied nearly three fold for the white zone over the first year for loblolly pine roots. This variation was due mainly to a doubling of the number of cells and to a 30 percent increase in cell diameter from June to December. The cells also decreased almost a 30 percent in length resulting in nearly 40 percent increase in the transverse plasmalemma surface area due to a greater number of cells in the longitudinal direction. The mycorrhizal CPSA increased equally due to increases in cell number and diameter. There may have been equally large differences among different orders of lateral roots at a given time, but the approach of the current study did not allow determination of anatomical differences among lateral roots of different orders. There is no information about genetic and environmental control of root anatomy in loblolly pine and the relation of root anatomy to root conductivity. Further studies should be done to fill this gap in knowledge.

The ratio of CPSA to leaf area reached a peak value in July, dropped sharply in August and then declined gradually until December (Fig. 24). The high ratio of CPSA to leaf area (> 2.5) during June through August could contribute to the seedling capacity to maintain a favorable water balance during the hottest period of the year. Apparently early growth favored the development of CPSA and led to a high ratio of absorptive capacity to demand capacity. This would

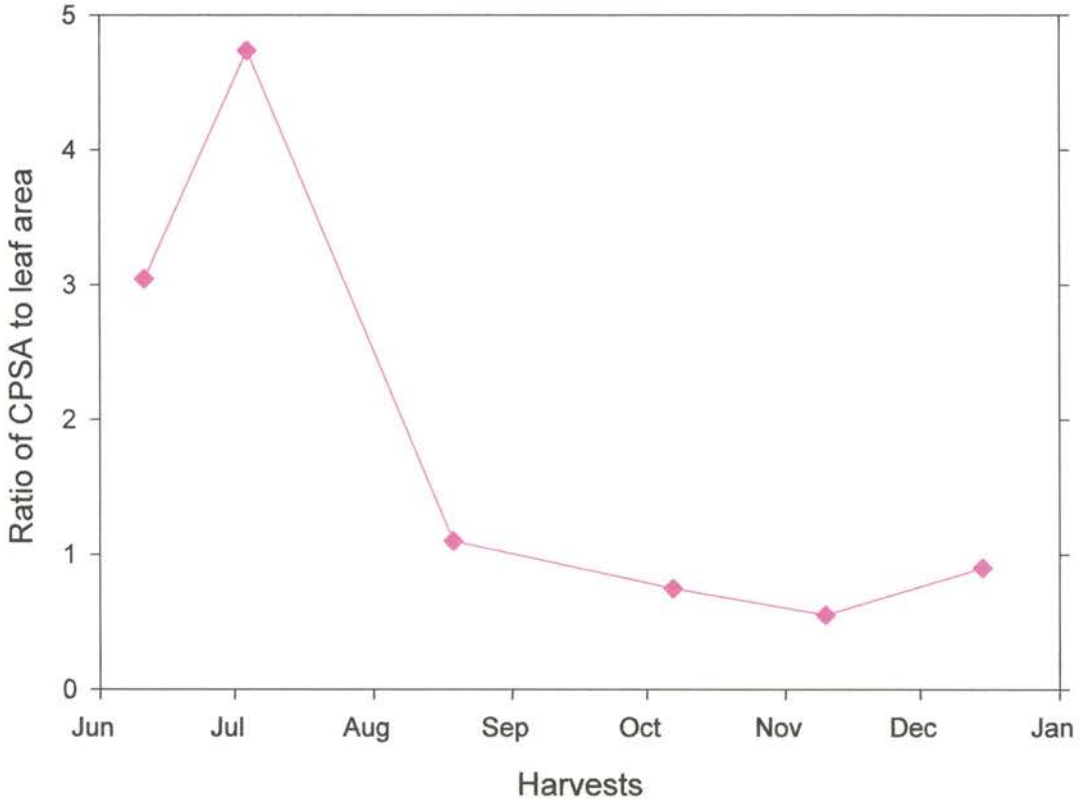

Fig. 24. Seasonal changes in the ratio of cortical plasmalemma surface area to needle surface area of first year nursery-grown loblolly pine seedlings, n = 3.

prepare the plant for the hot periods of high evaporation during the summer. Later in the summer there was a rapid increase in CPSA and a greater increase in leaf area which reduced the CPSA to leaf area ratio to its lowest value just as winter began. The low CPSA to leaf area ratio would reduce the capacity to supply water but low winter temperature would create a lower water demand than during the summer.

CONCLUSIONS

The following conclusions were made from the present research involving roots of loblolly pine seedlings in the first growing season:

- Roots continued rapid biomass growth at least one month later into the winter than shoots.

- The preponderance of roots (over 90 % of the root weight, root length, number of tips, root length density, surface area, and CPSA) was in the surface 20 cm of soil at the time of undercutting in October, most likely due to the fact that irrigation water did not reach deeper soil layers during the hot months of July and August. Although undercutting may have facilitated harvesting seedlings in mid-winter, it probably did not have an important impact on root parameters.

- The specific root length, SRL ($m\ g^{-1}$) which was highest at the beginning of the season decreased towards mid-December; mycorrhizae contained the highest SRL compared to other root types.

- Mycorrhizae were relatively late to develop in the nursery perhaps due to fumigation treatments and highly favorable growing conditions for tree seedlings. Mycorrhizae which were not found until late August began a very

large increase in number, surface area, and CPSA in October that continued through mid-December.

- Some aspects of white root anatomy remained unchanged and others changed over the first growing period for loblolly pine seedlings. The number of endodermal cells and stele diameter remained unchanged from June to December. The number of cortical cells increased two fold and their diameter increased 30 percent over the same period; as a consequence CPSA per root length increased nearly three fold.

- Over 98 percent of the CPSA was in the white roots and mycorrhizae, despite the fact that nearly 93 percent of root length was in the cork and CT zones.

- The CPSA per length of root in the CT zone declined by 75 percent from June to December. Passage cells are the only source of CPSA in the CT zone. In contrast, the passage cell CPSA per length of root in the white zone and mycorrhizae increased by 50 percent over the growing season.

- The ratio of CPSA to leaf area reached a peak in the hot summer months indicating a favorable balance of absorbing to loss capacity for water during the period of greatest demand. This ratio declined in the fall and winter when evaporative demand was decreasing.

IMPLICATIONS AND FUTURE RESEARCH

- The current research is very basic involving seedlings from one family only and did not include any treatments. Therefore, further studies should be conducted considering genetic and environmental controls of root anatomy.

- Attempts are underway to expand the range of loblolly pine to the west of its natural range where the conditions are drier. The information gained from the current research may be used to select traits for further study of genetic variation in drought resistance.

- Among the three zones the CT zone comprised the largest amount of root length and contained passage cells throughout the year that allowed entry of water. The adaptive advantage of this zone in the whole root system needs detailed investigation.

- There was a huge increase in the number of mycorrhizae towards the end of the year. As mycorrhizae need living cortex cells for nourishment, the cost of mycorrhizae needs further investigation. As cortex cells in the mycorrhizal zone are enclosed by a mantle, permeability of the mantle needs further research to learn whether it is a barrier to uptake.

- Although the absorbing surface of roots is confined to white, CT, and mycorrhizae, the vast amount of cork zone in large trees needs to be examined further considering the possibility of lenticels, wounds, and crevices created by lateral root emergence.

- Although the nursery environment is artificial, it is more representative of field conditions than pot-grown plants in a greenhouse. Information gained from this study may be extended with caution to plants in the field in defining better models of water uptake by allowing us to define more accurately effective root length and/or surface area.

- In the present research the absorbing surface was determined only during the initial year of seedling growth. Large ontogenetic changes may occur in the first year. Information on older trees is a high priority.

- Anatomical studies involving different root zones have been confined to a limited number of tree species such as jack pine, eucalyptus, and loblolly pine (Enstone *et al.* 2001, McKenzie and Peterson 1995a, b, Peterson *et al.* 1999, and Taylor and Peterson 2000). Therefore, research in similar lines may be extended to other species of commercial importance.

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APPENDIX - A

Identification of suberin lamellae with Fluorol Yellow 088 (0.005% w/v) – (Brundrett *et al.* 1991).

Preparation:

1. Dissolve sufficient amount of dye to make a 0.01 % (w/v) solution in polyethylene glycol 400 by heating at 90 °C for 1 h.

2. Add an equal volume of 90 % (v/v) glycerol (90 ml glycerol + 10 ml water) to the above solution and stir well.

Staining:

1. Place sections of fresh or 50 % alcohol-preserved tissues in section holders and place the holders in stain for 1 h at room temperature.

2. Rinse sections several times with water, blotting holders between rinses, and mount them in 75 % glycerol.

3. Observe the sections under UV epifluorescent illumination.

Results:

1. Fluorol Yellow 088 specifically stains lipids bright yellow and produces very high contrast images of suberin lamellae and other hydrophobic structures.

Caution:

1. Fading of the stain can occur as a result of excitation at high magnifications; so keep exposure times short at high magnifications.

2. The high intensity of staining can illuminate adjacent tissues; so examine them with fluorescent field diaphragm closed to see if staining is due to fluorescence or reflection.

APPENDIX - B

Detection of mycorrhizae and Casparian bands with Chlorazol Black E (Brundrett & Kendrick 1988 and Brundrett *et al.* 1984).

Preparation:

1. Dissolve 0.1 % (w/v) Chlorazol Black E (CBE) in a solution of 80 % lactic acid, glycerine, and distilled water (1:1:1).

2. Allow to settle down undissolved stain particles.

Staining:

1. Fix root segments overnight in FAA (or in 50 % ethanol-5 % lactic acid).

2. Rinse the roots to remove FAA (or ethanol-lactic acid).

3. Transfer them to 10 % KOH.

4. Clear in autoclave at 121 °C for 15 min.
   (less time for delicate tissues and more time for coarse tissues).

5. Rinse several times, first with tap water and then with deionized water.

6. Transfer to the CBE stain.

7. Stain for 1 h at 90 °C or autoclave again.

8. Transfer from staining solution to glycerine for storage or observation.

9. Destain overnight in the glycerine.

10. Mount in modified Hoyer's mounting fluid (chloral hydrate reduced to 2 %) or in PVLAG.

11. Observe under white light.

Results:

1. Fungal hyphae and Casparian bands appear black or bluish-black.

Caution:

1. Bleach roots containing too much tannin with 3 % alkaline H_2O_2.

VITA

Prem Kumar

Candidate for the Degree of

Doctor of Philosophy

Thesis: ANATOMICAL CHARACTERISTICS OF ROOTS OF LOBLOLLY PINE SEEDLINGS

Major Field: Plant Science

Biographical:

Education: Attended St. Thomas' College, Trichur, Kerala, under University of Calicut, Calicut, Kerala, 1971 - '76 and received Bachelor of Science and Master of Science degrees in Botany, April 1974 and April 1976 respectively; attended State Forest Service College, Coimbatore, Tamil Nadu, under Forest Research Institute and Colleges, Dehra Dun, 1986 - '87 and received Diploma in Forestry, December 1987; attended Oklahoma State University, Stillwater, OK, 1995 - '99 and received Master of Science in Forest Resources, July 1999. Completed the requirements for Doctor of Philosophy degree with a major in Plant Science at Oklahoma State University, Stillwater, OK, December 2003.

Experience: Research Fellow, Central Plantation Crops Research Institute, Calicut, Kerala, 1977 - '78; Clerical Assistant in a business organization, Cochin, Kerala, 1979 - '82; Research Support Assistant, Department of Plant Pathology, College of Horticulture, 1982 - '85 and Assistant Professor, College of Forestry, 1988 - '94, Kerala Agricultural University, Trichur, Kerala; Student Tech Paraprofessional, Department of Plant and Soil Sciences, 1995 - '98 and Graduate Research Associate, Department of Forestry, 1998 - '03, Oklahoma State University, Stillwater, OK.

Professional Memberships: Microscopy Society of America, Oklahoma Academy of Sciences, Society of American Foresters, and Xi Sigma Pi - National Honour Society.