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Root Growth, Root Function, and Water Relations of Shortleaf Pine (Pinus Echinata Mill.) Bare-Root Seedlings Transplanted Into Different Edaphic Environments.

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ROOT GROWTH, ROOT FUNCTION, AND WATER RELATIONS OF SHORTLEAF PINE (*Pinus echinata* Mill.) BARE-ROOT SEEDLINGS TRANSPLANTED INTO DIFFERENT EDAPHIC ENVIRONMENTS

A Dissertation

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by
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ABSTRACT

Root growth and its effects on water uptake and plant water relations of seedlings during the initial weeks after transplanting was studied. One-year old bare-root shortleaf pine seedlings were put into six different root zone environments defined by the factorial arrangement of two temperatures and three levels of soil water potential. Needle water potential and stomatal conductance were measured 28 days after planting. The next day, root system absorptive capacity was evaluated by forcing water at a constant hydrostatic pressure through detopped seedlings. The projected surface area of old and new roots was then measured. Also, the relative water content of each root system was determined. The experiments were repeated three times.

Under the conditions of the study, about one-half of the variation in new root growth was accounted for by the root zone environment. The amount of root growth was affected by the interaction between root zone temperature and soil water potential. In the most favorable root environment, new roots made up about 20% of the mean total root system surface area after 29 days. Root relative water content had a minimum level associated with the presence of new roots; however, it was not highly correlated with the amount of root growth. The amount of new root surface area had a positive, linear relationship with absorptive capacity. Each 10 mm^2 of new root projected surface area improved absorptive capacity

by about 5%. The amount of old root surface area did not influence absorptive capacity, probably because the experimental populations were restricted to a narrow range in initial root system size. The various measures of water status all improved with greater new root development. Under the conditions of these experiments, the water stress that was induced by transplanting was alleviated by the growth of approximately 500 to 550 mm^2 of new root projected surface area. Only among seedlings in the most favorable treatment did mean root growth exceed that amount.

CHAPTER 1

INTRODUCTION

World-wide the most common method of artificial regeneration of forest stands is by planting bare-root seedlings. During lifting from the nursery soil many of the fine roots are broken off and left behind. Roots often suffer further injury due to desiccation and mechanical damage during handling at the nursery, in storage, and during transport to the planting site. Shoots typically suffer less damage because seedlings are lifted during the winter when there is seldom any succulent tissue. Furthermore, the roots present when seedlings are transplanted will seldom have as good of contact with the soil as the roots of plants that germinated and grew in place. Therefore, transplanted seedlings have reduced absorbing capacity compared to what they had prior to lifting, but they have the same transpirational surface area.

The establishment phase for a transplanted seedling is that period of time after outplanting during which the rates of physiological processes are adjusting to the new environment. It is the same period of time during which all planted seedlings, even those planted under ideal conditions, suffer some degree of transplanting stress (Rietveld 1989a). Therefore, establishment may be considered that period of time after planting during which physiological processes recover to rates comparable to those of undisturbed plants of similar morphology that are growing on

the same site. Essentially all the plant's physiological processes are affected for some time after transplanting. The time required for the resumption of normal root function depends on seedling morphology and physiological condition, and on the environment of the planting site. Consequently, the establishment phase might last for several weeks, or for an entire growing season or longer.

This research had two goals: to provide a better understanding of environmental effects on pine seedling root growth during the first, critical weeks after outplanting; and to describe the impacts new roots have on water absorption and seedling water relations. Shortleaf pine (Pinus echinata Mill.) from the Interior Highlands was chosen because the drought-prone sites in the Ouachita and Ozark Mountains where it is planted are difficult to successfully reforest. The research had three major objectives: (1) describe the effects of root zone temperature and water availability on new root growth after transplanting; (2) determine the relative importance of old and new root surface area on the capacity of the root system to absorb water; and, (3) determine to what extent the amount of new root growth affects several measures of seedling water status.

The success of artificial regeneration depends primarily on four factors: the environment of the planting site, the quality of the planting stock, the care exercised in planting the seedlings, and the environmental conditions following planting. The planting site environment can be ameliorated through the silvicultural treatments applied while preparing the site for planting. Stock quality is determined by how the seedlings are produced and cared for before planting, and how well they are

genetically, physiologically, and morphologically matched to the planting site. Seedling care and handling is largely a matter of adequate knowledge and supervision. Many aspects of the environment following planting, such as the weather, cannot be predicted or altered. However, they can be anticipated and measures taken during site preparation, seedling production, and seedling handling to maximize the possibility of reforestation success. Of these four factors, seedling quality has received, and continues to receive, the most research attention.

Planting stock quality is an often used but seldom defined phrase used to provide some relative measure of how seedlings might perform after outplanting in the field. Thus, the survival and growth of "excellent" seedlings exceed expectations for a particular planting site, while "poor" seedlings do not meet expectations on that site. Meeting expectations for field performance is best assured by planting seedlings that are carefully matched to the planting site. Such matching includes being of an adapted genetic source, having the proper balance of morphological attributes, and being physiologically ready to grow when planted or when the environmental conditions are favorable.

After nearly 30 years of reforestation research, Wakeley (1954) concluded that initial survival of planted southern pines depends more on the formation of new roots than on any other factor. He estimated that 50% of a pine seedling's root system could be lost during lifting from the nursery without visible evidence of damage. Nambiar (1980) concluded that the total length of roots transplanted does not exceed 25% of that in the nursery. Reduced absorptive capacity because of loss of roots during lifting can lead to severe and often prolonged water deficits in

transplanted trees. In the Front Range of the Rocky Mountains of Colorado, and in Sweden, it took 2 years or longer for xylem water potentials of transplanted pine seedlings to recover to the level of established control seedlings (Baldwin and Barney 1976, Orlander 1986).

Shortleaf pine is found in pure natural stands in the Interior Highlands and in the northern portion of the Piedmont Province; elsewhere in its range it is found in mixture with loblolly pine (P. taeda L.) (Walker 1980). Shortleaf pine is the most important species used for artificial regeneration on the Ouachita and Ozark National Forests in Arkansas and Oklahoma (Kitchens 1986). On those national forests it is preferred over loblolly pine because of excellent wood quality, good yields when grown at long rotations, and a high potential for genetic improvement (Kitchens 1986).

Between 1980 and 1986 an average of 7 million seedlings were planted yearly on the two forests, resulting in an annual regeneration effort of about 4,000 hectares. The peak planting during that period was approximately 12 million seedlings planted on about 7,000 hectares. Since 1987 there has been a greater reliance on natural regeneration, especially on the Ouachita National Forest, and total planting has declined somewhat. Shortleaf pine seedling production for the two forests is expected to remain at the current level of about 6 million seedlings annually.

Shortleaf pine is adapted to and usually planted on south- and west-facing slopes where soil moisture is often limited (Barnett et al. 1986). Consequently, establishing successful plantations is often more difficult for shortleaf pine on mountain sites than for southern pine species planted in the Coastal Plain where the sites are typically flatter and

more moist. Between the beginning of large scale planting in the 1970s and the mid-1980s, first-year stocking averaged less than 50% of the number of seedlings that were planted. Although the planting sites are harsh, many foresters do not think that difficult site conditions alone explain the poor seedling performance. Other reasons often cited include poor planting stock quality, prolonged seedling storage, and poor handling and planting practices. Therefore, a better understanding of the interactions between the planting stock and the environment that determine how quickly seedlings become established will improve reforestation, especially on harsh sites.

The uptake of water by plants has been studied under a variety of conditions by numerous investigators (see Kramer 1983). In general, it has been found that water uptake is reduced as soil temperature decreases and as soil water is depleted. Under both low temperature and water stress, water uptake is affected by decreased root growth and increased resistance to water movement through the soil-plant system.

Most research on water uptake has used established plants, or container seedlings that have essentially undisturbed root systems. In loblolly and white pine (P. strobus L.) seedlings established in pots, root elongation and root dry weight increase are significantly reduced by soil water potentials less than about -0.2 MPa (Kaufmann 1968). Also, when root elongation is retarded by low soil water potential, the new roots become suberized in as few as 5 days (Kaufmann 1968). Root growth of container jack pine (P. banksiana Lamb.) is reduced about 50% at root medium water potentials of -0.8 MPa and root growth essentially stops at water potentials of -1.2 MPa (Buxton et al. 1985).

While root growth slows and stops with decreasing soil water potential, root system permeability also declines because resistance to water flow increases. The increased resistance to water flow is likely caused by reduced turgor which results in root shrinkage and concomitant loss of intimate contact between the root surface and the soil (Faiz and Weatherley 1978, Weatherley 1979).

Decreasing temperature inhibits root growth by limiting the metabolic activity necessary for growth. Root growth will stop at some temperature critical for a particular species. In established loblolly and shortleaf pines, intermittent root growth was observed throughout the winter as far north as Fayetteville, Arkansas and Durham, North Carolina (Reed 1939, Turner 1936). However, in neither case were the critical temperatures determined for either the cessation or resumption of root growth.

Root system permeability decreases with decreasing soil temperature because the membranes which water must cross to get into the xylem become less permeable and because the viscosity of water increases as temperature decreases (Kramer 1983). Thus, lower soil temperatures and reduced soil water potential both reduce root system permeability, but in somewhat different ways. Although both factors limit root growth, and thereby the surface area of more permeable unsuberized tissue, the mechanisms of reduced permeability are different. Soil drying results in greater resistance across the root-soil interface, whereas low temperatures limit movement across root membranes.

Although unsuberized portions of the root system are more absorptive than older, suberized root surfaces, woody plants must rely on suberized

roots for much of their water uptake (Kramer 1983). Using potometers on established trees, Kramer (1946) showed that shortleaf pine does absorb significant amounts of water through suberized roots, but at a rate of only 73% of that through suberized roots of dogwood (Cornus florida L.). Addoms (1946) showed that water enters the suberized pine roots only through tiny wounds and not around dead branch roots or through the tips of aborted roots. For loblolly pine seedlings growing in pots, Chung and Kramer (1975) found that water uptake through completely suberized root systems was about 70% of that through growing root systems with 40 to 50% of their surface unsuberized.

Water uptake by established Scots pine (P. sylvestris L.) seedlings was enhanced by mycorrhizal associations between fungi and the roots (Duddridge et al. 1980, Brownlee et al. 1983). The mycorrhizal rhizomorphs served as extensions to the pine root systems, effectively increasing the surface area available for absorption. Although mycorrhizae may increase the effective surface area of a root system, they had no effect on the permeability of loblolly pine roots (Sands et al. 1982).

Water uptake by transplanted seedlings has not been studied as much as in established plants. Sands (1984) compared water uptake of radiata pine (P. radiata D. Don) seedlings planted into either aerated nutrient solution or soil soon after lifting from the nursery. He concluded that transplanting stress in radiata pine is caused by air gaps around the planted roots resulting in a large root-soil interface resistance that can limit water uptake even in wet soils.

Root system permeability also can be affected by conditions within the xylem that may be independent of, or influenced by, the root-soil interface environment. Between water uptake from the soil at the root surface and water vapor loss to the atmosphere through the stomates, the xylem forms a complex maze of pathways for water movement. Water in the xylem is held under tension while it is pulled through the plant as the result of transpiration. Water can move both longitudinally and radially within and between the tracheids of conifer xylem, or the tracheids and vessel elements found in the xylem of angiosperms. An embolism caused by an air bubble forming in a tracheid or vessel element can break the continuity of the water molecules in the xylem and block water flow beyond that point (Zimmermann 1983). When embolisms occur water flow must follow alternative routes through the xylem. Embolisms may eventually dissolve and allow that potion of the xylem to function again, or they may be permanent. In either case, the cavitation caused by embolisms can greatly reduce the permeability of a root system. It has been suggested that even established woody plants have only a small margin of safety protecting them from catastrophic xylem dysfunction because of embolisms (Tyree and Sperry 1988). In northern white-cedar (Thuja occidentalis L.), eastern hemlock (Tsuga canadensis (L.) Carr.), and sugar maple (Acer saccharum Marsh.) water stress has been shown to induce xylem embolism (Tyree and Dixon 1986). In that study, a 50% reduction in conductance due to embolisms occurred at xylem water potentials of -4.1, -3.4, and -3.1 MPa for maple, cedar, and hemlock, respectively. Thus, disturbance of the severity encountered during seedling lifting, handling, and planting is

likely to result in some amount of cavitation. Cavitation may explain the observation by Chung and Kramer (1975) that suberized roots of loblolly pine seedlings that were lifted and stored under operational conditions were only about one-half as permeable as undisturbed suberized roots maintained in aerated Hoagland's solution.

Sutton (1980) emphasized the importance of reestablishing intimate contact between the transplanted root system and the soil through renewed root growth. The root growth response of transplanted seedlings entails two separate processes, elongation of undamaged root tips, and initiation and elongation of adventitious roots (Ritchie and Dunlap 1980). Root initiation is a complex process that results from an interaction among the major classes of plant hormones and the physical and chemical environments of the whole plant (Torrey 1986). Likewise, root elongation depends on a number of factors, notably the availability of assimilates and a favorable growing environment. Two key elements of the environment are temperature and water.

Both initiation and elongation of roots are affected by temperature. Above 10°C, elongation of radiata pine roots was much more sensitive to temperature than was initiation (Nambiar et al. 1979). In red pine (P. resinosa Ait.) elongation was also more sensitive to temperature than was initiation (Andersen et al. 1986). The total number of new root tips 27 days after transplanting did not differ significantly over a range of root zone temperatures between 8 and 20°C, while new root length had significant increases between 12 and 16°C, and again between 16 and 20°C. In shortleaf pine, the number of new roots greater than 1 cm long after 28 days at

constant root zone temperatures of 10, 15, or 20°C increased linearly with temperature; the number of roots averaged 0.6, 6.0, and 11.7, respectively (Brissette and Carlson 1987).

The effects of soil moisture on the new root growth response of transplanted seedlings has not been investigated as much as the effect of temperature. Larson and Whitmore (1970) transplanted red oak (Quercus rubra L.) seedlings into vermiculite and controlled the osmotic potential of the medium with polyethylene glycol. After 6 weeks, seedlings from the -0.03 MPa treatment had an average of 4.5 new roots, those at -0.4 MPa averaged 0.9 new roots, and the seedlings at -0.6 MPa did not produce any new roots. However, the authors found that actively growing roots subjected to -0.6 MPa conditions continued to grow at a reduced rate for several weeks. Ritchie and Dunlap (1980) cited unpublished data showing that loblolly pine seedlings produced new roots at initial soil water potentials as low as -1.3 MPa.

Reduced root growth under conditions of either low temperature or low water potential is at least partially a result of water stress. Cell enlargement is curtailed by even mild water stress (Hsiao 1973). Cell growth occurs when the cell wall is elastic enough to yield to turgor pressure, thus allowing cell enlargement. Cosgrove (1986) reviewed numerous studies that support the hypothesis that cell wall yielding depends on turgor pressure in excess of a critical level, the yield threshold. Based on the limited data available, he speculated that the yield threshold for plant tissues lies in the range of 0.2 to 0.4 MPa. For the current study, the implication is that if transplanted roots are to resume growth, they must absorb sufficient water to maintain turgor

above the yield threshold, and supply the transpirational demand of the shoot.

Interpolating data presented by Pallardy et al. (1982) about tissue water relations for shortleaf pine seedlings suggests that the yield threshold for roots is at a relative water content (RWC) of about 65%. Relative water content is defined by Kramer (1983) as

(1.1) RWC = fresh weight - ODW / turgid weight - ODW

where ODW is ovendry weight.

An often used measure of a seedling's capacity for root initiation and elongation is root growth potential (RGP). To measure RGP, seedlings are put into a controlled environment, usually favorable for root growth, for a period of time, typically 7 to 28 days. At the end of the test period new root development is measured. RGP is often measured as the number of new roots, the length of new roots, or some index value that reflects the relative amount of new root growth. Sutton (1987) stated that the ideal measure of RGP might be the increase in root surface area during the test, or the rate of increase, but noted the difficulty in determining root surface area. The use of RGP to evaluate seedling vigor or physiological quality was first proposed by Stone (1955). He hypothesized that RGP measures the potential for new root growth after outplanting, and hence, the ability to tolerate subsequent drought. Because RGP is correlated with the bud dormancy cycle it varies seasonally, and therefore can change before conditions permit new root growth (Ritchie and Dunlap 1980). Although in many climates soil temperatures during the planting season severely limit root growth for

weeks or even months, RGP is often a good predictor of field survival and sometimes growth (Feret and Kreh 1985, Ritchie 1985). Therefore, Ritchie (1985) proposed the working hypothesis that RGP is related to field performance because it is correlated with cold hardiness and other forms of stress resistance, not necessarily with the ability to extract water from the soil.

Burdett (1987) found only circumstantial evidence to support either Stone's (1955) or Ritchie's (1985) hypothesis. He concluded that either might be correct, depending on the species and the planting site conditions. He stressed, however, that the distinction between the interpretations is important. If RGP is related to water uptake after planting, then defining the relationship among seedling attributes, the planting environment, and root function will provide valuable insights for improving reforestation success. But, if RGP predicts performance because it measures seedling health or vigor, direct tests of the appropriate physiological functions will provide more exact methods of assessing stock quality.

Clearly, transplanted seedlings have at least two major disadvantages in water uptake when compared to established seedlings of similar shoot size. First, for a period of weeks and perhaps months, they have less total root and mycorrhizae surface area for water absorption. Secondly, the root system they do have is less permeable. A third, possibly critical factor, is even mild stress may lower their root cell turgor pressure below the yield threshold and prevent or retard new root growth.

Root system absorptive capacity (L_R) is a measure of the ability to absorb water and depends on the root system permeability (L_p) and root surface area (A_R) (Kramer 1983), such that

(1.2) $$L_R = L_p \times A_R$$

where L_R is measured as amount of water absorbed per unit time per unit of pressure applied; for example, L_R in this research is reported in mmol s^{-1} MPa^{-1}. Root system permeability (L_p) is L_R divided by the surface area of the root system. Consequently, L_p will be expressed as mmol m^{-2} s^{-1} MPa^{-1}, when total A_R is estimated in m^2.

As equation 1.2 indicates, L_R changes when either L_p of A_R change. Moreover, neither root system permeability nor surface area are constant. Because unsuberized roots are more permeable than suberized roots, permeability will increase dramatically with a small increase in surface area due to new root growth. When growth stops and the new roots become suberized, permeability will decline. Absorptive capacity follows the same pattern; however, because of the increase in A_R, it will not decline to the rate prior to new root production. If resistance to water movement in the soil and across the root-soil interface increases, such as happens when soils dry to less than field capacity, total water uptake may be less than absorptive capacity.

Water transport through roots is driven by osmotic and pressure forces that interact to affect the flow rate (Boyer 1985). As mass flow of water increases, the concentration of solutes in the xylem becomes diluted until it has a negligible effect on water flux in rapidly transpiring plants. In experimental systems, external pressures in the

range of 0.2 to 0.5 MPa are usually great enough to induce flow rates similar to rapidly transpiring plants, thus minimizing the effect of the osmotic force (Boyer 1985). Therefore, the relationship between the applied pressure and the flux of water from the roots is typically linear at high flow rates but is curved at low flow rates (Passioura 1988). The pressure at which the flow rate becomes linear can depend on a number of factors; including the species, age and condition of the plants studied, and the experimental conditions. In a study of loblolly pine seedlings, Sands et al. (1982) found in an initial trial that the flow rate became linear at pressures greater than 0.3 MPa. Subsequently they determined absorptive capacity from the slope defined by the steady state flow rates at 0.533 and 0.667 MPa. In a study with black spruce (Picea mariana Mill.) seedlings, Colombo and Asselstine (1989) found that water flux became linear with pressures above 0.5 MPa.

Plant hydraulic conductivity (G_p) is a measure of the rate of water movement through the soil-plant system (Passioura 1982), such that

$$G_p = q / \psi_s - \psi_l \tag{1.3}$$

where q is the water flux per unit leaf area, ψ_s is the effective soil water potential in the major part of the rooting zone, and ψ_l is the average leaf water potential. Thus, G_p can have the same units as L_p, namely mmol m^{-2} s^{-1} MPa^{-1}. The effective soil water potential in the rooting zone is often assumed to equal the average predawn leaf water potential (ψ_{pd}) (Slatyer 1967).

Because G_p can be measured in the same units as L_p, the ratio between the rates of water flux from the leaves and the rate of water uptake by

the roots on an unit area basis can be calculated. If the transpirational surface area is known then the total plant water uptake (G_T) can be determined. Therefore, G_T and L_R can be compared. These ratios, L_R/G_P and L_R/G_T, should vary with the morphological and physiological attributes of shoots and roots, and with the shoot and root environments. Thus they can provide insights into how well root systems can supply transpirational demands under a variety of seedling and environmental conditions.

The capacity for water uptake can also be evaluated indirectly by measuring various aspects of seedling water status. Nambiar et al. (1979) found a significant, positive relationship between midday needle water potential (ψ_n) and new root elongation in radiata pine. They concluded that measuring ψ_n was useful for assessing new root growth of outplanted seedlings. Orlander and Rosvall-Ahnebrink (1987) used G_P, and Grossnickle (1988) used the inverse of G_P, water-flow resistance, to evaluate water uptake by transplanted conifer seedlings. The recovery rate of ψ_n as it returns towards its ψ_{pd} level after stomatal closure should also provide a meaningful measure of new root growth. The faster ψ_n approaches its predawn level, the greater one might expect new root growth to be, either in absolute amount or as the proportion of the root system that is new. Furthermore, one would expect xylem water potential recovery to be positively related to absorptive capacity and permeability also.

Assessing new root growth using indirect methods is valuable because the measurements can then be made nondestructively using commercially available instruments. What is not known, however, is how much new root development is required to make a substantial improvement in any of the attributes of water relations that have been discussed. Therefore, first

it is important to know how much root growth can be expected during the early, critical weeks after outplanting into typical field environments. Then it also becomes important to understand how the amount of root growth affects the degree of improvement in water uptake and water relations and, thereby, seedling establishment.

To meet the objectives of this research, several experiments were conducted. The first objective was to describe the effects of the root zone environment on new root growth during the first few weeks after outplanting. An experiment was designed to measure the interactive and main effects of root zone temperature and soil water potential on root development. It required designing and constructing a system of root environment chambers to control both temperature and water availability in the root zone. The experiment was repeated three times over the course of a planting season. In each repetition the following hypothesis was tested: Root zone temperature and soil water potential interact to determine the amount of root growth that occurs after transplanting. When the interaction was significant the next step was to describe how the two environmental factors interacted. This phase of the research had two secondary objectives. One was to determine the minimum level of root tissue hydration, measured by relative water content, required for new root development. The other was to determine if the experimental treatments had an effect on the surface area of the roots that were present when the seedlings were planted.

The second major objective was to determine the relative importance of old and new roots to the capacity of a root system to absorb water. The experiment designed to evaluate absorptive capacity was also repeated

three times, using the seedlings grown in the root environment chambers. Measurement of absorptive capacity required designing and constructing an apparatus, and developing the methods needed to collect all the water that flowed through seedling root systems over a measured time interval. It also required characterizing the amount of both old and new roots. For that purpose, the projected surface area of the roots was measured. The projected surface areas of the old and new roots were then used as predictor variables to model root system absorptive capacity and permeability.

The last major objective was to determine to what extent the amount of new root growth affected seedling water status. Attributes which describe plant water relations, such as needle water potential and stomatal conductance, were measured on the seedlings in the root environment chambers 4 weeks after transplanting. Again, these measurements were made on seedlings in all three repetitions of the root growth experiment. The measurements were made using commercially available instruments and standard procedures. The next day the projected surface areas of the old and new roots were measured. The projected root surface areas were then used to model several water relations response variables. Besides measures of water status, specific leaf area (SLA), a morphological attribute that is affected by root growth and plant water relations, was also assessed. A secondary objective in this portion of the research was to examine the relationship between the degree of seedling water stress and stomatal conductance.

These experiments were conducted under controlled environmental conditions. Plants were grown under controlled conditions so that the

physiological processes could be examined in greater detail than is normally possible in the field. Under typical field conditions so many factors are interacting that it is difficult to accurately separate plant responses to a single, or to a particular set of environmental parameters.

CHAPTER 2

MATERIALS AND METHODS

Plant Material

Seedlings for this research were grown from a single half-sib family lot collected at the United States Department of Agriculture, Forest Service (USDA-FS) Ouachita-Ozark Seed Orchard near Mount Ida, Arkansas. Several families were sown in the nursery to allow for selection of the family with the most uniform seedlings at the time of lifting.

The seed orchard is organized by geographic sources. Separate blocks in the orchard consist of selections from the Ozark National Forest and the east and west sides of the Ouachita National Forest. The geographic source of a family can be determined from the identification number; the 100s are east Ouachita, the 200s are west Ouachita, and the 300s are from the Ozark. However, for operational reforestation, the seeds produced in the orchard are bulked into one seedlot and used on both national forests. Seedlings from the bulked seedlot exhibit a lot of variation in size. Therefore, half-sib family seed collections were made in the orchard to reduce the variation among seedlings used in this and other research into problems of shortleaf pine artificial regeneration.

Because no growth data were available when the families were selected, seed orchard clones were chosen on the basis of the survival of their progeny in full-sib tests planted on both forests. To be

considered, a clone had to be the female parent in at least three tests. Progeny survival was compared to the average survival of each test. Thus, families with better than average survival in a test had relative survivals of greater than 100, and those with below average survival had relative survivals of less than 100. The overall relative survival for progeny of the same female was the selection criterion.

In 1985, seeds were collected from several ramets of clones with an average relative survival greater than 100 and from clones with an average less than 100. Seed processing was done at the USDA-FS Southern Forest Experiment Station laboratory in Pineville, Louisiana. The seeds were stored in sealed plastic containers in a freezer until they were needed.

Four of the selected families were sown for this research. Families 115, 219, and 322 were selected as relatively good survivors. Family 342 was selected as a relatively poor survivor. Family 322 was selected for this research at the first lift date because it had the most uniform appearing seedlings. The counties where the ortets (the original trees from which the seed orchard clones were derived) were selected and a comparison of some seedling attributes of the families sown for this research are presented in Table 1.

The seeds were sown in mid-April, 1988, at Weyerhaeuser Company's Fort Towson Forest Regeneration Center in southeastern Oklahoma. Seeds were sown to produce approximately 20,000 seedlings of each family. Many of the seedlings were destined for use in other research; however, the large population size insured that a representative sample could be selected for this study. The seeds were sown with a Weyerhaeuser-designed precision vacuum seeder. The machine sows 8 drills with approximately

Table 1. County of origin of the half-sib families of shortleaf pine grown for this research, and some seedling attributes from the 1986 crop

Family	County[1/]	Relative Survival[2/] (%)	Shoot Length (mm)	Root Collar Diameter (mm)	Root Volume (mm^3)
115	Polk	103	294	5.4	3400
219	Scott	109	202	3.8	1700
322	Pope	108	283	5.1	3000
342	Yell	88	202	4.0	1700

[1/] All counties are in Arkansas.

[2/] Compared to the mean survival in several progeny tests.

5 cm between a double row of seeds that make up each drill and about 15 cm between drills. The two outside drills on both edges of the 1.3-m wide nursery beds were planted with a seed orchard mix. The selected families were assigned at random to the interior drills and re-randomized for each of six blocks. Each block was 15.2 m long.

Germination tests were not conducted on the seedlots. Therefore, a germination rate of 90% was assumed, and the seeds were sown to achieve a density of about 220 seedlings per square meter of nursery bed space. Nursery cultural practices, such as fertilization and root pruning, were applied based on the best judgment of the nursery manager. Top pruning was not done.

At least 1,000 seedlings of Family 322 were carefully hand lifted for the three repetitions of this research on December 5, 1988, January 17, 1989, and February 27, 1989. The chilling hours (0-8°C at 20 cm above the soil) that had accumulated by the morning of those lift dates were 301, 715, and 1077, respectively. The seedling root systems were thoroughly wetted at the nursery, then packed in kraft-polyethylene (K-P) bags. They were kept in cold storage (about 3°C) for either 7 or 9 days before they were used in the experiments.

Before they were put in cold storage, the seedlings were root pruned to a maximum root length of 150 mm. The root pruning was needed to trim any long lateral roots to the length at which most taproots were undercut in the nursery. After pruning, root system morphology was measured as projected surface area. The complex surface of a root system is very difficult and time consuming to measure precisely, but the projected surface area can be readily estimated using a photoelectronic image

analyzer (Decagon Devices, Inc., Pullman, WA). The estimated projected surface area of a seedling root system has been termed its root area index (RAI) (Morrison and Armson 1968). In this research, RAI was used to nondestructively quantify root system morphology before the experiments began and, at the end of the experiments, to separate the root system into new roots, old lateral roots, and old taproot.

The image analyzer uses a high-resolution video camera mounted vertically over a light table upon which the object to be measured is laid. The image produced by the camera is shown on a video monitor. An area meter electronically counts the number of video lines on the monitor that are crossed by the image of the object. It then converts the number of intersections to an arbitrary numerical value which can be calibrated to projected surface area. Video-imaging is an accurate method of quantifying both root systems (Burke and LeBlanc 1988, Harris and Campbell 1989, Lebowitz 1988, Rietveld 1989b), and leaf surface area (Diebolt and Mudge 1988).

Accurate measurement of projected surface area required careful calibration. Besides being of known projected surface area, the calibration targets also had to be of similar size and shape to the objects that were measured. However, because of extensive overlap among roots, comparing intact root systems can provide only relative differences among seedlings. Therefore, a single calibration target with 2,670 mm^2 was used to set the area meter component of the image analyzer for whole root systems. Different calibration targets, which are described later, were used when measuring root system components at the end of the experiments.

To reduce the variation in root system size, seedlings were selected on the basis of initial root area index (IRAI). First, the IRAI of 100 root pruned seedlings from each lift date was nondestructively measured. Subsequently, seedlings were selected for the experiments only if their IRAI was within $\pm$ 1.0 standard deviation of the sample mean. To minimize the effect of the variation caused by overlapping lateral roots on IRAI, the IRAI of each seedling was recorded as the mean measurement of three different images of the root system.

Besides the IRAI, other measurements of morphology taken at the start of each repetition of the experiment were: shoot length (seedling height), root collar diameter, and whether an overwintering bud was present. A terminal bud was considered overwintering if it had brown bud scales. Shoot length was measured to the nearest 1 mm with a rule, and root collar diameter to the nearest 0.1 mm with a micrometer.

Among the three lift dates there were differences in mean morphological attributes (Table 2). The differences in mean seedling height were very small and most likely due to sampling because there was no new height growth observed in the nursery between early-December and late-February. Also, there was no trend to the differences in height, seedlings lifted in January were tallest. There was a definite trend in root collar diameter, with the mean increasing as the lifting season progressed. Continued diameter growth of southern pine nursery stock is well documented (Huberman 1940, Zimmermann and Brown 1971). The seedlings lifted in late-February had a much greater mean IRAI than the earlier-lifted stock. No root elongation was observed when the seedlings were lifted in February. Therefore, the larger root system size was because

Table 2. Seedling morphological attributes after lifting on 3 dates

Lift Date	N	Shoot Length (mm)	Root Collar Diameter (mm)	Root Area Index (mm^2)	Bud Present (%)
5 Dec 88	126	307b[1/]	4.6c	7390b	72.2a
17 Jan 89	126	315a	4.7b	7330b	77.8a
27 Feb 89	126	309ab	5.1a	9030a	77.8a
Mean	378	310	4.8	7920	75.9

Note: The F-test$_{(2;375)}$ for each attribute had a critical value= 3.00 at p= 0.05. The MSEs were: shoot length, 740.817; root collar diameter, 0.239; root area index, 82.721; presence of bud, 0.184.

[1/] Lift date means for a given attribute followed by the same letter are not significantly different at the 0.05 level.

of either sampling error or secondary growth that occurred after mid-January. Secondary root growth was more likely because of the continued diameter growth that was measured.

Environmental Controls

The environment in the growth chamber was set for a constant air temperature of 20°C with a 14 hour light period and 10 hour dark period. The growth chamber did not control relative humidity but the floor was kept flooded so that evaporating water would moderate fluctuations in relative humidity. The conditions in the growth chamber were monitored electronically with a data logging and recording system (Easy Logger Model EL-824, Omnidata International, Inc., Logan, UT). Air temperature, relative humidity, and photosynthetically active radiation (PAR) were measured at seedling height near the center of the chamber. The sensors were scanned on the hour and half-hour and the means recorded hourly. There were not enough sensors available to adequately monitor root zone temperature. However, it was checked daily at several locations with mercury thermometers.

During the first repetition of the experiment, air temperature varied less than 1°C, and relative humidity ranged between 65% and 90% with an average of about 75%. About midway through the second repetition, the air temperature rose to a maximum of 25.3°C over a 4 hour period, and dropped to a low of 18.2°C at night. The relative humidity was greater than 60% throughout that repetition of the experiment, and again averaged about 75%. For a two day period during the last repetition, the air temperature ranged between 18.2°C and 27.6°C because a malfunctioning

compressor switch made only intermittent temperature control possible. During this period, the relative humidity dropped to a low of 39% for 2 hours and was below 60% for 31 hours; it was greater than 60%, and again averaged about 75%, throughout the rest of the experiment. No marked changes in root zone temperature were observed. Between the three repetitions, new light bulbs were installed as needed to keep the PAR at the top of the seedling crowns above 750 μmol m^{-2} s^{-1}.

The growth chamber had approximately 1.22 m x 2.44 m of bench space. Two root zone temperature control water baths were constructed from 19 mm exterior plywood, each approximately 1.09 m x 1.14 m x 0.45 m deep. The baths were finished with a marine paint and the seams were sealed with silicone caulk. The two root zone temperatures used were 15°C and 20°C. The 20°C temperature was selected because it is often used for testing root growth potential (RGP). The 20°C water bath was maintained by the ambient conditions of the growth chamber. The 15°C temperature was selected based on the results of Brissette and Carlson (1987) who studied the effects of root zone temperature on new root growth of bare-root shortleaf pine seedlings. They showed that, at 15°C, about one-half the number of new roots greater than 10 mm long were present after 28 days when compared to 20°C. The 15°C bath was maintained by circulating water between the bath and a reservoir where the water was chilled enough to keep the root zone at the desired temperature. During all three repetitions of the experiment, the root zone temperatures were within $\pm$ 0.5°C of the target in both water baths.

The soil water potential in the root zone was controlled by a system that maintained the plants' growing medium at a constant height above a

water column. Moinat (1943) first described such a system for plant research. More recently, Snow and Tingey (1985) described a highly modified version of the method. For this research, the system described by Snow and Tingey (1985) was further modified, including the addition of root zone temperature control. There were 63 root environment chambers constructed for each water bath. The chambers consisted of an outer sleeve to isolate the seedling roots from the water baths, a seedling pot with sand growing medium, a column with uniform hydraulic conductivity, and a supply of irrigation water (Figure 1).

The root environment chambers were made from sections of Polyvinyl Chloride (PVC) pipe. These sections were about 450 mm long with a nominal inside diameter of 101.6 mm. A flat-bottomed PVC pipe cap was glued to each section. Water was supplied by 6.35 mm inside diameter plastic tubing between irrigation water reservoirs and a connector installed near the base of each section of pipe. The root environment chambers were buoyant in the water baths. Therefore, to hold them in place, holes were drilled in the capped ends and they were attached to the bottom of the water baths with brass screws with rubber washers. A glob of silicone caulk was also put between the floor of the water bath and the bottom of each section of pipe to ensure a good seal around the screw. The depth of the water in the temperature control water baths was kept 10-20 mm below the top of the PVC pipe sections. Thus, the root environment chambers were isolated from the water in the water baths.

The pots into which the seedlings were planted were made from 200 mm long sections of PVC pipe that, like the outer sleeves of the root environment chambers, also had a nominal diameter of 101.6 mm. Therefore,

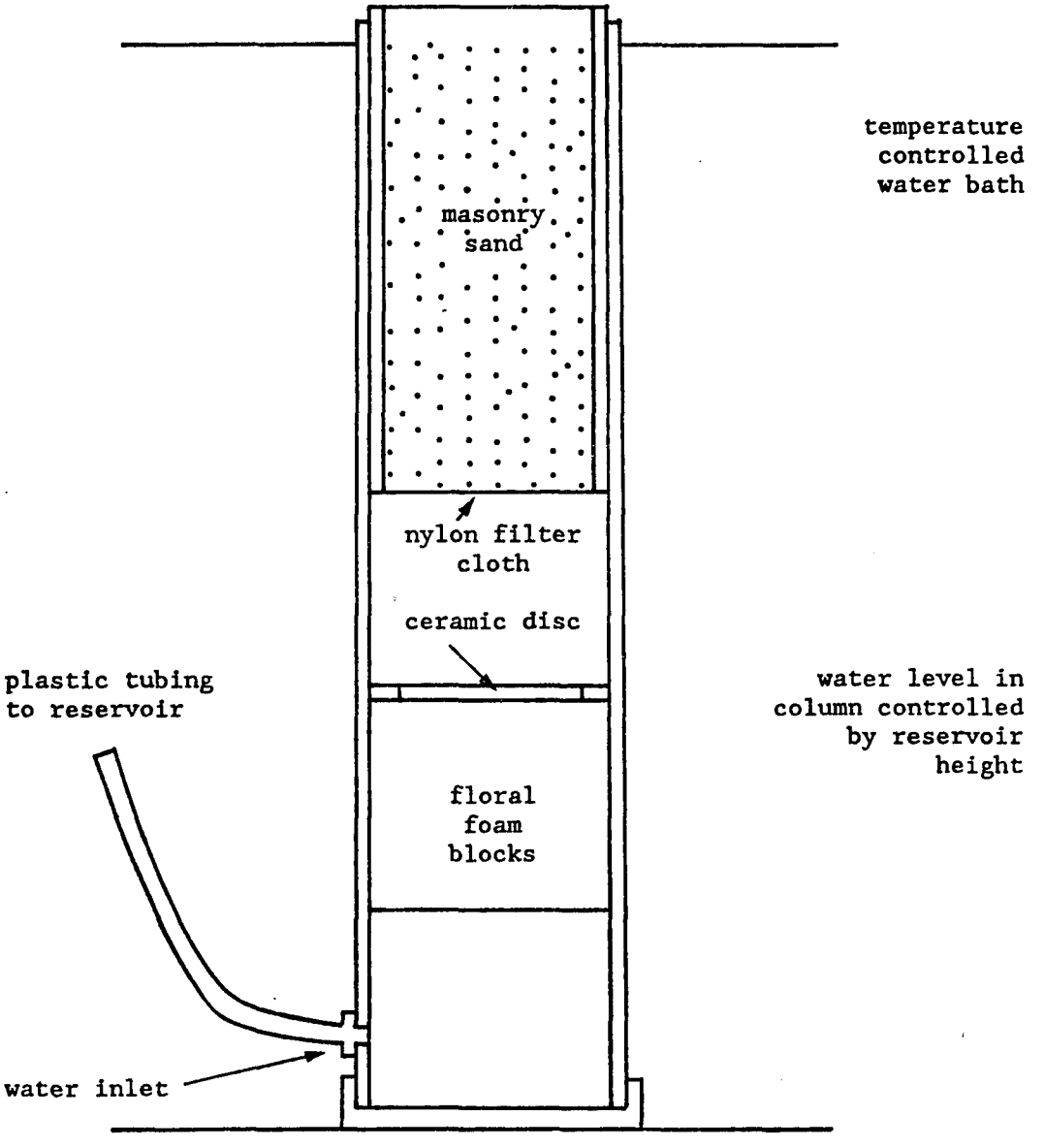

Figure 1. Diagram of one of the 126 root environment control chambers.

a lengthwise segment was cut out of the seedling pots to reduce the diameter so that they would fit snugly into the outer sleeves. The seedling pots were then sealed back together with duct tape. Root growth downward to the water source was prevented by nylon filter cloth (Tetko, Inc., Elmsford, NY) glued and taped to the bottom of the seedling pots. The cloth suggested by Snow and Tingey (1985), and which was used in this research, had a 20 μm weave.

Masonry sand was used as the growing medium because of its uniform physical and chemical properties, and because it has been used extensively for RGP research. When the sand was delivered it was first air dried to a uniform moisture content. Gravimetric samples were taken of the sand at various moisture contents as it dried. We determined that the desired moisture content for potting the seedlings was about 2% on a dry weight basis, and the amount of water needed to bring the sand to that moisture content was calculated. After the sand was wet to approximately 2%, it was stored in sealed plastic bags until used in the experiments. Thus, all the treatments in each of the repetition of the experiment started with sand at the same initial moisture content.

Three levels of soil water potential were compared in this research; one was a well-watered control and the other two were at less than field capacity. The water potential of the sand was controlled by adjusting the height of the water column and the hydraulic conductivity of the medium in the column. The level of the water columns was the same as the level of water in the respective reservoirs that supplied the irrigation water. The reservoirs were checked daily and tap water added as needed to maintain the level within 5 mm of that desired. The hydraulic

conductivity of the medium in the column depends on the material used. Following Snow and Tingey (1985), this research used a series of highly absorbent commercial floral foam blocks (No. 6 cylinders; Smithers-Oasis, Kent, OH). The blocks were approximately 100 mm in diameter and 80 mm tall. Before the floral blocks were used, they were thoroughly rinsed under a garden hose, even after they became saturated, to remove dust-like particles of the foam. Snow and Tingey (1985) found rinsing was necessary to remove the soluble material, which presumably could inhibit water flow through the pores of the blocks. After washing, the blocks were allowed to air dry before they were used in the root environment chambers.

The well-watered control treatment was similar to RGP test conditions when sand is used as the medium. The water level was maintained in the topmost block; about 40 mm below the filter cloth, or about 80 mm from the bottom of the planted root systems. There were two water stress levels, Level 1 was to be considered "moderate," and Level 2 "severe." The water level for both treatments was in the bottom block about 200 mm below the filter cloth, or about 240 mm from the bottom of the planted root systems. The difference between these two treatment levels was the placement of a ceramic disk of uniform hydraulic conductivity (Soil Moisture Equipment Corp., Santa Barbara, CA) between the top and middle blocks of the Level 2 stress treatment, which altered the conductivity of those columns. The disks were 7 mm thick and 80 mm in diameter. Their conductivity was 2 $cm^3\ h^{-1}\ cm^{-2}$ at 0.1 MPa.

Water was supplied to the roots by capillarity, first through the foam blocks (and ceramic disk if present), then across the nylon filter cloth, and through the sand. The locations of the 21 pots in each of the

three soil water potential treatments were assigned completely at random within each temperature treatment when the root environment chambers were secured in the water baths.

Water levels in the root environment chambers were initially chosen based on work reported by Snow and Tingey (1985) and Faiz and Weatherley (1978). The levels were then checked for applicability to this research in a preliminary experiment. Snow and Tingey (1985) used a commercial peat-vermiculite rooting medium in their study. With three stress levels they obtained a range of midday leaf water potentials for sunflower (Helianthus annus L.) plants after 21 days of -0.1 $\pm$ 0.02, -0.73 $\pm$ 0.04, and -2.35 $\pm$ 0.17 MPa. These levels corresponded to distances between the filter cloth and the water of 80 mm and 200 mm, without and with a ceramic disk between the two upper blocks. Faiz and Weatherley (1978) describe a similar system for imposing water stress except they used a sand column rather than foam blocks. They had soil water potentials of -0.23 and -0.32 MPa in soil and sand, respectively, when the nylon gauze separating the rooting medium from the sand column was 270 mm above the water level.

The preliminary experiment was started on September 28, 1988, using container seedlings. The three levels of stress resulted in mean water potentials of -0.63, -1.41, and -1.84 MPa for the Control, Level 1, and Level 2 stresses, respectively. The differences were all significant ($p=0.05$). The mean moisture contents for the stress levels were 14.50%, 2.13%, and 1.69% for the Control, Level 1, and Level 2, respectively. The moisture content of the sand in the control treatment was significantly different from the stress treatments. However, Level 1 and Level 2 stresses did not differ. Based on these preliminary results and the

previous cited research, the three stress levels used in the preliminary trial were used when the actual experiments began.

However, because of excessive mortality, some changes in the water levels within the columns were made after the first and second repetitions of the experiment. After the first repetition, it was discovered that several of the root environment chambers malfunctioned. During insertion of the foam blocks, air was forced into some of the supply tubes, thus preventing water flow to those chambers. Corrections were made and the system functioned well during the second repetition. However, after the second repetition it was obvious that for bare-root seedlings there was not enough difference between Level 1 and Level 2 stress, and that both levels were too severe. Therefore, before the last repetition, the water level for the stressed treatments was raised 80 mm to bring the height of the water level up to the center of the second foam block.

To facilitate the various measurements of water status, RGP, and absorptive capacity, the start of each experiment was split over two days. For each temperature-stress combination there were 21 root environment chambers. Therefore, on the first day 11 seedlings of each treatment combination were started and on the second day the other 10 were put into the experiment. The first repetition was started on December 12 and 14, 1988. The second repetition began on January 24 and 26, 1989. The last repetition started on March 6 and 8, 1989. Measurements of needle water potential and stomatal conductance were made 28 days after the seedlings were put into the growth chamber. Measurements of RGP and absorptive capacity were made 29 days after the experiments began.

Measurement of Root Growth

New roots are easily distinguished from old roots by color and surface texture. Old roots are dark brown and typically have a rough surface. The distal portions of new roots will generally be pearly white, although closer to the old root they may grade to tan and then brown as they become suberized. The point of transition is usually easily distinguished by a fairly abrupt change in color and surface texture. Because new roots are somewhat translucent, their image is not distinct on the monitor of the image analyzer and, therefore, not accurately measured by the area meter. Consequently, new roots were separated from the root system and dipped in Paragon multiple stain (7.3 g toluidine blue + 6.8 g basic fuchsin in 1 L 30% ethanol) before their projected surface area was measured (Rietveld 1989b).

The occasional new growth that originated from the taproot was kept separate from new lateral root development. Also, to facilitate accurate measurement of the root system, the old lateral roots were cut from the taproot so that they could be spread out on the light table without overlap. Then a measurement was obtained of the total projected surface area of the laterals, the taproot, and new roots from both sources.

For the new roots and the old lateral roots the calibration targets were 10 pieces of copper or steel wire of various lengths and diameters that were similar in size to actual roots. They ranged in diameter from 0.635 mm to 1.70 mm, and in length from 44 mm to 96 mm; the total projected surface area was 1,012 mm^2. The resolution of the image analyzer was the nearest 10 mm^2; therefore, the area meter was set to a projected surface area of 1,010 mm^2. The calibration target for the taproots was a

single piece of steel rod 6.23 mm in diameter and 102 mm long. Its actual projected surface area was 635 mm^2, so the area meter was set to 640 mm^2.

Even with the root system separated into its component parts, projected surface area measurement is at best an index of the actual absorbing surface area. Therefore, for this research, surface area measurements of new root growth were termed "new root area index" (NRAI), and was the total of both new lateral root and taproot development. The projected surface areas of the components of the root system that were planted were termed "old lateral root area index" (OLRAI), and "old taproot area index" (OTRAI). The sum of OLRAI and OTRAI was the "old root area index" (ORAI). The sum of NRAI + OLRAI + OTRAI was the "total root area index" (TOTRAI). For determining absorptive surface area, roots are usually considered cylindrical in shape (Sutton and Tinus 1983). Therefore, the absorptive surface area of any of the root system components was estimated by multiplying the appropriate RAI by π.

Measurement of Root Function

Measuring absorptive capacity requires a method of collecting all the water that flows through a root system. One technique is to confine a detopped root system, force water through it under pressure, and collect the water as it exudes from the cut stem. A vessel was manufactured locally (Hayes Manufacturing Co., Pineville, LA) for sealing seedling root systems under hydrostatic pressure so that as water came through it could be collected. The vessel was made with 12.7 mm thick aluminum and was cylindrical, 315 mm in diameter. The bottom was slightly concave, making

the vessel 255 mm deep at the sides and 275 mm deep in the center. The water holding capacity of the vessel was approximately 20,500 cm^3.

The top was designed to accommodate eight seedlings. The ports in the top consisted of a 6.35 mm holes through the lid centered in recesses that were 25.4 mm in diameter and 9.525 mm deep. The top was held in place by eight, 9.525 mm bolts with wing nuts. A rubber "O" ring between the body of the vessel and the top ensured a water-tight seal.
A valve was fitted through the top of the vessel so that trapped air could be removed during pressurization. A gauge on the side of the vessel was used to monitor the internal pressure.

Tap water was used in the system and was circulated through a temperature controlled reservoir so that the water and root systems in the vessel were kept at 20°C $\pm$ 0.5°. Water was pumped from the reservoir by a rotary gear water pump (Teel Industrial Series Model 1P783, Dayton Electric Manufacturing Co., Chicago, IL) driven at 1725 rpm by an electric motor and delivering approximately 190 $cm^3\ s^{-1}$ at a maximum of 690 kPa. Based on the operation of similar systems (Carlson 1986, Johnsen et al. 1988, Oosterhuis and Wullschleger 1987, Ramos and Kaufmann 1979, Sands et al. 1982, Smit and Stachowiak 1988), the pressure desired when using the vessel was 300 kPa; therefore, controls were used to reduce the pressure from the pump. A pressure relief valve (Model M3, Watts Regulator Co., Franklin NH) rated at 552 kPa was installed in the line after the pump with its discharge going back into the reservoir. It was followed by an in-line valve to further reduce the pressure and provide a means of regulating the flow. Water diverted from the valve also went back into the reservoir. A water flow regulator (Model D36, Spence Engineering

Inc., Walden, NY) that could be adjusted for any pressure between 172 kPa and 517 kPa provided a final, fine adjustment of pressure before the water entered the vessel. At 300 kPa, the flow rate through the vessel was approximately 240 cm^3 s^{-1}; consequently, the water in the vessel was exchanged about every 85 seconds. The system resulted in very stable pressure within the vessel. While measuring absorptive capacity in these experiments the hydrostatic pressure was 300 kPa $\pm$ 0.5 kPa.

When water left the vessel it flowed through a filter to remove any soil or organic debris that may have been washed from the root systems. As water returned to the reservoir it fell through air for approximately 100 mm which provided agitation and ensured an adequate supply of oxygen in the water pumped to the vessel. Water from both the pressure relief valve and the in-line valve also helped provide agitation and oxygenation. The oxygen content of the water was 93.4% that of the air above the reservoir.

To use the apparatus, a seedling shoot was severed about 25 mm above the topmost lateral root and the remaining stem was inserted through a rubber stopper. The stopper was then seated in one of the recesses in the vessel's top. The cut stem protruded through the top and the intact root system was suspended in water inside the vessel. With the top secured, water was allowed to flow through the vessel with the valve in the top open. Once all the air was removed from the vessel, the valve was closed. With that valve closed, the hydrostatic pressure was stabilized at 300 kPa, and water was forced through the root system, out the cut stem, and was collected in wicks. The wicks for water collection were

constructed from pieces of plastic tubing, approximately 60 mm long and 6.35 mm inside diameter, filled with absorbent tissue paper.

In an initial trial with eight seedlings a constant rate of flow was reached and maintained after no more than 10 minutes. A 15 minute equilibration period was used in the experiments. After the equilibration period, water was collected four times at approximately 5 minute intervals; the actual time was recorded to the nearest second. The exuded water was collected in the wicks which were pre-weighed within 5 minutes of use. The weight of the wick and water was measured to the nearest 1 mg. These measurements were made in a laboratory at room temperature; therefore, 1 g of water was assumed to be 1 cm^3, and 1 mg equal to 0.0556 mmol.

Absorptive capacity was measured the day following the needle water potential and stomatal conductance measurements. Root system permeability of a seedling was calculated by entering its absorptive capacity and estimated total root surface area into equation 1.2.

Measurements of Water Relations

At 28 days after seedlings were put into the growth chamber, before they were disturbed for root growth or absorptive capacity measurements, several attributes of water status were measured. Needle xylem pressure potential, stomatal conductance, and water flux were each measured several times. Needle xylem pressure potential was assumed to equal the water potential of the cells (Kramer 1983). Eight seedlings in each treatment combination were chosen at random for these measurements. The order in which seedlings were evaluated was determined by random selection and was

followed for all subsequent measurements taken during that repetition of the experiment. The first xylem pressure potential measurement was before the lights of the growth chamber came on. That is, it was predawn and, therefore, assumed to equal the average soil water potential in the rooting zone. Needle xylem pressure potential was also measured beginning approximately 2 hours, and again at about 4.5 hours, after the lights came on in the growth chamber. After all the seedlings were measured twice in the light, the photoperiod was interrupted. A final xylem pressure potential measurement was then taken starting after approximately 2 hours of darkness. That measurement was used to estimate the rate at which needle water potential was recovering towards its predawn value. After all the seedlings were measured the lights were turned on to return to the programed photoperiod.

To estimate needle water potential, xylem pressure potential of needle fascicles was measured with a pressure chamber (PMS Instrument Co., Corvallis, OR). Needle water potential was recorded as the negative value equal to the pressure required to force water to the cut surface of the fascicle sheath. The pressure at the time water is observed is called the balance pressure or the end point. Replicate measurements were made; in most cases two or three per seedling, depending on the uniformity of the measurements.

When very low plant water potential is measured, high pressure is required to reach the end point. Measuring low water potential on seedling fascicles was difficult. In many fascicles at low water potential the end point was hard to identify because there was relatively little water in the needles. However, the most serious problem occurred

when high pressure caused needles to break in the stopper that sealed them in the pressure chamber. When needles broke, no measurement was obtained and another fascicle had to be sampled. Such repeated sampling was destructive to the seedlings, which were needed for repeated measurements, and time consuming. Furthermore, the propensity for needles to break at high pressure tended to be a consistent characteristic within seedlings. Therefore, I decided that if the end point was not observed by the time a positive pressure of 4.00 MPa was applied, the needle water potential was to be recorded as -4.00 MPa.

Measurements of stomatal conductance (g_n) and the flux of water transpired (q) were paired with water potential measurements taken in the light. A steady state porometer (Model LI-1600M; Li-Cor, Inc., Lincoln, NE) was used to measure stomatal conductance and water flux. Model LI-1600M expresses both stomatal conductance and water flux in mmol water m^{-2} leaf surface area s^{-1}, as defined by Cowan (1977). For other Li-Cor porometers, which express stomatal conductance in cm s^{-1}, it is assumed that diffusion is the only driving force for transpiration (Li-Cor, Inc. 1987). The LI-1600M considers both diffusion and temperature to be driving forces for transpiration. Therefore, the conversion from conductance as defined by Cowan (1977) to the more common units varies with temperature; at 25°C a conductance of 1 mole m^{-2} s^{-1} is equivalent to 2.5 cm s^{-1}.

All Li-Cor porometers enclose a foliage sample in a cuvette system which includes an air mixing fan. There is a very predicable boundary layer resistance of about 0.15 s cm^{-1}, which is subtracted by porometers that calculate stomatal resistance (or conductance) in those units (Li-

Cor, Inc. 1987). The LI-1600M does not subtract the boundary layer resistance in its calculations.

If the leaves being sampled do not fill the chamber the operator must determine their surface area. If a constant sample size is used, it can be set and the porometer will use it in its calculations of conductance and water flux. The leaf chamber used in this research was 20 mm x 20 mm square and all measurements were made on the needles from two, 3-needle fascicles. Therefore, the mean total surface area sampled was determined for a 20 mm long, 6-needle sample. Total surface area was used because pine needles have stomata on all surfaces; therefore, transpiration is from the total needle surface area.

The total surface of an individual needle can be calculated from an equation by Johnson (1984):

$$SA_T = 2r + \left[\frac{2\pi r}{n}\right] L \qquad (2.1)$$

where r is the radius of the fascicle, n is the number of needles in the fascicle, and L is the length of the needle. Shortleaf pine seedlings rarely have other than three needles per fascicle. Using 3-needle fascicles from Family 322, the total surface area of needles from 142 fascicles from 18 seedlings was calculated by equation 2.1. The fascicle radius was measured to the nearest 0.0254 mm under a binocular microscope using an eyepiece micrometer. Needle length was measured to the nearest 1 mm. Fascicle diameter ranged from 0.445 mm to 0.787 mm. Needle length ranged from 39 mm to 108 mm. The mean total surface area was 504 mm^2 with a standard error (SE) of 13 mm^2. For the 20 mm x 20 mm porometer chamber,

the mean total surface area of the enclosed portion of the needles was 305 mm^2. That value was set in the porometer and used in all three repetitions of the experiment.

The projected surface areas of groups of those needles were measured on the image analyzer so that they compared with the calculated total surface areas. There were 26 groups that had 12 needles, 6 groups that had 9, and 4 that had 15 needles. To measure the projected needle surface area, 10 pieces of copper wire were used as calibration targets. They were all 0.635 mm in diameter and ranged in length from 50 mm to 87 mm; the total projected surface area was 436 mm^2 and the area meter was set to 440 mm^2. With projected needle surface area as the independent variable, there was a significant ($p=0.0001$) linear regression with total needle surface area:

$$SA_T = -0.2728 + 4.0141SA_p \tag{2.2}$$

where SA_T and SA_p are total and projected surface areas, respectively. The coefficient of determination (r^2) was 0.945.

The projected surface area of a sample of 36 needles was measured for each seedling in the growth chamber experiments. Because equation 2.2 was based on mostly 12-needle samples, it was used to determine the total surface of only one-third of the 36-needle sample from each seedling. That value was then multiplied by three to obtain the total surface area of the sample. The ovendry weight of the sample was obtained, as well as the ovendry weight of the rest of the needles. The specific leaf area (the ratio of total surface area to ovendry weight) of the sample was

multiplied by the total ovendry weight of the needles to obtain an estimate of the total needle surface area.

At the time conductance was measured, the driving force for water movement was assumed to be the difference between the predawn needle water potential and the water potential corresponding to that flux measurement. Plant hydraulic conductivity (G_p) was calculated as measured flux divided by the driving force and was expressed in mmol m^{-2} s^{-1} MPa^{-1}, the same units as root system permeability. Total plant water uptake (G_T) was calculated as the product of G_p and the estimated total surface area of needles. The units of G_T were the same units used to measure root system absorptive capacity.

The relative water contents of the roots and needle samples were determined by equation 1.1 for the seedlings used to measure absorptive capacity. For roots, the fresh weight was obtained just before absorptive capacity was measured. It was assumed that full turgor would be reached during the 35 minutes that the roots were under 300 kPa hydrostatic pressure for equilibration and measurement of absorptive capacity. Therefore, the root systems were blotted on absorbent towels and weighed immediately after they were removed from the vessel. That weight was considered the turgid weight. After the turgid weight was measured, the RGP, lateral roots, and taproot were separated to facilitate measuring projected root surface area. The total ovendry weight of the root systems was measured after 24 hours at 70°C $\pm$ 5°.

The fresh weight of a sample of needles large enough to fill a 10 cm^3 test tube was obtained from the seedlings for which root relative water content was measured. Water was added to the test tubes to a level

about half the length of the needles, and the tubes were capped with marbles. After 24 hours, the surface water was blotted dry and the turgid weight was measured, and 24 hours later the ovendry weight was obtained.

During the growth chamber experiments some of the seedlings had extensive needle mortality. Brix (1960) found needle moisture content (expressed as a percentage of ovendry needle weight) a reliable index of loblolly pine seedling mortality. Under the conditions of his experiment, when needle moisture content reached 110% the seedlings did not recover after rewatering. However, the needles themselves could regain turgidity at moisture contents as low as 76%. Furthermore, he found that when needle moisture content fell below 76% there was a significant decrease in the needle respiration rate. For both loblolly and shortleaf pine seedlings, Stransky and Wilson (1964) determined that seedlings might live or die when their needle moisture content ranged between 65% and 105%. However, their experiments did not evaluate physiological changes that accompanied decreasing needle moisture contents. In this study, the data collected to determine needle relative water content was also used to determine needle moisture content on a dry weight basis. Mortality in this study was defined as needle moisture content $< 76\%$.

Experimental Design and Analyses

Analysis of variance (ANOVA) was used to determine the effects of root zone temperature and soil water potential on new root growth (NRAI). Treatment effects on the proportion of the root system that was new (PNRA) were also evaluated. Regression analysis was used to describe the

relative importance of old root area index (ORAI) and new root area index (NRAI), on root system absorptive capacity and seedling water status.

Within each of the three repetitions of the experiment used to evaluate root growth, there was a factorial arrangement of the two factors, two levels of temperature and three levels of soil water potential. The temperatures used were kept constant. Moreover, temperature was a quantitative factor. Soil water potential, however, was not quantified for every seedling. Thus, it was a qualitative factor with respect to root growth. The physical layout of the treatments resembled a split plot experimental design. The water potential treatments were assigned at random to the root environment chambers within each of the water baths. However, the water baths were not replicated; therefore, it had to be assumed that the root zone temperatures were maintained at the desired levels without error. Without replication of the temperature treatments, there was not an appropriate error term for temperature as the whole plot factor in a split plot design. Consequently, the experimental design was considered completely random (Tommy R. Dell, personal communication, June 1989). The systems used for controlling the two temperatures were effective and the root zone temperatures were monitored closely. Fluctuations in root zone temperatures were negligible. Therefore, the assumption that the desired conditions were maintained was considered valid.

The experimental units were the individual seedlings in the root environment chambers. There were 21 experimental units in each treatment combination; however, a random selection of 16 of those were used for data collection and analysis. Seedlings were randomly assigned to the root

environment chambers, and measurements were taken on a random sequence of the seedlings. Because the levels of both factors were chosen based on previous research, both factors were considered fixed. Therefore, the F-tests compared the interaction and the main effects with the experimental error.

Seedlings that were considered dead (needle moisture content < 76%) were deleted from the data. Consequently, all the ANOVA models were unbalanced. Therefore, least squares means were used to compare the factor levels.

Regression analysis modeled the relationships between root morphology and root system absorptive capacity and seedling water status. Measurements of root system morphology, as described by the various root area indices, were used as independent variables in those regressions. The appropriateness of the regression models was checked by lack of fit tests and residual analysis, as outlined by Neter et al. (1985).

In situations where a decision was needed about further analysis, such as the use of means separation procedures or for determining the importance of an independent regression variable, a significance level of $p= 0.05$ was used.

CHAPTER 3

RESULTS AND DISCUSSION

Seedling Survival

Seedlings from the December lift suffered greater mortality than seedlings in the later repetitions of the experiment. Some mortality was anticipated, especially in the water stressed treatments, but excessive mortality became apparent about 2 weeks into the experiment. Of the 96 seedlings that were evaluated, only 49 survived the 29-day long experiment (Table 3). Survival of the seedlings in the January repetition was greater than for the December-lifted seedlings, but still not as good as desired. The overall survival of the January-lifted stock was 62.5%. After raising the water level of the two stressed treatments for the last repetition of the experiment, the overall survival of the February-lifted seedlings was 93.75%. At 15°C, one seedling in the Level 2 stress treatment died, and at 20°C, four seedlings in the Level 2 stress treatment died.

During the December repetition a number of the root environment chambers did not function properly. The affected seedlings simply did not get any water and consequently desiccated. After correcting the problem, the poor survival in the January repetition was a result of underestimating the impact of the root loss that occurred during lifting. Levels of soil water potential similar to those initially chosen for this

Table 3. Seedling survival, based on needle moisture contents ≥ 76% (ODW basis), after 29 days under different root zone environments for all three repetitions of the experiment

Repetition / Water Stress Level	Root Zone Temperature 15°C (%)		Root Zone Temperature 20°C (%)
December			
Control	87.50[1/]		81.25
Level 1	56.25		6.25
Level 2	68.75		6.25
December Mean		51.04	
January			
Control	100.00		100.00
Level 1	75.00		12.50
Level 2	75.00		12.50
January Mean		62.50	
February			
Control	100.00		100.00
Level 1	100.00		100.00
Level 2	93.75		68.75
February Mean		93.75	

[1/] N= 16 for each treatment combination.

research were not too severe for plants with established root systems, such as those used in studies by Faiz and Weatherly (1978), or Snow and Tingey (1985). Furthermore, the selected levels of stress were not too severe when the system designed for this research was tested using container shortleaf pine seedlings.

Because the roots of container plants are not disturbed before they are planted, properly grown container seedlings often have a better mean shoot to root balance than do bare-root seedlings. In one study, container shortleaf pine seedlings had an average ratio between shoot and root volume of about 0.9, compared to approximately 2.3 for bare-root seedlings (Brissette and Barnett 1989). The lower ratio for the container seedlings suggests that they had more absorptive surface area per unit of leaf area than did the bare-root seedlings. The container seedlings used to test the root environment chambers for this research were grown by the same methods and to similar specifications as those used in the study by Brissette and Barnett (1989). Consequently, the container seedlings in the test did not encounter the extreme water stress which the experimental bare-root stock received.

In all the repetitions of the experiment, survival was better at 15°C than at 20°C. Increasing temperature increases the rates of evaporation and transpiration, but the shoot environment was the same for all the treatments. Therefore, the greater mortality of the seedlings at 20°C compared to 15°C was probably due to differences in the rates of evaporation from the surface of the sand. Thus, a mulch may be beneficial in this experimental system.

Root Growth

Although there was a restriction put on the range of acceptable initial root area index (IRAI), there was still variation in that attribute, both within and between the repetitions of the experiment. Therefore, to eliminate any influence root system size may have had on the amount of new root growth, the percentage of the root surface area that was new (PNRA) was used in the analyses. The PNRA was calculated using the following equation:

$$PNRA = (NRAI / (NRAI + ORAI)) \times 100 \quad (3.1)$$

where NRAI = new root area index and OLRAI = old root area index as measured by the image analyzer at the end of the experiments (see Table 14 in the Appendix for a summary of the abbreviations, their derivations and units of measure).

The December Repetition

New root initiation and elongation occurred only in the Control water stress treatments during the December repetition of the experiment. Of the 14 surviving Control seedlings at 15°C, the PNRA ranged from 0 to 21.0%, with a mean of 2.2% and a standard error (SE) of $\pm$ 1.5%. The PNRA for the 13 living Control seedlings at 20°C ranged between 0 and 37.9%, and had a mean and SE of 17.0% $\pm$ 4.0%. When compared using a t-test, the difference between the means for the control seedlings from the two temperatures was significant ($t_{(.95;15.3)} = -3.50$, $p = 0.003$).

The greatest amount of root growth on one seedling at 20°C was 1,170 mm^2 of NRAI, all as lateral root growth. At 15°C, the most NRAI was

460 mm^2. Of the 17 seedlings with new root growth only 3 had any new taproot development; the most growth was 360 mm^2 on a seedling at 20°C. One seedling had just taproot and no lateral root growth, and it was less than 10 mm^2.

Bare-root shortleaf pine seedlings had twice the average number of new roots greater than 10 mm long after 28 days at 20°C, compared to the same period at 15°C (Brissette and Carlson 1987). However, in the first repetition of this 29-day experiment, the mean PNRA was over 16-times greater at 20°C than at 15°C. Moreover, the difference in mean NRAI was 23-fold, 460 mm^2 versus 20 mm^2. Thus, the root zone temperature had a marked effect on the development of new root absorptive surface area after outplanting.

The effects of root growth on water relations will be discussed in detail later. However, predawn needle water potential data from a subsample of the seedlings used for root growth measurements are presented here to provide an indication of the soil water potential levels that were imposed by the treatments. Because some treatment combinations had poor survival, the number of seedlings available for predawn water potential measurements was not equal. Therefore, least squares means were used for comparing treatment effects (Table 4). The interaction between root zone temperature and stress could not be evaluated because there were no predawn water potential measurements from seedlings at Level 1 or Level 2 stress at 20°C. However, there was no main effect of temperature ($p= 0.7$). Based on the data available from the 15°C treatments, the main effect of stress was significant ($p= 0.0001$).

Table 4. Mean predawn needle water potentials of seedlings in the December repetition of the experiment

Water Stress Level	Root Zone Temperature 15°C (MPa)	20°C (MPa)	Mean (MPa)
Control	-1.58a[1/] (11)[2/]	-1.66 (10)	-1.62 (21)
Level 1	-3.80b (5)	-- (0)	
Level 2	-4.00b (4)	-- (0)	
Mean	-3.12p[3/] (20)	-1.66p (10)	-2.30 (30)

Note: The MSE= 0.7994, for temperature $F_{(1;26)}$= 0.05, and for stress $F_{(2;26)}$= 16.57.

[1/] Stress level means for 15°C followed by the same letter are not significantly different at the 0.05 level.

[2/] Numbers in parentheses are base Ns for the adjacent least squares means.

[3/] Temperature least squares means followed by the same letter are not significantly different at the 0.05 level.

Because of the malfunctioning root environment chambers, the water potentials of the stressed seedlings were very low, low enough to result in almost total mortality of the 20°C stressed seedlings. The very low soil water potential probably also explains why there was no root growth among seedlings in the 15°C stressed treatments. Moreover, the overall mean water potential for the control seedlings appeared to be very low. However, the mean predawn needle water potential was higher for the 16 seedlings with root growth than for the 4 without new roots; -1.33 $\pm$ 0.18 MPa compared to -2.24 $\pm$ 0.66 MPa, respectively.

The January Repetition

Seedlings in four of the six treatment combinations produced new roots in the January repetition of the experiment (Table 5). There was a temperature x water stress interaction ($p= 0.003$) affecting PNRA. The mean PNRA from the Control seedlings at 20°C was almost 6 times that at 15°C. However, among the stressed treatments, root growth was inhibited regardless of the root zone temperature. Consequently, the interaction was significant. The ANOVA model explained 60.1% of the total variation in PNRA; the interaction explained 9.3%, while the main effects of temperature and stress accounted for 40.5% and 10.3%, respectively.

The maximum PNRA was 39.9% for one of the seedlings in the 20°C control treatment. The PNRA of many other seedlings in that treatment exceeded 25%. Among the seedlings at 15°C, the most PNRA was 31.6%, but the PNRA was less than 8.5% for the remainder of the seedlings. From the 20°C control treatment, two seedlings had greater than 1,550 mm^2 of NRAI, all in lateral roots. Of the other seedlings in that treatment, three had

Table 5. Mean PNRA (percentage of new root surface area) of seedlings in the January repetition of the experiment

Water Stress Level	Root Zone Temperature 15°C (%)	20°C (%)	Mean (%)
Control	3.9 (16)[1/]	22.5 (16)	13.2 (32)
Level 1	0.1 (12)	0.0 (2)	0.1 (14)
Level 2	0.0 (12)	0.2 (2)	0.1 (14)
Mean	1.3 (40)	7.6 (20)	7.1 (60)

Note: The MSE= 65.5462, for the interaction $F_{(2;54)}$= 6.31, for temperature $F_{(1;54)}$= 4.13, and for stress $F_{(2;54)}$= 12.56.

[1/] Numbers in parentheses are base Ns for the adjacent means.

more than 1,200 mm^2 NRAI. At 15°C, the greatest amount of NRAI was 1,000 mm^2, all the other seedlings had NRAI below 230 mm^2. Only one seedling in this repetition of the experiment had any new taproot development and it had just 10 mm^2. Just one seedling in either of the 20°C stressed treatments in this repetition produced any new roots, and it had < 10 mm^2 of NRAI. Among the water stressed seedlings at 15°C, one had 30 mm^2 of NRAI.

The least squares mean predawn needle water potentials tended to be higher in the January repetition than in the December repetition (Table 6). Neither the interaction between temperature and stress ($p= 0.4$), nor temperature alone ($p= 0.2$) were important. Although the control treatments differed from the stress treatments ($p= 0.0001$), the two stress levels were similar ($p= 0.7$). These data show that during the January repetition there were only two statistically different levels of soil water potential, the well watered controls and a single level of extreme stress composed of all the seedlings from both Level 1 and Level 2.

The February Repetition

Some new root growth occurred in all the treatments during the February repetition of the experiment (Table 7). As in the previous repetition, there was a temperature x stress interaction ($p= 0.002$). The interaction resulted because under stress Level 2 there was little new root growth at either temperature, but at the Control and stress Level 1 the amount of new root growth at 20°C greatly exceeded that at 15°C. The ANOVA model explained 43.8% of the total variation in PNRA. The temperature x stress interaction explained 9.2% of the variation in PNRA,

Table 6. Mean predawn needle water potentials of seedlings in the January repetition of the experiment

Water Stress Level	Root Zone Temperature 15°C (MPa)	20°C (MPa)	Mean (MPa)
Control	-1.22 (8)[2/]	-1.11 (8)	-1.17a[1/] (16)
Level 1	-3.43 (5)	<-4.00[3/] (2)	<-3.72b (7)
Level 2	-3.71 (6)	<-4.00 (1)	<-3.85b (7)
Mean	-2.79p[4/] (19)	<-3.04p (11)	<-2.34 (30)

Note: The MSE= 0.1977, for the interaction $F_{(2;24)}$= 1.30, for temperature $F_{(1;24)}$=1.35, and for stress $F_{(2;24)}$= 99.71.

[1/] Stress level least squares means followed by the same letter are not significantly different at the 0.05 level.

[2/] Numbers in parentheses are base Ns for the adjacent means.

[3/] By definition, -4.00 MPa was the lowest water potential recorded; see text.

[4/] Temperature least squares means followed by the same letter are not significantly different at the 0.05 level.

Table 7. Mean PNRA (percentage of new root surface area) of seedlings in the February repetition of the experiment

Water Stress Level	Root Zone Temperature 15°C (%)	20°C (%)	Mean (%)
Control	1.8 (16)[1/]	19.4 (16)	10.6 (32)
Level 1	0.5 (16)	10.1 (16)	5.3 (32)
Level 2	0.1 (15)	1.0 (11)	0.6 (26)
Mean	0.8 (47)	10.2 (43)	5.8 (90)

Note: The MSE= 71.9097, for the interaction $F_{(2;84)}$= 6.88, for temperature $F_{(1;84)}$= 27.11, and for stress $F_{(2;84)}$= 10.04.

[1/] Numbers in parentheses are base Ns for the adjacent means.

nearly the same as the 9.3% accounted for by the same interaction in the January repetition of the experiment. The main effect of temperature explained 22.8%, and the main effect of stress accounted for 11.8% of the variation in PNRA.

Although the mean NRAI and PNRA from the two control treatments were less than in January, there were more seedlings with new root growth in this repetition of the experiment. In three of the treatment combinations--the 15°C and 20°C Controls, and the 20°C Level 1--the majority of the seedlings produced some new roots. From the 20°C Control treatment, five seedlings had more than 1,200 mm^2 of NRAI. The maximum NRAI was 1,730 mm^2, and the only new taproot development in this repetition was the 90 mm^2 on that seedling. From stress Level 1 at 20°C, two seedlings had greater than 900 mm^2 of NRAI. The maximum NRAI in the 20°C Level 2 treatment was 160 mm^2. In the 15°C Control treatment the maximum NRAI was 280 mm^2. For the 15°C Level 1 treatment the maximum NRAI was 70 mm^2. Two seedlings from Level 2 stress at the 15°C had NRAIs of 10 and 20 mm^2.

The seedling from the 20°C Control treatment that had the maximum amount of new root growth also had the greatest PNRA, 46.6%. Four other seedlings from the 20°C Control treatment had PNRAs greater than 33.3%. From the 20°C Level 1 treatment, the two seedlings with the most NRAI had over 30% PNRA. The maximum PNRA for a seedling from Level 2 at 20°C was 8.3%, the other seedlings had less than 2% PNRA. The maximum PNRA at 15°C was 11.5% for the control seedling with the most NRAI. From Level 1 at 15°C the greatest PNRA was 2.9%. For the two seedlings in the Level 2 treatment that grew roots their PNRA was < 1.0%.

As with the earlier repetition, there was no effect on the mean predawn needle water potential due to the temperature x stress interaction ($p= 0.8$), or due to the main effect of temperature ($p= 0.9$) (Table 8). Again, the main effect of water stress was highly significant ($p= 0.0001$). Furthermore, the Control treatments differed from Level 1 ($p= 0.01$), and Level 1 differed from Level 2 ($p= 0.005$). Thus, there were three distinct levels of water potential imposed during the last repetition of the experiment.

Comparing and Contrasting the Results from the Three Repetitions

The treatment most favorable for root growth was the 20°C root zone temperature with the sand maintained at the control water stress level. The changes made in water stress levels between the repetitions of the experiment did not affect the control treatments. Thus, the 20°C Control treatment remained comparable to conventional RGP test conditions throughout the study. The results (mean and SE) from that treatment were: 17.0% ± 4.0%, 22.5% ± 3.3%, and 19.4% ± 4.0% PNRA, for lifting after 301, 715, and 1077 hours of accumulated chilling, respectively. These data show the expected peaking of RGP in mid-winter (Ritchie and Dunlap 1980), which is often, but not always observed in the southern pines (Barden et al. 1987, Brissette and Roberts 1984, Brissette et al. 1989, Hallgren and Tauer 1989). However, in this case the January mean was not significantly greater than the earlier and later means. Therefore, during the season in which this research was conducted, not only did the pattern of the root growth response to soil temperature and water availability remain similar,

Table 8. Mean predawn needle water potentials of seedlings in the February repetition of the experiment

Water Stress Level	Root Zone Temperature 15°C (MPa)	20°C (MPa)	Mean (MPa)
Control	-1.48 (8)[2/]	-1.33 (8)	-1.41a[1/] (16)
Level 1	-2.18 (8)	-2.20 (8)	-2.19b (16)
Level 2	-2.99 (8)	-3.10 (5)	-3.04c (13)
Mean	-2.22p[3/] (24)	-2.21p (21)	-2.15 (45)

Note: The MSE= 0.7436, for the interaction $F_{(2;39)}$= 0.09, for temperature $F_{(1;39)}$= 0.00, and for stress $F_{(2;39)}$= 12.57.

[1/] Stress level least squares means followed by the same letter are not significantly different at the 0.05 level.

[2/] Numbers in parentheses are base Ns for the adjacent means.

[3/] Temperature least squares means followed by the same letter are not significantly different at the 0.05 level.

but the amount of root growth was also the same under favorable conditions.

When the results of the repetitions are considered together it is clear that both soil temperature and water potential limited root growth. The interaction between the two factors was significant in the two repetitions during which root growth occurred on seedlings in the water stressed treatments. It accounted for about 9% of the total variation in root growth in both the January and February repetitions of the experiment. That is, about 16% and 21% of the variation that was explained in the two repetitions, respectively. There was always more root growth at 20°C than at 15°C. However, as soil water potential dropped between levels, the amount of root growth fell much more rapidly at 20°C than at 15°C. At the most severe level of stress, the seedlings could not generate much root growth at either temperature.

Whether root zone temperature or soil water potential had a greater impact on root growth in these experiments depended on which factor was most limiting. A 5°C difference in root zone temperature made as much as a 23-fold difference in mean NRAI under well watered conditions. Nevertheless, the lower temperature did not prevent root development. Soil water potential was not measured directly in this research. However, at 20°C, a mean predawn needle water potential of about -1.3 MPa for the well watered control seedlings had no apparent negative effect on root growth. With an average predawn needle water potential for the Level 1 stress seedlings of -2.2 MPa at 15°C, and -2.3 MPa at 20°C, NRAI was 30% and 42% that of the respective control treatments. Once the mean predawn needle water potential fell below -3.0 MPa, as it did for the Level 2

stress seedlings, NRAI was only 5% or less that of the controls. Ritchie and Dunlap (1980) reported new root growth on loblolly pine seedlings during RGP tests conducted at an initial soil water potential of -1.3 MPa. Therefore, bare-root southern pine seedlings are clearly capable of sustaining new root growth in soils at 15°C or above, and under soil moisture conditions well below field capacity.

The results of this research have implications for the use of RGP as a predictor of field performance. Measuring RGP under conditions favorable for root growth has become an accepted method of evaluating seedling quality. The results of such tests frequently correlate well with field survival but sometimes they do not (Brissette and Roberts 1984, Burdett 1987, Sutton 1987). When RGP does not relate to field performance it is usually because planting site conditions either impose no significant stress or so severe a stress that no seedlings will do well, regardless of their quality (Burdett 1987). Testing RGP in favorable environments is analogous to seed germination tests, which are also conducted under ideal laboratory conditions. Seed testing laboratories, however, follow strict international rules to ensure uniformity of procedures. The procedures used for conducting and measuring the results of RGP tests vary with the objectives and facilities of those seeking the results. Because of the impact the environment has on root growth after outplanting, RGP measured under optimal root growth conditions may not be a realistic predictor of field performance. The results of RGP tests conducted in environments more similar to field planting conditions should relate more strongly to actual plantation survival and perhaps growth.

The tradeoff is that such testing will usually take longer to yield meaningful results.

The current measures of RGP are all indicators of the amount of root growth that occurred during the time period of the test. Also, in this research, root growth was measured at only one point in time. How quickly root growth begins after planting, and at what rate new roots elongate may be more meaningful predictors of field performance. However, the test environment will certainly affect those parameters just as it does total root growth.

This study also provides some insights about root growth after planting in the field. Most of the recent regeneration research has focused on the role of the seedling because of the importance of new roots to establishment and subsequent field performance. The current research has shown, however, that the root zone environment may be at least as important as seedling quality to successful establishment. The ability of site preparation techniques to moderate soil temperature and improve soil moisture availability is very important because of the sensitivity of root growth to both of those factors.

Predawn xylem water potential of well watered vegetation is a good estimate of the equilibrium point between plant and soil water potential (Slayter 1967). However, for plants that are water stressed, the night period is often not long enough for equilibration to occur (Landsberg 1986). This is often true in dry soils which have low hydraulic conductivities, and even more so in sand. Furthermore, in transplanted seedlings predawn water potential is also affected by root damage resulting from lifting and handling, and by new root development after

outplanting. As new absorptive surface area develops, additional sources of water become available and the predawn water potential increases markedly, even though the overall soil water potential may not change.

When soil is moist its hydraulic conductivity is such that the water potential gradient across the roots, from the epidermis to the xylem, is much steeper than the gradient through the soil to the surface of the roots (Passioura 1988). In that case, water flows through the soil to the roots. However, as the soil dries its hydraulic conductivity is reduced until the water potential across the roots is not strong enough to maintain liquid water flow through the soil. Water may still move in the soil as vapor, but the flux of water as vapor is many times slower than that of liquid water in moist soil. Furthermore, the transition of water flow from liquid to vapor occurs at much higher moisture contents in sand than in soil (Passioura 1988). The rapid draining of the relatively large pores in sand, and the lack of small pore space are the reasons why the water-filled pores become discontinuous sooner in sand than in field soils.

In these experiments, the mean water potential of the sand in the root environment chambers within a given treatment combination probably did not differ much. However, the mean predawn needle water potential of each individual seedling depended on the rate of water flow to the roots, and on the amount of root growth, if any. That is, the predawn needle water potential reflected soil water potential for only those seedlings that absorbed enough water to bring the plant to equilibration with the soil during the night. As will be discussed in detail later, that occurred only when the NRAI exceeded a minimum level.

Root Relative Water Content

One secondary objective regarding root growth was to determine the minimum level of root system relative water content required for new root growth. Cosgrove (1986) speculated that the minimum turgor required for plant cell growth is in the range of 0.2 to 0.4 MPa. The results of Pallardy et al. (1982) suggest that a relative water content greater than about 65% is required if turgor is to remain above such a threshold in shortleaf pine seedling root systems. In all three repetitions of this study, root growth occurred only when the root relative water content was greater than about 85% (Figure 2).

The study by Pallardy et al. (1982) was designed to examine the components of water potential in both stem and root tissue. It was not designed to measure the relative water content necessary for growth. Also, the data presented were for a single seedling with results typical of their study. That seedling could have differed in numerous ways, both physiologically and morphologically, from the population of seedlings used in this study. Another possible explanation for the apparently high relative water content required for root growth in this study, is that root initiation may require more turgor than that required to drive enlargement of developing cells. Nevertheless, the results of this research showed that root growth required a rather distinct minimum level of root relative water content of about 85%.

Treatment Effects on Old Root Morphology

Another secondary objective was to determine if the treatments affected the morphology of the old root system. The literature about the

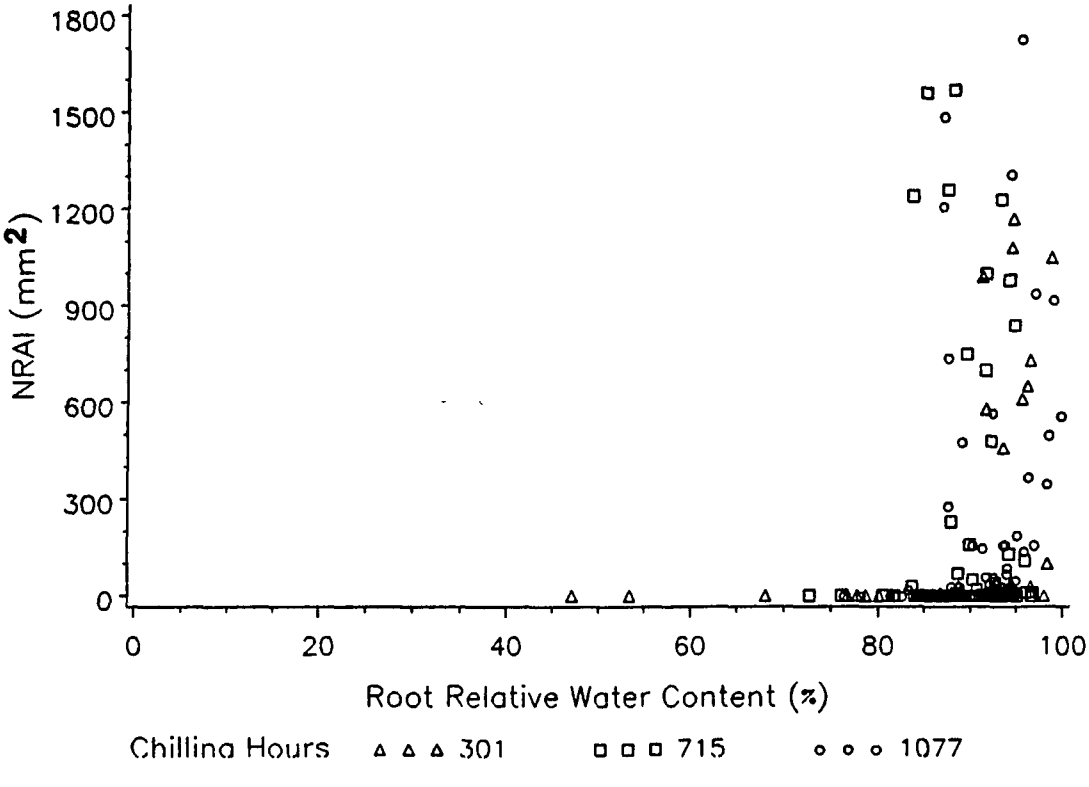

Figure 2. The relationship between new root area index (NRAI) and root relative water content among seedlings from all three repetitions of the experiment.

influence of seedling morphology on RGP has been contradictory. In practice, seedling morphology refers to the easily measured parameters of size; for example: height, root collar diameter, root volume or RAI, and ovendry weight. Burdett (1987) agreed with Feret and Kreh (1985) that seedling morphology is unrelated to RGP. However, in studies by Brissette and Roberts (1984), Carlson (1986), and South et al. (1989) a positive relationship existed between measures of root system size and RGP. Carlson (1986) concluded that there is a relationship because larger root systems have more sites for new root growth.

Among all the seedlings studied in this research, NRAI was correlated ($N = 199$, $r = 0.25$, $p = 0.0003$) with ORAI. These results support the hypothesis that healthy, old lateral roots provide the sites for new lateral root initiation and support elongation of both new and old roots. However, ORAI was affected by the treatments so that a definite relationship between root system size and new root growth cannot be established from the results of this study.

The IRAI was measured before the experiments were started while OLRAI and OTRAI were measured at the end. Therefore, the ratio of the ORAI to IRAI provided a measure of how much the surface area of the root systems were affected by the various treatment combinations. This ratio was termed change in RAI (CRAI). Because the IRAI was a nondestructive measurement while OLRAI and OTRAI were more accurately measured after the root systems were separated into component parts, the ratio did not equal 1.0. Nevertheless, if root surface area was not affected by the treatments, then the mean CRAI should have been constant among the treatments within each repetition of the experiment. The smaller the mean

value of the CRAI, the greater the average loss of root surface area during the experimental period, probably due to root shrinkage of death of fine roots.

The December Repetition

Among seedlings in the December repetition, the CRAI was affected by the interaction between temperature and water stress ($p= 0.005$). At 15°C there was little difference in CRAI among the levels of water stress, but at 20°C the CRAI was much higher for the control than for the two stress treatments (Table 9). The ANOVA model explained 34.6% of the total variation in CRAI. The temperature x stress interaction accounted for 18.2% of the variation. However, there was only one living seedling in each of the two 20°C water stressed treatments. Therefore, although the interaction was statistically significant, it may not accurately describe how the root systems were affected by the root zone environments. The main effect of temperature, which was based on 34 seedlings at 15°C and 15 seedlings at 20°C, explained 7.9% of the total variation in CRAI. Moreover, there was a greater loss of old root surface area among the seedlings at 15°C than among those at 20°C.

The January Repetition

Among seedlings in the January repetition, the temperature x stress interaction did not affect CRAI ($p= 0.8$). However, the main effects of both temperature ($p= 0.04$) and water stress ($p= 0.004$) did influence CRAI (Table 10). As in the earlier repetition of the experiment, the seedlings at 15°C suffered more old root surface area loss than did the seedlings at 20°C. Seedlings in the stressed treatments lost more root surface area

Table 9. Mean ratio of the index of old root surface area at the end of the experiment to the root area index prior to treatment application for seedlings in the December repetition of the experiment

Water Stress Level	Root Zone Temperature 15°C	20°C	Mean
Control	0.79 (14)[2/]	0.85 (13)	0.82a[1/] (27)
Level 1	0.79 (9)	0.65 (1)	0.72b (10)
Level 2	0.77 (11)	0.68 (1)	0.73b (12)
Mean	0.78p[3/] (34)	0.73p (15)	0.80 (49)

Note: The MSE= 0.00374, for the interaction $F_{(2;43)}$= 5.99, for temperature $F_{(1;43)}$= 3.29, and for stress $F_{(2;43)}$= 7.81.

[1/] Stress level least squares means followed by the same letter are not significantly different at the 0.05 level.

[2/] Numbers in parentheses are base Ns for the adjacent means.

[3/] Temperature least squares means followed by the same letter are not significantly different at the 0.05 level.

Table 10. Mean ratio of the index of old root surface area at the end of the experiment to the root area index prior to treatment application for seedlings in the January repetition of the experiment

Water Stress Level	Root Zone Temperature 15°C	20°C	Mean
Control	0.88 (16)[2/]	0.93 (16)	0.90a[1/] (32)
Level 1	0.82 (12)	0.85 (2)	0.84b (14)
Level 2	0.84 (12)	0.87 (2)	0.86b (14)
Mean	0.85p[3/] (40)	0.89q (20)	0.87 (60)

Note: The MSE= 0.00247, for the interaction $F_{(2;54)}$= 0.17, for temperature $F_{(1;54)}$= 4.40, and for stress $F_{(2;54)}$= 6.07.

[1/] Stress level least squares means followed by the same letter are not significantly different at the 0.05 level.

[2/] Numbers in parentheses are base Ns for the adjacent means.

[3/] Temperature least squares means followed by the same letter are not significantly different at the 0.05 level.

than seedlings in the control treatments. However, the mean CRAIs did not differ between the two stress treatments. The ANOVA model accounted for 41.4% of the total variation in CRAI in the January repetition. The main effect of temperature explained 25.7%, and the main effect of stress explained 15.3% of the variation.

The February Repetition

The temperature x stress interaction did not affect the CRAI of seedlings in the February repetition ($p = 0.2$). The main effect of temperature had a marked impact ($p = 0.0001$), but the main effect of water stress was marginally significant ($p = 0.054$). In a reversal of the results in the two earlier repetitions, in this case the seedlings at 20°C had greater root surface area loss than the seedlings at 15°C (Table 11). Among the three stress levels, the mean CRAI of the control seedlings was greater than that of the Level 2 seedlings, but not of the Level 1 seedlings. Furthermore, the mean CRAI of the Level 1 seedlings was not significantly different than the mean CRAI of the Level 2 seedlings. The ANOVA model accounted for 55.1% of the total variation in CRAI, and the main effect of temperature alone explained 50.5%. The reduced impact of water stress on CRAI in the February repetition was probably a reflection of the changes made to the levels of soil water potential before the final repetition.

Comparisons Among the Repetitions

Overall, the CRAI was much lower among the seedlings in the February repetition of the experiment than in either of the earlier repetitions. The CRAI was smallest in the last repetition because the IRAI of the

Table 11. Mean ratio of the index of old root surface area at the end of the experiment to the root area index prior to treatment application for seedlings in the February repetition of the experiment

Water Stress Level	Root Zone Temperature 15°C	20°C	Mean
Control	0.76 (16)[2/]	0.66 (16)	0.71a[1/] (32)
Level 1	0.73 (16)	0.65 (16)	0.69ab (32)
Level 2	0.74 (15)	0.61 (11)	0.68b (26)
Mean	0.74p[3/] (47)	0.64q (43)	0.70 (90)

Note: The MSE= 0.00235, for the interaction $F_{(2;84)}$= 1.59, for temperature $F_{(1;84)}$= 98.77, and for stress $F_{(2;84)}$= 3.02.

[1/] Stress level least squares means followed by the same letter are not significantly different at the 0.05 level.

[2/] Numbers in parentheses are base Ns for the adjacent means.

[3/] Temperature least squares means followed by the same letter are not significantly different at the 0.05 level.

February lifted seedlings was much larger than those lifted earlier (Table 2), while the ORAI did not vary much among the repetitions. The ORAIs averaged 1,970 ± 50, 2,140 ± 40, and 2,090 ± 30, respectively, for the three repetitions of the experiment.

Unlike the data for water stress, which indicated that greater stress results in a lower mean CRAI, the temperature data provide no indication as to why the results reversed between the January and February repetitions. Perhaps the difference in IRAI between the seedlings lifted in January and those lifted in February may have some bearing on that question. Measuring CRAI as it was done in this research appears to provide a method for assessing the effects of stress on root system morphology. However, additional research, designed to address this aspect of seedling establishment, is needed to determine the value of CRAI.

Root Function

This research did not examine the relationship between changing pressure and water flux. A constant hydrostatic pressure of 0.3 MPa was used. Although the water flux induced at 0.3 MPa should have minimized the osmotic effect, the flow rate may not have been linear at that pressure. Therefore, data were expressed in units "at 0.3 MPa", rather than "per MPa." The units of root system absorptive capacity are mmol of water s^{-1} at 0.3 MPa, and for permeability they are mmol m^{-2} of root surface area s^{-1} at 0.3 MPa.

In the three repetitions of the experiment, absorptive capacity was measured and permeability calculated for a total of 199 root systems of seedlings that were alive at the end of the experiments. One seedling

from the December lift had such anomalous results that it was removed from the data set. Among the other 198 seedlings, absorptive capacity ranged from 0 to 0.01440 mmol s^{-1} at 0.3 MPa, with an overall mean and SE of 0.00237 $\pm$ 0.00021 mmol s^{-1} at 0.3 MPa. The average permeability was 0.300 $\pm$ 0.025 mmol m^{-2} s^{-1} at 0.3 MPa, with a maximum of 1.614 mmol m^{-2} s^{-1} at 0.3 MPa.

Although absorptive capacity and permeability are similar attributes, they can provide different insights into root function because of variation among seedlings in the amount of root system surface area. Root system absorptive capacity is a measure of whole plant water uptake and root system permeability measures uptake on a unit surface area basis. Reporting root system function in terms of permeability removes the confounding effect of root system size. However, in this research the selection of seedlings from a narrow range of IRAIs resulted in uniform experimental populations. Consequently, absorptive capacity and permeability were highly correlated ($p= 0.0001$) in each of the three repetitions of the experiment ($r= 0.92$ to 0.95). Furthermore, regression analyses with both absorptive capacity and permeability resulted in the same interpretations. Moreover, in each case, the analysis with absorptive capacity was more significant than the similar analysis with permeability. The lower significance associated with root system permeability was probably due to measurement error when calculating total root system surface area. Therefore, because the results of the analyses for absorptive capacity had more statistical power, only they are presented.

Effect of Time Under Pressure

Absorptive capacity was measured after approximately 20, 25, 30, and 35 minutes in the pressure vessel. To examine the effect of time in the pressure vessel on root function, the absorptive capacity was regressed against TOTRAI, time (in minutes), and their interaction. When the TOTRAI x time interaction and the main effect of time were tested simultaneously, the test was not significant in any of the repetitions ($0.5 < p < 0.8$). In other words, the length of time under pressure did not significantly affect absorptive capacity in these experiments. Therefore, the mean absorptive capacity of each seedling was used in all subsequent analyses.

Effects of New and Old Root Surface Area

Regression analysis was used to estimate the effects of old and new root area on the absorptive capacity of seedling root systems. Two independent variables were used in the regressions, ORAI and NRAI.

The December Repetition

The absorptive capacity of 49 root systems was evaluated from the first repetition of the experiment. However, one was deleted from the data because its absorptive capacity was so much higher than any other seedling in any of the repetitions. The mean of the remaining seedlings was 0.00197 mmol s^{-1} at 0.3 MPa. The CV was 85.3%. There was a significant linear regression for absorptive capacity with NRAI and ORAI which explained 49.8% of the total variation in absorptive capacity. However, the ORAI did not have a significant ($p= 0.2$) effect on absorptive capacity.

The simple linear regression (Table 12) of absorptive capacity with NRAI alone explained 48.0% of the total variation:

$$(3.2) \qquad L_R = 1.212 \times 10^{-3} + 4.793 \times 10^{-6} \text{ NRAI}$$

In this model, both the intercept ($p = 0.0001$) and NRAI ($p = 0.0001$) were highly significant. The model predicted that for every 10 mm^2 of NRAI, absorptive capacity increased $48 \pm 7.4 \times 10^{-6}$ mmol s^{-1}. That represents an average increase of 4.0% for each additional 10 mm^2 of NRAI.

The January Repetition

The absorptive capacity of 60 seedlings was measured during the second repetition of the experiment. The mean was greater than it had been in the first repetition, 0.00269 versus 0.00197 mmol s^{-1} at 0.3 MPa, while the CV was smaller, 73.1% versus 85.3%. The linear regression of absorptive capacity with NRAI and ORAI explained 71.7% of the total variation. However, as in the December repetition, the ORAI did not significantly ($p = 0.2$) impact absorptive capacity.

In a simple linear regression (Table 12), NRAI alone accounted for 70.7% of the total variation in absorptive capacity:

$$(3.3) \qquad L_R = 1.066 \times 10^{-3} + 7.049 \times 10^{-6} \text{ NRAI}$$

As in the December repetition, both the intercept ($p = 0.0005$) and NRAI ($p = 0.0001$) were significant. This model predicted that for every 10 mm^2 of NRAI, absorptive capacity increased $70 \pm 6.0 \times 10^{-6}$ mmol s^{-1}, or about 6.6%.

Table 12. Significant regressions; variables and statistics

Model	Variables[1/] Dependent	Independent	Statistics MSE	df	F	p	r^2
3.2	Dec L_R	NRAI	2.8667 (x 10^{-6})	1;46	42.5	0.0001	0.480
3.3	Jan L_R	NRAI	3.9216 (x 10^{-6})	1;58	140.0	0.0001	0.707
3.4	Feb L_R	NRAI	5.0288 (x 10^{-6})	1;88	65.4	0.0001	0.465
3.5	Dec ψ_{pd}	ln(NRAI + 1)	0.6815	1;28	50.3	0.0001	0.642
3.6	Jan ψ_{pd}	ln(NRAI + 1)	0.4679	1;28	83.8	0.0001	0.749
3.7	Feb ψ_{pd}	ln(NRAI + 1)	0.5064	1;43	52.0	0.0001	0.547
3.8	Jan $\Delta\psi_{rec}$	ln(NRAI + 1)	0.0138	1;21	39.7	0.0001	0.654
3.9	Feb $\Delta\psi_{rec}$	ln(NRAI + 1)	0.0095	1;35	50.2	0.0001	0.589
3.10	Dec g_n	NRAI	22.6318	1;20	9.2	0.006	0.316
3.11	Jan g_n	NRAI	12.7684	1;28	89.6	0.0001	0.762
3.12	Feb g_n	NRAI	21.7243	1;43	38.6	0.0001	0.473
3.13	Dec g_n	ln(| ψ_{pd} |)	10.9786	1;20	40.3	0.0001	0.668
3.14	Jan g_n	ln(| ψ_{pd} |)	16.9476	1;21	50.1	0.0001	0.705
3.15	Feb g_n	ln(| ψ_{pd} |)	29.8395	1;35	20.2	0.0001	0.366

[1/] Abbreviations are defined in the Appendix, Table 14.

The February Repetition

During the last repetition of the experiment, absorptive capacity was measured on 90 seedlings. The mean was intermediate between the two earlier repetitions, 0.00238 mmol s^{-1} at 0.3 MPa, but the CV was the highest at 94.0%. Because of the greater variation in absorptive capacity, the linear regression with NRAI and ORAI accounted for less total variation, 43.4%. As in the other repetitions, the ORAI was not significant ($p = 0.3$).

The NRAI alone accounted for 42.6% (Table 12) of the total variation in absorptive capacity:

(3.4) $$L_R = 1.488 \times 10^{-3} + 5.280 \times 10^{-6} \text{ NRAI}$$

As in the simple linear models for the other repetitions, the intercept ($p = 0.0001$) and NRAI ($p = 0.0001$) were both highly significant. For each additional 10 mm^2 of NRAI, the model predicted an increase in absorptive capacity of $53 \pm 6.5 \times 10^{-6}$ mmol s^{-1}, which is equivalent to an incremental increase of 3.5%.

Comparing the Three Repetitions

The NRAI had a significant impact on the rate of water uptake in each repetition of the experiment. The intercept values represent the estimated absorptive capacity of those seedlings with no new root tissue. The intercepts varied among the repetitions from a low in the January experiment of 0.00106 ± 0.00029 mmol s^{-1} at 0.3 MPa, to a high of 0.00149 ± 0.00026 mmol s^{-1} at 0.3 MPa for the February repetition (Figure 3). Furthermore, the models estimated that for every additional 10 mm^2 of NRAI,

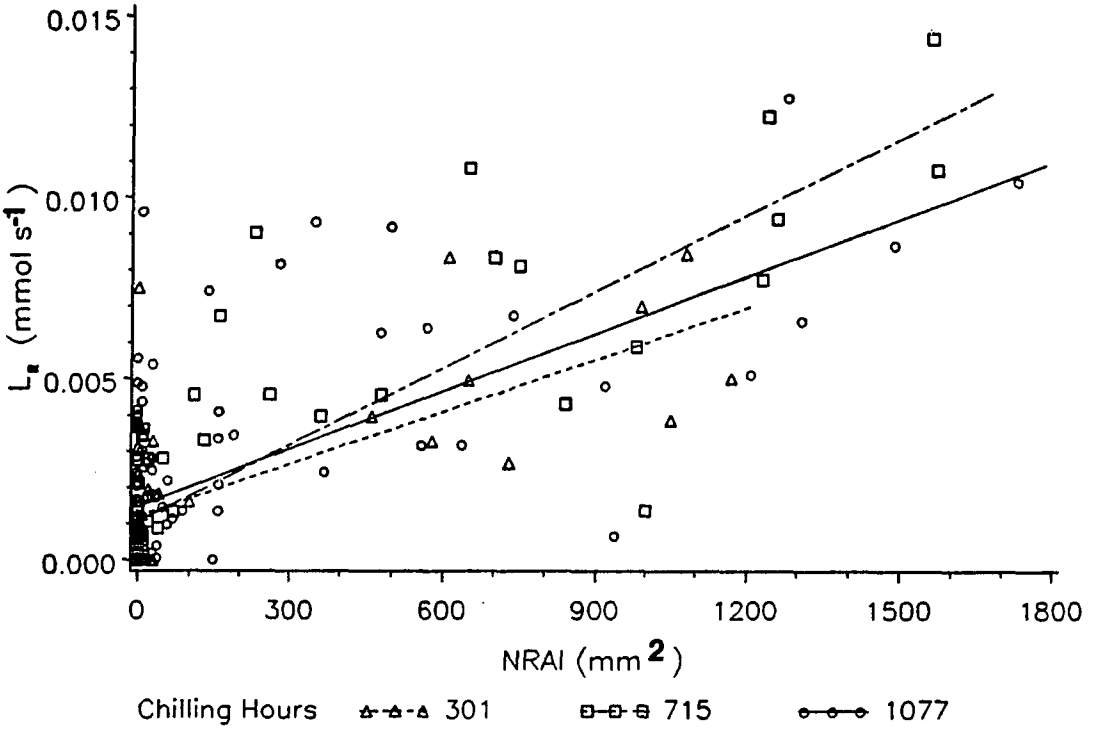

Figure 3. Linear regressions of absorptive capacity (L_R) with new root area index (NRAI) for seedlings in each of the three repetitions of the experiment.

absorptive capacity increased from 3.5% to 6.6%. The percentage increase in absorptive capacity depended on both the rate of increase and on the intercept. For example, the rate of increase in absorptive capacity with NRAI was intermediate in the February experiment; however, the percentage increase was less than in the other repetitions because the intercept was greater than in the other two models.

The relationship between NRAI and absorptive capacity is best illustrated by some examples. The average NRAI among the 20°C Control seedlings from the January repetition was 750 mm^2. With a percentage increase in January of 6.6% for each 10 mm^2 of NRAI, that amount of root growth provided an estimated increase in absorptive capacity of nearly 5-fold compared to the 0.00107 mmol s^{-1} expected of seedlings that did not grow any new roots. Using the February model, which had the lowest percentage increase, a seedling with 1,000 mm^2 of NRAI would be expected to have an absorptive capacity 350% greater than a seedling with no root growth.

Moreover, those increases were based on a relatively moderate driving force of 0.3 MPa. The relative impact of each mm^2 of NRAI depends on the rate of water absorption. When soil water availability is not limited, the rate of absorption increases as the water potential gradient that drives transpiration increases. In this study, the driving force for transpiration was calculated as the difference between the needle water potentials measured predawn and when stomatal conductance was measured. The maximum transpirational driving force calculated was 0.9 MPa, and driving forces of $\geq$ 0.5 MPa were not uncommon. Therefore, in rapidly

transpiring seedlings, the amount of new root growth should have an even larger impact on absorptive capacity than in the examples given above.

The regression models showed that the surface area of the old roots did not affect the absorptive capacity of seedlings in these experiments. Selecting only seedlings with an IRAI of $\pm$ 1 standard deviation of a sample mean resulted in relatively little variation in root system size among seedlings in each repetition of the experiment. That lack of variation most likely explains why ORAI did not significantly contribute to absorptive capacity in these experiments. If a broader range of ORAI had been evaluated, a positive relationship between absorptive capacity and ORAI should have been evident.

Carlson (1986) found a significant, positive relationship between absorptive capacity and the volume of old roots of loblolly pine seedlings. Although Carlson (1986) used root volume to characterize root system morphology and this study used projected root surface area, there is a strong correlation ($r = 0.83$, $p = 0.0001$) between root volume and IRAI (Brissette et al. 1989). Therefore, over a range of root system sizes, absorptive capacity should correlate with IRAI or ORAI, as well as root volume.

The procedures used in this research to evaluate absorptive capacity were modeled after those of Carlson (1986) for loblolly pine seedlings. In his study, linear regressions of absorptive capacity as a function of root volume, both before and after new roots, had r^2 values of 0.51 and 0.34, respectively. For eastern white pine (*P. strobus* L.), Johnsen et al. (1988) explained 56% of the variation in absorptive capacity with the percentage of the root system that was new roots. In this study with NRAI

as the independent variable, r^2 values of between 0.43 and 0.71 were calculated for the three repetitions of the experiment. Thus, the amount of variation in absorptive capacity that could be explained in this and similar studies was about the same. The reasons for the extent of the variation were not evident.

As mentioned, one seedling in the December repetition was deleted from the data because of its extremely high absorptive capacity. There were also several others that had little or no new root growth but had relatively high water flow rates. Normally, water movement into roots is restricted. The impermeable Casparian band in the endodermis of unsuberized roots forces water to enter the symplast before it reenters the apoplast in the xylem (Kramer 1983). In older roots, the outer suberized layer and the vascular and cork cambiums provide potential barriers to water uptake (Chung and Kramer 1975). Addoms (1946) showed that water enters suberized roots of pine predominately through tiny wounds, often small enough to go unnoticed. Such wounds provide direct access to the xylem. Although care was taken in this research to minimize root disturbance while washing root systems from the sand, it is possible that some breaks in the impermeable layers of old roots did occur, and perhaps some wounding of new roots as well. Apoplastic flux is less than 1% of the total flux for well aerated, intact red pine root systems (Hanson et al. 1985). Thus, if root damage resulted in a large proportion of the total water flux coming directly through the apoplast, greater than expected root system absorptive capacity could be the result.

In addition to those seedlings with absorptive capacities higher than expected, there were many that had absorptive capacities near zero.

One possible explanation for low absorptive capacity is xylem dysfunction caused by disruption of the water column in the xylem. Water in the xylem is normally under tension. Consequently, for water to be continuous in the xylem it must remain liquid at pressures below its vapor pressure. This is possible because of the hydrogen bonds among the water molecules.

Embolisms, which disrupt the continuity of the xylem water column, develop as the result of water stress (Sperry and Tyree 1988) and winter freezing, and may be pathogen induced (Tyree and Sperry 1989). When an air bubble forms in a tracheid or vessel it will rapidly expand and complete embolization can occur in less than 20 minutes (Tyree and Sperry 1989). Air can enter the xylem through wounds caused by mechanical damage or herbivory. In this research, it is possible that embolisms developed due to the water stress treatments, or perhaps as a result of any damage which might have occurred during washing and handling seedling root systems.

Embolisms may explain reduced absorptive capacity of planted seedlings where the xylem water column is under tension. However, air bubbles are unstable in water at atmospheric pressure because surface tension puts the bubble under pressure (Tyree and Sperry 1989). In kPa this pressure is 140/r, where r equals the radius of the bubble in μm (Tyree and Sperry 1989). The average tracheid diameter in shortleaf pine wood is 60 m (Panshin and de Zeeuw 1970). Thus, a bubble with a 30 μm radius contains air at a positive 4.7 kPa, when the water is at atmospheric pressure. The positive hydrostatic pressure applied in this study was 300 kPa. That pressure should have been sufficient to dissolve both large and small air bubbles. Once embolisms dissolve, xylem function

is restored (Tyree and Sperry 1989). Furthermore, in this study absorptive capacity did not increase after the equilibration period. Increasing absorptive capacity should have been measured if embolisms continued to dissolve during the time the roots were in the pressure vessel. Therefore, although embolisms may have been present in the xylem, they were probably dissolved by the time absorptive capacity was measured. Additional research is needed to understand why the absorptive capacity was so low for many of the water stressed seedlings in these experiments.

Water Relations

Predawn Needle Water Potential

Among the three repetitions, the predawn needle water potential was estimated for 105 seedlings. It ranged from a high of -0.55 MPa to the minimum defined for this research of -4.00 MPa. When plotted, it appeared that predawn needle water potential increased exponentially with NRAI in each repetition of the experiment (Figure 4). Therefore, a logarithmic transformation of NRAI was used to linearize the function for analysis (Neter et al. 1985). Because many seedlings had NRAI= 0 , 1 was added to each value of NRAI before taking its logarithm (Steel and Torrie 1980). Predawn needle water potential was strongly correlated with both NRAI ($p= 0.0001$) and ORAI ($p= 0.004$), as well as with a number of other attributes describing plant water relations ($p= 0.002$ to 0.0001) (Table 13).

The December Repetition

Predawn needle water potential was estimated on 30 seedlings in the December repetition of the experiment. The highest value was -0.72 MPa for a seedling from the 20°C Control treatment. In a simple linear

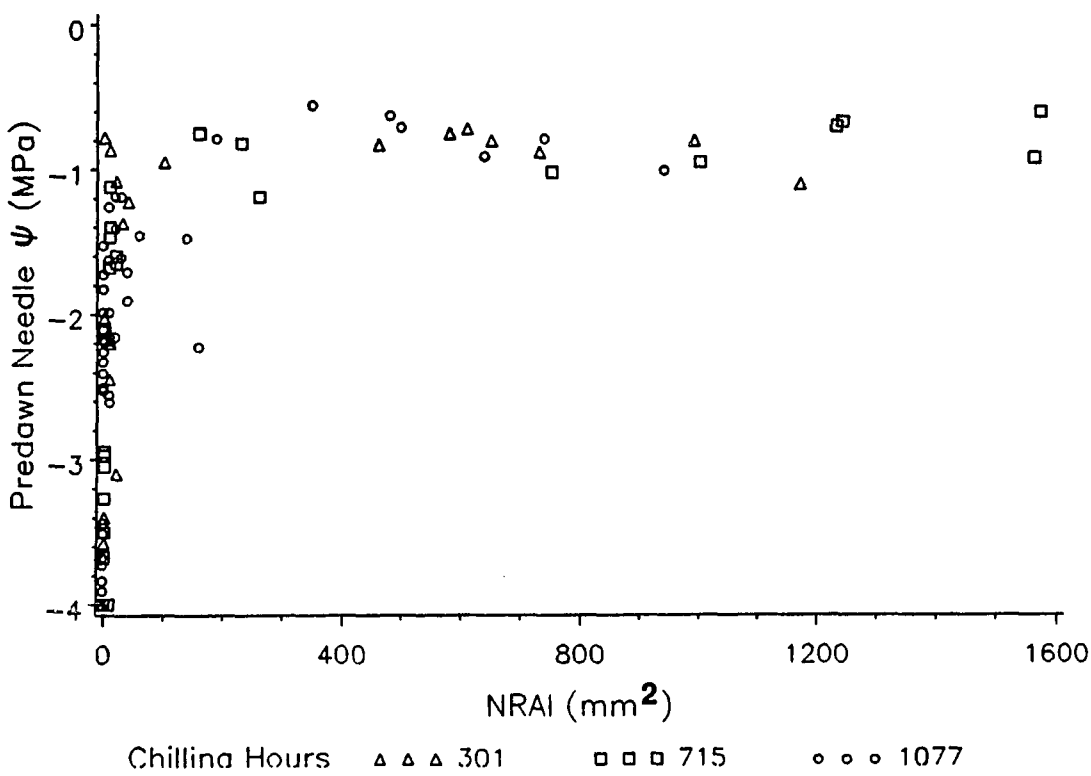

Figure 4. The relationship between predawn needle water potential and new root area index (NRAI) for seedlings in each of the three repetitions of the experiment.

Table 13. Correlation coefficients (r), levels of significance (p), and the numbers of observations (n) among several seedling attributes

Attribute		NRAI[1/]	ORAI	ψ_{pd}	$\Delta\psi_{recov}$	g_n	G_p
SLA	(r)	-0.3686	-0.4764	-0.5220	-0.0799	-0.3815	-0.2807
	(p)	0.0001	0.0001	0.0001	0.4782	0.0001	0.0128
	(n)	198	198	104	81	96	78
G_p	(r)	0.3528	0.1773	0.4017	-0.0109	0.5851	
	(p)	0.0015	0.1205	0.0003	0.9259	0.0001	
	(n)	78	78	78	75	78	
g_n	(r)	0.7178	0.1897	0.6824	0.3753		
	(p)	0.0001	0.0628	0.0001	0.0005		
	(n)	97	97	97	82		
$\Delta\psi_{recov}$	(r)	0.4068	0.0658	0.3435			
	(p)	0.0001	0.5568	0.0016			
	(n)	82	82	82			
ψ_{pd}	(r)	0.5470	0.2790				
	(p)	0.0001	0.0040				
	(n)	105	105				
ORAI	(r)	0.2538					
	(p)	0.0003					
	(n)	199					

[1/] Abbreviations are defined in the Appendix, Table 14.

regression (Table 12), transformed NRAI explained 64.2% of the total variation in predawn needle water potential:

(3.5) $$\psi_{pd} = -3.28 + 0.4029 \ln(NRAI + 1)$$

Both the intercept ($p = 0.0001$) and the ln(NRAI + 1) ($p = 0.0001$) were highly significant terms in the model.

The data shown in Figure 4 suggest that, under the conditions of this experiment, a high value for predawn needle water potential was about -0.8 MPa. For the December repetition, the model predicted that predawn needle water potential would be -0.8 MPa if a seedling had about 470 mm^2 of NRAI.

The January Repetition

The predawn needle water potential was also estimated for 30 seedlings in the January repetition. As in the December repetition, the highest value was for a seedling in the 20°C Control treatment; it was -0.60 MPa. The regression of predawn water potential with transformed NRAI (Table 12) accounted for 74.9% of the total variation:

(3.6) $$\psi_{pd} = -3.37 + 0.4048 \ln(NRAI + 1)$$

Both the intercept ($p = 0.0001$) and the transformed NRAI ($p = 0.0001$) had significant impacts on the model. This model predicted that about 570 mm^2 of NRAI were required to achieve a predawn needle water potential of -0.8 MPa.

The February Repetition

There were 45 seedlings in the February repetition which had predawn needle water potential estimated. The highest value, -0.55 MPa, was the highest among all the seedlings. Transformed NRAI explained 54.7% of the total variation in predawn needle water potential (Table 12):

(3.7) $$\psi_{pd} = -2.84 + 0.3248 \ln(NRAI + 1)$$

As in the other models, both the intercept ($p = 0.0001$) and transformed NRAI ($p = 0.0001$) were highly significant. This model predicted that approximately 530 mm^2 of NRAI would result in a predawn needle water potential of -0.8 MPa.

Comparing the Three Repetitions

The models relating predawn needle water potential to NRAI for the December and January repetitions had very similar estimates of the regression parameters (Figure 5). Probably because of the changes made in the water stress levels before the February repetition of the experiment, the predicted intercept was higher, and the slope of the relationship between water potential and ln(NRAI + 1) was less, than in the previous models (Figure 5). However, the amount of NRAI required to bring predawn needle water potential to a predicted level of -0.8 MPa in the February model was intermediate between the other two models. That is, seedlings in all three repetitions required a similar amount of new root growth to bring their predawn needle water potentials to a level that was optimum for the experimental conditions. The amount of NRAI required

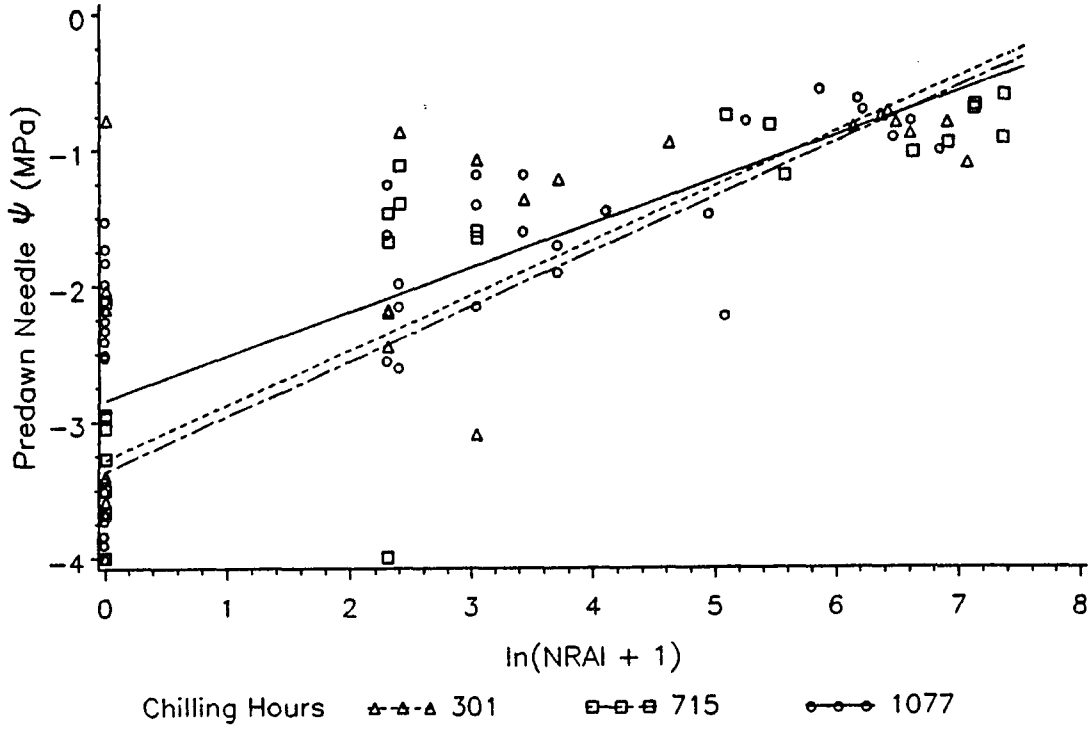

Figure 5. Linear regressions of predawn needle water potential with the logarithm of new root area index plus one (NRAI + 1) for seedlings in each of the three repetitions of the experiment.

to optimize predawn water potentials in these experiments ranged between 470 and 570 mm^2.

In each repetition, the mean NRAI for the 20°C Control root environment treatment exceeded the amount needed to predict that predawn needle water potentials would be greater than -0.8 MPa. Also, individual seedlings in some of the other treatments had NRAIs in excess of the amount required to optimize predawn water potential. However, the average NRAIs for all the 15°C treatments, and for the 20°C water stressed treatments, were at least 50% less than that required to predict a mean predawn needle water potential of -0.8 MPa. Thus, only when seedlings were grown under the most favorable conditions was the average amount of root growth sufficient to alleviate water stress by 28 days after planting.

Water Potential Recovery

Among the three repetitions, the rate of needle water potential recovery during the first 2 hours in the dark was computed for 82 seedlings. The minimum rate was -0.35 MPa h^{-1}. That is, instead of increasing after the lights were turned off, the needle water potential dropped before the final measurement was taken. The continued decline of water potential occurred in about 20% of the seedlings. The maximum rate of recovery was 1.0 MPa h^{-1}. However, only two seedlings had recovery rates greater than 0.6 MPa h^{-1}. Similar to predawn needle water potential, the relationship between water potential recovery rate and NRAI tended to be exponential in two of the three repetitions. That is, a small increase in NRAI resulted in a great increase in the rate of water potential

recovery in the dark. The water potential recovery rate was correlated with NRAI ($p = 0.0001$), predawn needle water potential ($p = 0.002$), and stomatal conductance ($p = 0.0005$) (Table 13).

The December Repetition

The model for the 22 seedlings evaluated in the December repetition was not significant (MSE= 0.0705, $F_{(1;20)} = 0.2$, $p = 0.7$). Figure 6 shows that the water potential recovery rate in the December repetition was essentially the same, regardless of the NRAI.

The January Repetition

In the January repetition, the water potential recovery rates of 23 seedlings were evaluated. The simple linear regression with the logarithm transformed NRAI was significant (Table 12) and explained 65.4% of the variation in recovery rate:

$$\Delta\psi_{recov} = -0.0904 + 0.0538 \ln(NRAI + 1) \quad (3.8)$$

The intercept differed from zero ($p = 0.02$), and the transformed NRAI had a highly significant ($p = 0.0001$) impact of the recovery rate after 2 hours in the dark. The model suggests that among seedlings with no new roots needle water potential continued to decline for at least 2 hours after the lights in the growth chamber were turned off. However, for seedlings with root growth, the water potential recovery rate was positive and increased exponentially with NRAI (Figure 6).

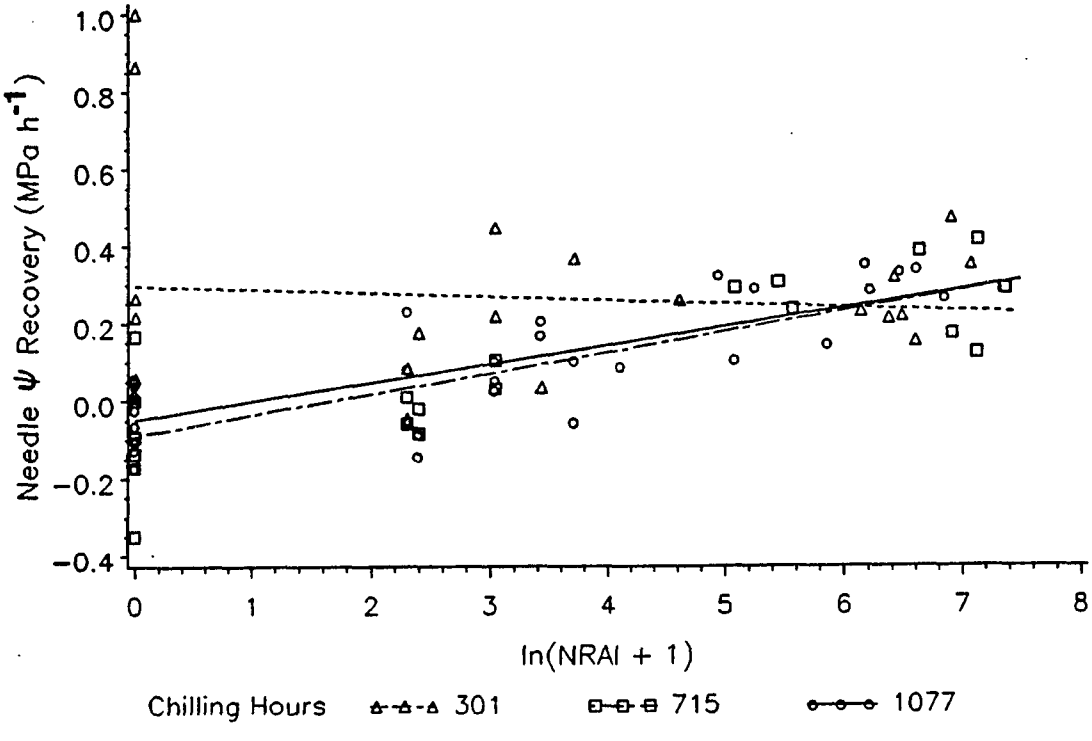

Figure 6. Linear regressions of needle water potential recovery rate during the first 2 hours in the dark with the logarithm of new root area index plus one (NRAI + 1) for seedlings in each of the three repetitions of the experiment.

The February Repetition

In the last repetition of the experiment, the water potential recovery rate was evaluated for 37 seedlings. The logarithm of the NRAI accounted for 58.9% of the total variation (Table 12) in the water potential recovery rate:

(3.9) $$\Delta\psi_{recov} = -0.0492 + 0.0482 \ln(NRAI + 1)$$

Similar to the model in the January repetition, the intercept was significantly different from zero ($p = 0.05$), and the transformed NRAI was highly significant ($p = 0.0001$). The February model also predicted that unless seedlings had some NRAI needle water potential would not begin recovering within 2 hours in the dark. Among seedlings with NRAI, the rates of water potential recovery were similar to those in the January repetition (Figure 6).

Comparing the January and February Repetitions

As discussed previously, NRAIs of 570 mm^2 and 530 mm^2, respectively, were required to optimize predawn needle water potential under the experimental conditions of the January and February repetitions. When applied to the water potential recovery models, those amounts of NRAI predicted mean recovery rates of 0.25 ± 0.06 MPa h^{-1} in the January repetition, and 0.25 ± 0.04 MPa in the February repetition. These results suggest that if needle water potential increased at a rate of about 0.25 MPa h^{-1} after about 2 hours in the dark, then seedling root growth was sufficient to alleviate the water stress induced by transplanting.

NRAI and Stomatal Conductance

The stomatal conductance of each sample seedling was measured twice for this study, at the times corresponding to the measurements of needle water potential taken in the light. The overall means and SEs were 6.36 ± 0.73, and 5.71 ± 0.66 mmol m^{-2} s^{-1}, respectively, for the measurements taken after an average of 2.2 and 4.7 hours of light. For each of the repetitions, the means for the two measurements were not different ($0.18 < |t|_{(.95;42\text{-}88)} < 0.92$, $0.4 < p < 0.9$). Therefore, the mean of the two measurements was used in subsequent analyses. Mean stomatal conductance was correlated with NRAI and the other attributes of seedling water relations (p= 0.0005 to 0.0001); however, it was only weakly correlated with ORAI (p= 0.06) (Table 13). When ORAI was used as an independent variable along with NRAI in regression models for stomatal conductance, it was not significant for any of the three repetitions (p= 0.4 to 0.9). Therefore, only the simple linear models of stomatal conductance with NRAI are discussed.

The December Repetition

For the 22 seedlings in the first repetition of the experiment, NRAI accounted for 31.6% of the total variation (Table 12) in mean stomatal conductance:

(3.10) $$g_n = 5.4071 + 0.00850 \text{ NRAI}$$

Both the intercept (p= 0.0003) and NRAI (p= 0.006) were significant. This model predicted that each 10 mm^2 of NRAI increased mean stomatal conductance by 0.085 ± 0.028 mmol m^{-2} s^{-1}, or by 1.6% (Figure 7).

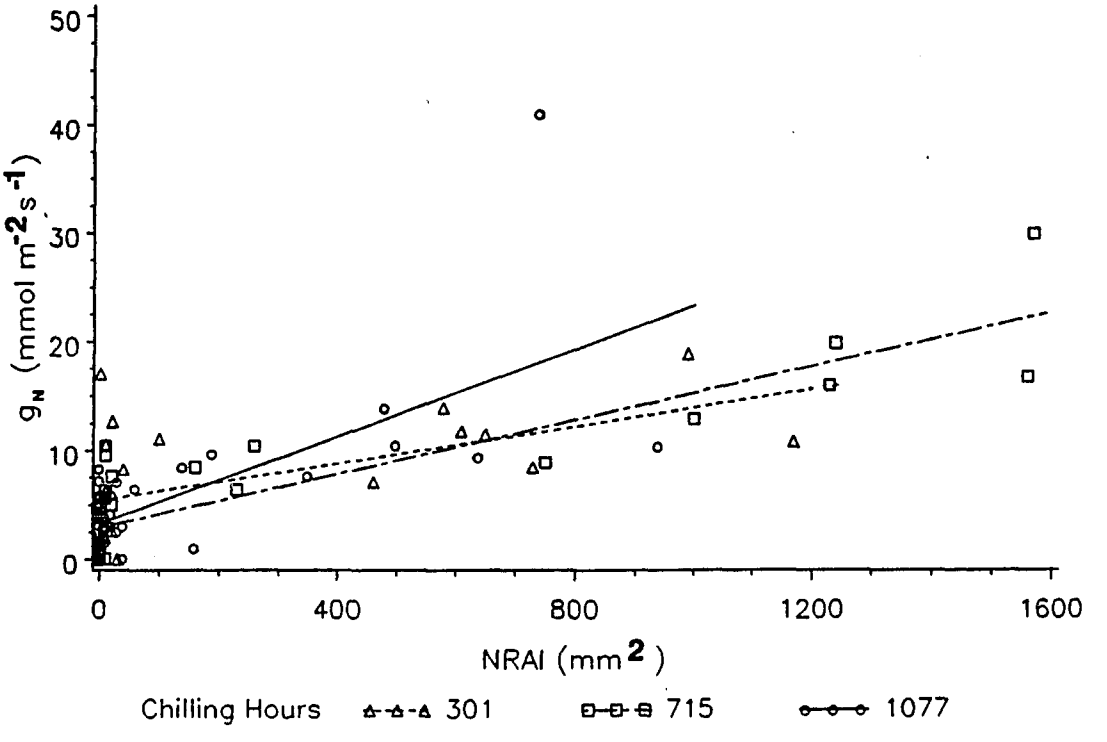

Figure 7. Linear regressions of mean stomatal conductance (g_n) with new root area index (NRAI) for seedlings in each of the three repetitions of the experiment.

The January Repetition

The stomatal conductance of 30 seedlings was measured during the January repetition of the experiment. There was a strong relationship between mean stomatal conductance and NRAI:

(3.11) $$g_n = 2.9131 + 0.01237 \text{ NRAI}$$

In this model, NRAI explained 76.2% of the total variation in conductance (Table 12). As in the earlier repetition, both the intercept ($p = 0.0005$) and NRAI ($p = 0.0001$) were highly significant. In this model, each 10 mm^2 of NRAI was predicted to increase mean stomatal conductance by 0.124 ± 0.013 mmol m^{-2} s^{-1}, or 4.2% (Figure 7).

The February Repetition

In the February repetition, stomatal conductance was measured on 45 seedlings. As in the other repetitions there was a significant linear relationship between mean conductance and NRAI:

(3.12) $$g_n = 3.2744 + 0.02002 \text{ NRAI}$$

The strength of this relationship was intermediate between the other models, explaining 47.3% of the total variation in conductance (Table 12). Both the intercept and NRAI were highly significant ($p = 0.0001$). Among the three repetitions, this model predicted the greatest increase in mean stomatal conductance with NRAI; however, the rate of increase was affected by a single influential observation (Figure 7). Nevertheless, the model predicted an increase in mean stomatal conductance of 0.200 ± 0.032 mmol m^{-2} s^{-1}, or 6.1%, for each 10 mm^2 of NRAI (Figure 7).

Comparing the Three Repetitions

The positive relationship between NRAI and stomatal conductance was significant for each repetition. The rate of increase in conductance was about 5% for each 10 mm^2 of new roots. Consequently, the effect that NRAI was predicted to have on stomatal conductance was of the same magnitude as it was for absorptive capacity.

Stomatal conductance of well established trees is often much higher than the values obtained in this study. For example, Carlson et al. (1988) measured maximum conductances of 150 to 200 mmol m^{-2} s^{-1} on six-year old loblolly pines in the field. On three of their five measurement days, mean stomatal conductance was less than 20 mmol m^{-2} s^{-1}, and on the other days the means were about 40 and 60 mmol m^{-2} s^{-1}.

One reason for the relatively low conductances in this research was the light level in the growth chamber. For loblolly pine seedlings, Teskey et al. (1986) showed a marked response of stomatal conductance to increasing irradiance up to the maximum they tested, 1,450 μmol m^{-2} s^{-1}. Interpolating from the data presented by Teskey et al. (1986) suggests that the light level used in this research, 750 - 800 μmol m^{-2} s^{-1}, resulted in a mean stomatal conductance about 75% of that at 1,450 μmol m^{-2} s^{-1}.

Predawn Needle Water Potential and Stomatal Conductance

A secondary objective was to examine the relationship between predawn needle water potential and stomatal conductance. Among two-year old loblolly pine seedlings growing in pots in a greenhouse, stomatal closure occurred at approximately -2.0 MPa (Teskey et al. 1986). In a

field experiment with six-year old loblolly pine, Carlson et al. (1988) found that minimal conductance levels of 3 - 5 mmol m^{-2} s^{-1} coincided with predawn needle water potentials of -1.6 to -2.3 MPa. Both studies showed that stomatal conductance increased with increasing needle water potentials (Carlson et al. 1988, Teskey et al. 1986).

Carlson et al. (1988) also showed that stomatal conductance is more closely related to predawn needle water potential than to the water potential at the time conductance is measured. The same relationship was found in this research. When stomatal conductance was measured at about 2.2 hours after the lights came on in the growth chamber, it was better correlated with predawn needle water potential ($r = -0.63$) than with the water potential measured at the same time as conductance ($r = -0.50$). Similarly, after an average of 4.7 hours of light, conductance had a stronger relationship with predawn water potential ($r = -0.62$) than with the corresponding water potential measurement ($r = -0.56$). The four correlations were all highly significant ($p = 0.0001$).

Predawn needle water potential predicted stomatal conductance better than the water potential which coincided with the conductance measurement for a number of possible reasons. Because water absorption lags behind transpiration, midday water potential is not as good an indicator of water status as is predawn water potential (Kramer 1983). Furthermore, plant water status is only one of several factors that influence stomatal movements (Cowan 1977).

In all three repetitions of this research, mean stomatal conductance increased exponentially as predawn needle water potential increased. Therefore, the logarithm of the absolute value of predawn needle water

potential was used to analyze the effect of water potential on stomatal conductance.

The December Repetition

In the December repetition there was a significant linear relationship (Table 12) between stomatal conductance and transformed predawn needle water potential:

(3.13) $$g_n = 10.292 - 7.9020 \ln(|\psi_{pd}|)$$

This model explained 66.8% of the total variation in stomatal conductance, and both the intercept and the independent variable were highly significant ($p = 0.0001$). The model predicted that a minimal mean daytime conductance level of 5 mmol m^{-2} s^{-1} occurred if the predawn needle water potential was -1.95 MPa (Figure 8).

The January Repetition

Transformed predawn water potential accounted for 70.5% of the variation in conductance (Table 12) in the January repetition:

(3.14) $$g_n = 12.278 - 10.1119 \ln(|\psi_{pd}|)$$

As in December, the regression parameters were both important ($p = 0.0001$). The model predicted that a predawn needle water potential of -2.05 MPa would result in a mean stomatal conductance of 5 mmol m^{-2} s^{-1} (Figure 8).

The February Repetition

As in the previous repetitions, conductance was related to transformed predawn needle water potential (Table 12), but in this case just 36.6% of the variation was accounted for:

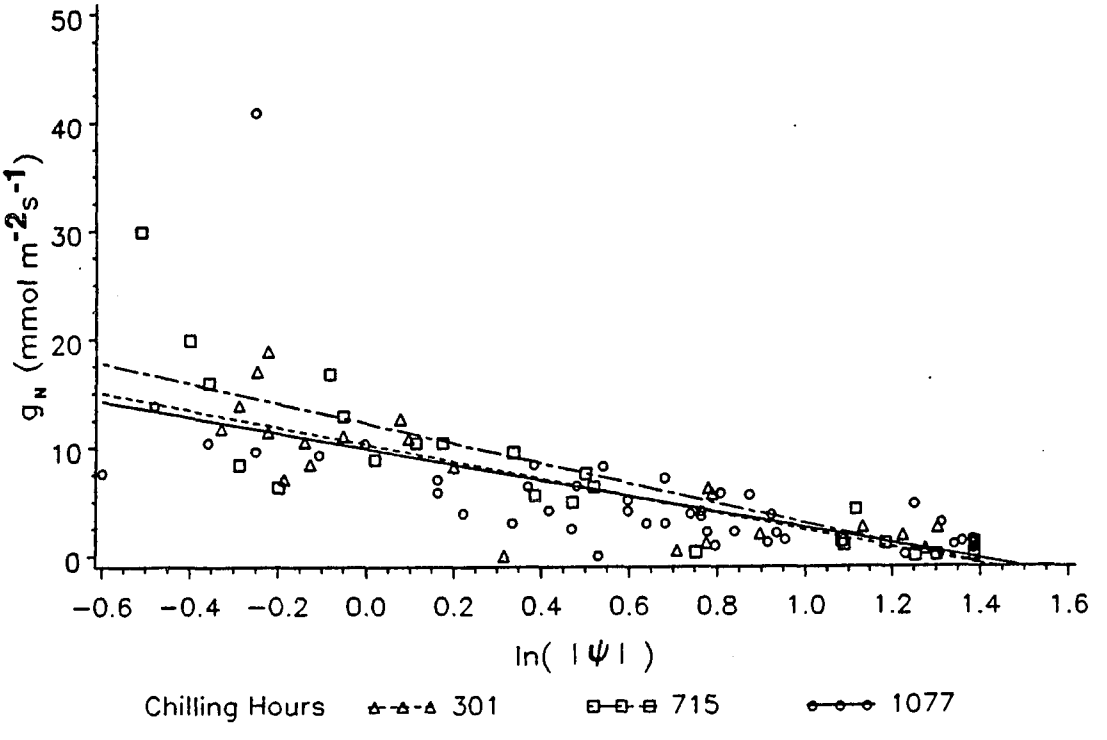

Figure 8. Linear regressions of mean stomatal conductance (g_n) with the logarithm of the absolute value of predawn needle water potential ($|\psi_{pd}|$) for seedlings in each of the three repetitions of the experiment.

(3.15) $$g_n = 10.260 - 8.8259 \ln(|\psi_{pd}|)$$

Although the regression was not as strong as in the earlier repetitions, both the intercept and the transformed water potential were highly significant ($p = 0.0001$). Among these seedlings, a predawn needle water potential of -1.81 MPa was expected to yield a mean conductance of 5 mmol m^{-2} s^{-1} (Figure 8).

Root Growth, Water Potential, and Stomatal Conductance

In all repetitions of this research, the predawn needle water potential that resulted in minimal stomatal conductances was similar to those reported for loblolly pine (Carlson et al. 1988, Teskey et al. 1986). The relationships among predawn needle water potential, NRAI, and conductance suggest that the root system, especially if root growth occurred, exerted a strong influence on stomatal movements. There are at least two mechanisms by which roots could influence the stomates. The most obvious is the direct impact new roots have on improving needle water potential, which would include the stomatal guard cells. Another mechanism whereby the root system can affect stomatal conductance is through an interaction among plant hormones. Recently Mansfield (1987) reviewed the literature showing that cytokinin, which is produced in the roots, interacts with auxin and abscisic acid to provide an elegant and precise mechanism for stomatal control. Such a hormonal control system could be very sensitive to the external plant environment, including water availability in the soil.

Plant Hydraulic Conductivity

Many of the stressed seedlings had low predawn needle water potentials, and water potential did not drop further during the day. Therefore, the driving force for water movement in those seedlings was zero. Thus, there was a total of 78 seedlings for which the driving force and, consequently, plant hydraulic conductivity and total uptake could be calculated. For plant hydraulic conductivity the mean and SE were 0.29 $\pm$ 0.028 mmol m^{-2} s^{-1} MPa^{-1}, which corresponded to a mean and SE for total water uptake of 0.016 $\pm$ 0.0016 mmol s^{-1} MPa^{-1}.

The ratio of root system permeability to plant hydraulic conductivity (L_p/G_p)--or water absorption by each m^2 of root surface to water loss from each m^2 of needle surface--averaged 6.3 with an SE of $\pm$ 1.13. However, the estimated total needle surface area (TNSA) of a seedling was always larger than the TOTRAI. Estimated TNSA averaged 0.0542 $\pm$ 0.0012 m^2, while the TOTRAI averaged 0.0071 $\pm$ 0.0002 m^2. Consequently, the mean transpirational surface area to absorptive surface area ratio was 7.9 $\pm$ 0.2, and therefore, the mean ratio of absorptive capacity to total plant water uptake (L_R/G_T) was 0.94 $\pm$ 0.18. There was a lot of variation associated with L_R/G_T because it ranged from 0, for those seedlings with absorptive capacity equal to 0, to a high of 8.7. Likewise, the L_p/G_p was highly variable because of how much more permeable new root tissue was than old roots, and because of the variation in the amount of root growth that occured.

Because of the relatively uniform IRAIs in these experiments, plant hydraulic conductivity and total water uptake were closely related attributes ($r = 0.96$, $p = 0.0001$). Plant hydraulic conductivity was also

highly correlated with stomatal conductance (p= 0.0001) and predawn needle water potential (p= 0.0003) (Table 13). It was less strongly related to NRAI (p= 0.002) and specific leaf area (p= 0.01), and was not significantly correlated with ORAI (p= 0.1) or water potential recovery rate (p= 0.9) (Table 13).

Specific Leaf Area

For this research, SLA was defined as the ratio of estimated total needle surface area (in m^2) of a sample of needles to the dry weight of that sample (in g). SLA is a morphological attribute, but it was correlated with most of the response variables evaluated in this research. It was related to predawn needle water potential (p= 0.0001), stomatal conductance (p= 0.0001), and plant hydraulic conductivity (p= 0.01), but not with water potential recovery rate (p= 0.5) (Table 13). It was also correlated with both NRAI (p= 0.0001), and ORAI (p= 0.0001) (Table 13). SLA was always negatively correlated with other variables. That is, high values of SLA were associated with low water potentials; low conductance or plant conductivity values; little new root growth; and fewer, or shrunken, old roots.

The negative correlations between either ORAI or NRAI and SLA show that the relationship between root system size and SLA was an inverse one. As stored carbohydrates are used and not replaced, and if the surface area does not change, the ratio of needle surface area to ovendry weight will increase. In this study, high SLA coincided with root systems that had relatively less initial surface area and that did not grow new roots. The relationship between ORAI and SLA suggests that seedlings planted with

larger root systems depleted fewer of the reserves stored in the needles than did seedlings with less root mass. Furthermore, the relationship between NRAI and SLA suggests that the reserves stored in the needles were not expended on new root growth.

Carbohydrates stored in the foliage of seedlings are important for meeting the respiration demands of the plant until photosynthesis resumes after outplanting (Marshall 1985). In Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco) new root growth depends on current photosynthate (Philipson 1988, van den Driessche 1987). For other species, such as Sitka spruce (Picea sitchensis (Bong.) Carr.) and ponderosa pine, the data are either contradictory or inconclusive (Philipson 1988, van den Driessche 1987). Thus, in shortleaf pine it may be that stored reserves are not sufficient to sustain vigorous root growth and meet the respirational demands of the rest of the seedling as well. If such is the case, those seedlings that had high SLAs may have depleted their reserves on respiration, thereby making them incapable of the root growth that markedly improved overall water status.

CHAPTER 4

SUMMARY AND CONCLUSIONS

This research was designed to study the establishment phase of artificial regeneration using bare-root shortleaf pine seedlings. Lifting bare-root seedlings from a nursery results in a significant loss of roots; especially the fine, higher order lateral roots that are important for water and nutrient uptake. Therefore, new root development after transplanting is essential if seedlings are to regain normal physiological functioning. The ability of a seedling to grow new roots is determined both internally by its physiological condition and externally by the root zone environment.

In this research, several related experiments focused on new root development after transplanting, the impact of root growth on the ability of root systems to absorb water, and the effects of new roots on several aspects of seedling water relations. The research had two goals: to provide a better understanding of environmental effects on pine seedling root growth during the first four weeks after outplanting and to describe the impacts new roots have on water absorption and seedling water relations.

To address the goals of the research, the study had three major objectives: (1) describe the effects of root zone temperature and water availability on new root growth after transplanting; (2) determine the

relative importance of old and new root surface area on the capacity of the seedling root systems to absorb water; and, (3) determine to what extent the amount of new root growth affects several measures of seedling water status. There were also several secondary objectives: (1a) determine the minimum level of root tissue hydration, measured by relative water content, required for new root development; (1b) determine to what extent the surface area of old roots, those that were present when seedlings were planted, were affected by the root zone environment; and, (3a) examine the relationship between stomatal conductance and predawn needle water potential.

The root growth and water relations portions of the research were conducted in a growth chamber where the shoot and root environments were controlled. The experiments designed to determine the effects of old and new roots on root system absorptive capacity were conducted in a laboratory to ensure that the experimental conditions were reproducible. Controlled, rather than field, conditions were used to eliminate the environmental variation that would otherwise confound the expression of the physiological responses that were of interest. The experiments were repeated three times between December 1988 and April 1989. They were repeated because the growth chamber provided limited space; therefore, repeating the experiments increased the sample size, and thereby the power of the statistical tests used to analyze the results.

To evaluate environmental impacts on root growth, the experimental treatments consisted of six root zone environments. Those environments were defined by the factorial arrangement of two root zone temperatures and three levels of soil water potential. The root zone temperature was

held constant at either 15°C or 20°C. Soil water potential was kept at three qualitative levels by controlling the distance that the seedling root systems were above water in a conductive column, and by controlling the conductivity of the column.

The root zone temperatures were maintained by water baths. The conductive columns were constructed from sections of plastic pipe into which a series of three floral foam blocks were placed to provide uniform hydraulic conductivity. The conductivity of the column was reduced by inserting a ceramic disk of known conductivity between two of the blocks. The seedlings were potted in masonry sand in pots that rested atop the conductive columns. The sand was separated from the foam blocks by nylon filter cloth which allowed water movement but was too fine for roots to penetrate. Water was supplied to the conductive columns through plastic tubing between the base of the columns and reservoirs. The water in the reservoirs was maintained at the levels desired in the columns. Water was supplied to the seedlings by capillary action; first through the foam blocks and ceramic disk where it was present, then across the nylon cloth, and finally through the sand. The shoot environment in the growth chamber was maintained at a constant 20°C with a 14 hour photoperiod.

Among the three levels of soil water potential, one was a well watered control treatment and two were considered water stress treatments. In the control treatments the water level was nearer to the seedling root systems than in the stress treatments. For the two stress treatments the distance between the root systems and the water level was the same. The stress levels differed because the conductive columns for the more moderate treatment were composed just of the floral foam blocks, while the

more severe treatment had a ceramic disk between the middle and top blocks to restrict water movement.

After 29 days in the growth chamber, the seedlings were carefully washed from the sand. Root system absorptive capacity was then measured by collecting the water that exuded from the detopped root systems while they were under hydrostatic pressure. Positive hydrostatic pressure was used to force water through the root systems in a manner analogous to the negative pressure that pulls water through a transpiring plant. The detopped seedlings were sealed in the lid of a water-filled vessel so that the roots were suspended in the water. Water was circulated between the vessel and a reservoir in which the water was kept at 20°C. The water that exuded from the root systems was collected in wicks during measured intervals of time and then weighed to determine absorptive capacity.

After absorptive capacity was measured the root systems were separated into new and old roots. New roots are readily distinguished from old roots by their appearance. The amount of new root growth was measured as its projected surface area using an image analyzer. This measure was called the new root area index (NRAI). The projected surface area of the old roots was measured in the same way, and it was called the old root area index (ORAI). From those data, the percentage of new root area (PNRA) was calculated.

Before the experiments began, a nondestructive measurement of each seedling's overall initial root area index (IRAI) was obtained. It was used as a selection criterion to narrow the range of acceptable root system sizes that were included in the study. Furthermore, the ORAI was divided by the IRAI as an index to determine how the treatments affected

the planted root systems. This value was called the change in root area index (CRAI).

Several aspects of water status were measured the day before absorptive capacity and root growth were measured; that is, when the seedlings had been undisturbed in the root environment chambers for 28 days. The water relations parameters that were measured included needle water potential, stomatal conductance, and water flux or transpiration on a unit area basis. Water potential was estimated as xylem pressure potential which was measured using a pressure chamber. Stomatal conductance and water flux were measured with a steady state porometer. Needle water potential was first determined before the lights in the growth chamber came on to represent a predawn measurement. It was later measured twice in the light to correspond with measurements of stomatal conductance and water flux. Finally, needle water potential was measured after about 2 hours in the dark. The rate of water potential recovery after transpiration was stopped by darkness was then calculated. Assuming that predawn needle water potential is a useful measure of soil water potential, the water potential gradients that drive transpiration were calculated. The measured water flux divided by the water potential gradient yields plant hydraulic conductivity which also provides an index of seedling water status.

Some other attributes were also measured. The relative water contents of the root systems and of a sample of needles were determined as indicators of tissue hydration. Needle moisture content was used as the criterion for defining seedling mortality. Finally, the specific leaf

area (SLA) of each seedling was determined as the ratio of needle surface area to ovendry weight of a sample.

In general, the results of this study show that under controlled conditions the root zone environment accounts for about one-half of the variation in the new root growth that occurs soon after planting. The rest of the variation is probably the result of factors internal to the seedlings, such as genetic potential and physiological condition. Under field conditions, the root zone environment may be more or less important than it was in these experiments, depending on how limiting the environment is for root growth. Root growth at 15°C was limited compared to the amount of growth that occurred at 20°C, but the lower temperature did not prevent root growth. However, root growth was severely reduced and even prevented by the water stress treatments imposed in this study. Moreover, root zone temperature and soil water potential interacted in their effects on seedling root growth.

New root growth was the dominant factor correlated with root system absorptive capacity, and a number of measures of seedling water status as well. The amount of new root growth explained at least 50% of the variation in absorptive capacity, predawn needle water potential, and stomatal conductance when those attributes were measured 4 weeks after transplanting. Such results emphasize the importance of root growth to the establishment process.

This research yielded several significant findings that have not been reported previously. Important conclusions, listed by objective, include:

Objective 1

The root zone temperature x soil water potential interaction was measured in two repetitions of the 29-day growth chamber experiment. It accounted for 15% and 21% of the explained variation in the PNRA. There was always more root growth at 20°C than at 15°C. The interaction was important because as soil water availability declined, the amount of root growth decreased much more rapidly at 20°C than at 15°C.

Objective 1a

New roots were present only when the relative water content of the root tissue was greater than about 85%. That is, there was a sharply defined minimum level of tissue hydration required for root growth among the seedlings in these experiments. However, there was no relationship between the root relative water content and the amount of root growth that occurred within 4 weeks after transplanting. Furthermore, many seedlings with root relative water contents greater than 85% did not produce any new roots. Thus, although good hydration was required for root growth, it was no assurance that roots would grow.

Objective 1b

For this research, the projected surface area of the root systems was measured before the seedlings were transplanted and again at the end of the experiments. If the root zone environment had no affect on the old roots, then the average ratio between the two measurements of root surface area would not differ among the treatment combinations. However, the mean ratio did differ significantly among the treatments. The results suggest that the root systems of the seedlings in the water stress treatments

either shrank or suffered fine root mortality, most likely due to desiccation in either case.

Objective 2

The absorptive capacity and permeability of pine seedling root systems depended primarily on the amount of new root tissue. During the three repetitions of the experiment, the NRAI explained from 43 to 71% of the total variation in absorptive capacity. At a driving force of 0.3 MPa, each 10 mm^2 of NRAI increased absorptive capacity by about 3 to 5%. The ORAI did not significantly affect absorptive capacity, most likely because seedlings from a narrow range of IRAIs were selected for the research.

Objective 3

New roots resulted in a significant improvement in seedling water status. How new roots improved water relations, and to what degree, varied with the attribute studied. Among the variables used in this research to describe water status, those that were most influenced by new root growth were needle water potential, water potential recovery rate in the dark, and stomatal conductance. A morphological characteristic, SLA, correlated well with the measures of water status and with both ORAI and NRAI.

Needle Water Potential

There was an exponential increase in predawn needle water potential with NRAI. Among the repetitions, the logarithm transformed NRAI accounted for 55 to 75% of the variation in predawn water potential.

About 500 to 550 mm^2 of NRAI was sufficient to maximize predawn water potential at about -0.8 MPa in these experiments. By 28 days after planting many seedlings had new root growth in excess of that amount. However, only in the 20°C Control treatment did the average NRAI exceed that required for an optimal predawn water potential.

Water Potential Recovery Rate

The rate at which needle water potential increased during the first 2 hours in the dark was not related to NRAI in the first repetition, the repetition which included the most severely water stressed seedlings. Like predawn needle water potential, however, it was exponentially related to the amount of NRAI in the other repetitions. The amounts of NRAI required to optimize predawn water potential resulted in a water potential recovery rate of 0.25 MPa h^{-1} for both the January and February repetitions of the experiment.

NRAI and Stomatal Conductance

Stomatal conductance was linearly related to the amount of new root growth. Among the repetitions, NRAI explained from 32 to 76% of the variation in conductance. Each 10 mm^2 of NRAI increase conductance by 2 to 6%, which is of the magnitude of the predicted impact of NRAI on absorptive capacity.

Objective 3a

Stomatal conductance was more strongly related to predawn needle water potential than it was to water potential at the time conductance was measured. The relationship between predawn needle water potential and

stomatal conductance was an exponential one, accounting for 37 to 70% of the variation. Minimal stomatal conductance values of about 5 mmol m^{-2} s^{-1} coincided with predawn needle water potentials of -1.8 to -2.0 MPa.

Establishment is the complex process by which the physiological functions of seedlings return to normal after transplanting. The water stress that is brought about by the disturbances associated with lifting, handling, and planting is alleviated during the establishment process. This research has provided some of the information needed to better understand the physiological responses related to root growth and water relations of transplanted shortleaf pine seedlings.

APPENDIX

Table 14. Derivation and units of the abbreviations used in this research

Abbreviation or Symbol	Name	Derivation	Units
A_R	Total root surface area	pi x TOTRAI (x 10^{-6})	m^2
CH	Chilling hours	recorded at nursery	°C
CRAI	Change in root area index	ORAI/IRAI	none
g_n	Stomatal conductance	measured	mmol m^{-2} s^{-1}
G_p	Plant hydraulic conductivity	$q/\psi_{pd} - \psi_n$	mmol m^{-2} s^{-1} MPa^{-1}
G_T	Total plant water uptake	G_p x TNSA	mmol s^{-1} MPa^{-1}
IRAI	Initial root area index	measured	mm^2
L_p	Root system permeability	L_R/A_R	mmol m^{-2} s^{-1} MPa^{-1}
L_R	Root system absorptive capacity	measured	mmol s^{-1} MPa^{-1}
N	Number of observations	counted	none
NRAI	New root area index	measured	mm^2
ODW	Oven dry weight	measured	g
OLRAI	Old lateral root area index	measured	mm^2

ORAI	Old root area index	OLRAI + OTRAI	mm^2
OTRAI	Old taproot area index	measured	mm^2
PNRA	Percentage new root area	NRAI/(NRAI + ORAI) (x 100)	%
ψ_d	Needle water potential in the dark	estimated by measuring xylem water potential	MPa
ψ_n	Needle water potential in the light	estimated by measuring xylem water potential	MPa
ψ_{pd}	Predawn needle water potential	estimated by measuring xylem water potential	MPa
$\Delta\psi_{rec}$	Needle water potential recovery rate	$\psi_d - \psi_n$/time lapse	MPa h^{-1}
q	Water flux	measured	mmol m^{-2} s^{-1}
RAI	Root area index	measured	mm^2
RGP	Root growth potential	RGP = NRAI in this research	mm^2
RWC	Relative water content	fresh weight - ODW/ turgid weight - ODW (x 100)	%
SA_p	Sample needle projected surface area	measured	cm^2
SA_T	Sample needle total surface area	-0.2728 + 4.0141 SA_p (x 10^{-5})	m^2
SLA	Specific leaf area	SA_T/sample ODW	m^2 g^{-1}
TNSA	Total needle surface area	SLA x total needle ODW	m^2
TOTRAI	Total root area index	ORAI + NRAI	mm^2

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VITA

John Closs Brissette was born December 4, 1949 in Big Rapids, Michigan, the youngest of three children of Henry and Ruth Brissette. He attended public and Catholic schools in Stanwood, Big Rapids, and North Muskegon, Michigan. He graduated from Reeths-Puffer Senior High School in North Muskegon in June 1968.

On August 23, 1968 he enlisted in the United States Army. He attended the Army Aviation School and graduated as a Warrant Officer Aviator on July 28, 1969. After training in CH-47 cargo helicopters, he was assigned to Vietnam in October 1969. In Vietnam he flew combat support missions and was an aircraft maintenance officer and test pilot. In October 1970 he was reassigned to Fort Benning, Georgia, where he continued to serve as an aviator until released from active duty as a Chief Warrant Officer on July 27, 1972.

He entered The University of Michigan in September 1972, and graduated with a B.S.F. degree in December 1975. He served as a Teaching and Research Assistant in the School of Natural Resources at The University of Michigan and received a M.F. degree in April 1977. The results of his research are recorded in the thesis: Juvenile Height Growth of Families of Populus grandidentata, P. tremuloides, and P. xsmithii.

In April 1977 he accepted a position as a Supervisor of Research in the Department of Forestry at Michigan State University. In that position he supervised the field operations of the Michigan State Cooperative Tree Improvement Program. In November 1979 he accepted a position in Jackson, Mississippi, with the United States Department of Agriculture, Forest Service, Southeastern Area, as a Nursery and Tree Improvement Specialist. He provided technical assistance and training to state forestry agencies and forest industry in the South. In May 1984 he transferred to his current position as a Research Forester with the Forest Service's Southern Forest Experiment Station in Pineville, Louisiana. His research assignment is in the seedling production and establishment phases of artificial regeneration of southern pines.

He was admitted to the Graduate School at Louisiana State University in August 1986, and was accepted as a candidate for the Ph.D. degree in Forestry in October 1988. His graduate course work at Louisiana State University was supported by the Southern Forest Experiment Station through the Government Employees Training Act.

He married Linda Marie Gonzalez of North Muskegon, Michigan on February 1, 1969 in Mineral Wells, Texas. They have three children: Catherine Ayn, born June 21, 1972 at Fort Benning, Georgia; Emily Ruth, born February 27, 1979 in Lansing, Michigan; and Charles Closs, born November 28, 1980 in Jackson, Mississippi.

He is a member of the Society of American Foresters; the forestry honorary society, Xi Sigma Pi; and the honor society of agriculture, Gamma Sigma Delta. He is also a certified silviculturist with the Southern Region of the Forest Service.

DOCTORAL EXAMINATION AND DISSERTATION REPORT

Candidate: John Closs Brissette

Major Field: Forestry

Title of Dissertation: Root growth, root function, and water relations of shortleaf pine (Pinus echinata Mill.) bare-root seedlings transplanted into different edaphic environments.

Approved:

Major Professor and Chairman

Dean of the Graduate School

EXAMINING COMMITTEE:

Date of Examination:

March 22, 1990