EFFECTS OF MOISTURE STRESS CONDITIONING AND THE PRESENCE OF NEW ROOTS ON THE WATER RELATIONS PARAMETERS DERIVED FROM PRESSURE VOLUME CURVES

By

FERIT KOCACINAR

Bachelor of Forest Engineering

University of Istanbul

Istanbul, Turkey

1993

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
December, 1997

OKLAHOMA STATE UNIVERSITY

EFFECTS OF MOISTURE STRESS CONDITIONING AND THE PRESENCE OF NEW ROOTS ON THE WATER RELATIONS PARAMETERS DERIVED FROM PRESSURE VOLUME CURVES

Thesis Approved:

Thesis Advisor

Wayne B Powell

Dean of the Graduate College

ACKNOWLEDGMENTS

I would like to express my sincere appreciation to my major advisor, Dr. Stephen W. Hallgren for his great assistance, constructive guidance, understanding, inspiration, support, and friendship. I would also like to thank Dr. Bjorn Martin and Dr. Niels Mannes, my graduate committee members, for their helpful and careful suggestions.

I wish to express my sincere gratitude to Mr. David Ferris for his friendship and invaluable assistance in the experiment and in analyzing the data. Special thanks go to my friends Mr. Mustafa Mirik, Hasan Gök, and Mrs. Gulnara Medeubekova for their assistance and support. I thank the Department of Forestry at Oklahoma State University and the Faculty of Forestry, especially Dr. Adil Çaliskan at the University of Istanbul, Turkey.

Finally, I also wish to thank my family for their support, encouragement, and patience during my study.

TABLE OF CONTENTS

Chapter Page

LIST OF TABLES

Chapter Page

I. The effects of moisture stress conditioning on the water relations parameters of roots and shoots of bare-root loblolly pine (*Pinus taeda* L.) seedlings

II. Effects of new roots on pressure volume curve parameters of loblolly pine seedlings

LIST OF FIGURES

I. The effects of moisture stress conditioning on the water relations parameters of roots and shoots of bare-root loblolly pine (*Pinus taeda* L.) seedlings

II. Effects of new roots on pressure-volume curve parameters of Loblolly pine seedlings

Chapter I

The Effects of Moisture Stress Conditioning on the Water Relations Parameters of Roots and Shoots of Bare-Root Loblolly Pine (*Pinus taeda* L.) Seedlings

Introduction

As the population of the world rises and forested areas decrease, demand on wood and timber production and on secondary products of the forest increases. Approximately, 33% of the total land area of the United States, 737 million acres, is forest land which is 70% of the area that was forested in the year 1630. Between 1850 and 1910, about 190 million acres were converted to other land uses, mainly to agricultural uses (Powell et al., 1992). Planting might be preferable, and may be the only way, to reestablish deforested or unproductive forest land. Total US forest planting on all ownerships was 138,970 acres in 1930. This number has increased annually and reached a peak in 1988 with 3,394,478 acres (USDA, 1989).

The southern United States, with 182 million acres of timberland, of which 62 million acres or 34%, are pine types, is a major timber-growing region. Pine plantations currently form one-third of all pine stands in the South (Saucier and Cubbage, 1990). The proportion of pine to all other species is projected to increase to 56% of all forested areas by the year 2000 and by 2030, plantations are expected to contribute up to two-thirds of the South's pine forest.

Loblolly pine (*Pinus taeda* L.) is one of the most important commercial forest tree species in the southern United States and is widely planted by the USDA Forest Service and industry. It is now the dominant species on about 11.7 million ha (29 million acres) (Baker and Langdon, 1990), or 17 percent of the South's timberland, and its rapid growth and good form make it one of the best choices for more acres (Edwards, 1987). The native range of loblolly pine covers 14 states from southern New Jersey to central Florida in the south and to eastern Texas in the west, and it has been introduced to other countries with different degrees of success. Loblolly pine in the western region is slow-growing, and more rust and drought-resistant than the eastern loblolly pine. The species is dominant only on 7% of the forest land in pine-oak and loblolly pine-sweet gum forests in the Coastal Plain area of southeastern Oklahoma McCurtain County. Low winter temperature associated with damage from ice, snow, and sleet during flowering, and lack of adequate precipitation during the growing season, might be the main factors limiting northern and western extension of the species (Baker and Langdon, 1990).

Reforestation is one of the major components of silvicultural management. In artificial reforestation as well as natural regeneration, many physiological processes such as growth, photosynthesis, and respiration and abiotic factors, nutrition and stress relationships involving water, temperature and light, are involved during seed germination and subsequent root and shoot development. To supply the planting and reforestation activities, state and industrial nurseries in the southern United States are currently producing nearly one billion pine seedlings annually (Hennessey and Dougherty, 1984) which is almost half of the seedlings produced in the US. Oklahoma nurseries alone produced 75 million seedlings in 1989 (Forward et al., 1990).

Since plantation performance is based on the result of an interaction between the stock planted and its environment, planting stock characteristics are important for plantation performance (Hennessey and Dougherty, 1984). Although reforestation technology has constantly improved, it is reported by many public and private forestry organizations that in southern pine plantations, early survival and growth have recently decreased significantly because of poor seedlings treatments and care (USDA, 1989). Drought is a major reason for mortality of planted loblolly pine seedlings especially in areas with low precipitation during the growing season.

After lifting from the nursery, seedlings have to be handled and treated very carefully to prevent water loss. Otherwise even very short exposure, under many environmental conditions (extreme temperatures, sunlight, wind and dry air) might significantly reduce survival, growth and new root production. Improper care, handling, storage and planting of nursery stock, and insufficient site preparation for control of competing vegetation also decrease survival by indirectly increasing moisture stress (Baker and Langdon, 1990).

Plant water relations

Water has a significant role in plant growth and survival and in the distribution of plant species. Almost all forms of terrestrial life depend on their ability to take up water from their environment and to maintain biological and physiological life processes and to survive adverse conditions. Water is an essential component of green plants and forms 70-90% of the fresh weight of actively growing tissues in most non-woody species (Hale and Orcutt, 1987; Nilsen and Orcutt, 1996; Kozlowski and Pallardy, 1997). Water contributes approximately 50% of the total fresh weight of a tree or of freshly cut wood (Larcher, 1975; Ksontini, 1983). However, the actual water content varies widely within cells and tissue types, among parts of a plant and with species, age, site and season. The parts of plants containing the least water are mature dry seeds and they usually contain 10-15% water. In seed with large stores of fat, water may contribute only 5-7% of the weight (Larcher, 1975). Although there is a considerable variation in the distribution of total amount of water in a plant, the majority of the tissue water (70 % or more) is present in the symplast where it provides a suitable medium for many biochemical reactions (Nilsen and Orcutt, 1996). Water in the symplast plays significant roles in the physiology of plants by its physical and chemical properties. Details of these properties can be found in the reviews by Nobel (1991) and Nilsen and Orcutt (1996).

Water is a substrate in photosynthesis and it is necessary to maintain turgidity of cells and tissues. It provides the driving force for cell enlargement, stomatal opening for gas exchange, and maintenance of the form of young leaves and other slightly lignified structures. Plant water status is determined by the rate of exchange of water between soil and atmosphere through plants. Water is absorbed by roots from the soil and is translocated to transpiring leaves of the shoots, basically as a result of differences in the free energy content of water in different parts of the system. Long-distance transport of water requires that there is continuity of liquid throughout the pathway (Fitter and Hay, 1987). The free energy content of water can be expressed in terms of water potential (Ψ_w) and defined as the free energy per unit volume of water, assuming under standard conditions, pure water's potential to be zero. Determination of the seedling water potential (Ψ_w) is one recommended procedure for expressing the intensity of the desiccation and defining conditions that cause dehydration, and has permitted large expansion in our understanding of plant responses to water stress. Whenever the rates of water loss by transpiration exceed the rates of water absorption by roots, water potential inside the plant is lowered and the plant is under stress.

Many studies have reported on the effects of exposure time or desiccation of shoot, root or both on survival, growth, water potential, physiological processes and root-growth potential in many species. Hett (1980) studied water potential differences between soil, roots and shoots of *Pinus radiata* D. Don and *Pinus brutia* Ten. seedlings grown under controlled climatic conditions. He found in both species that shoot water potential (Ψ_s) and root water potential (Ψ_r) in cool climates were always higher (less negative) than in warm climates. Soil water potentials were higher than root water potentials, and root water potentials were higher than shoot water potentials suggesting a gradient of decreasing water potential from the soil through the roots to the shoots. In both species, Ψ_s was always lower than Ψ_r. While this difference in *P. radiata* was quite uniform with increasing moisture stress, it was not so in *P. brutia* (Heth, 1980). When bare-root red pine and white spruce seedlings were dried in various controlled ways before measurement, Ψ_r was directly proportional to, and highly correlated with, xylem water potential (Ψ_x) (Sucoff et al., 1985). There were large differences between Ψ_r and Ψ_s and the investigators recommended that Ψ_r might be the preferable predictor of postplanting seedling behavior in both red pine and white spruce. Exposure of bare-root loblolly pine seedlings to air resulted in reduced survival of about 0.43%/minute of exposure and 3-year losses of 0.22%/minute (Diearauf and Marler, 1971). Based on their results, Diearauf and Marler (1971) pointed out that exposure of seedling roots should be minimized during the period between lifting and planting.

Tabbush (1987) reported that 1 hour 44 minutes desiccation did not affect subsequent survival or growth, but 3 hours 18 minutes exposure decreased survival to 68% after two years and significantly diminished height growth in Sitka spruce (*Picea stichensis* Bong. Carr.).

Since the intensity of desiccation is affected by many environmental factors, such as temperature, wind, and air moisture content and species, each study could give different results for each condition. Water potential or water stress and water content of a seedling provides good information about the intensity of desiccation. Knowing the relationships between tissue water content and tissue Ψ_w and its osmotic and turgor components are needed to understand plant response to water stress (Weatherley, 1970; Wiebe, 1972). After a range of desiccation treatments in Sitka spruce, if root moisture content did not fall below 180% of dry weight, survival was high. But small reductions in root moisture content or Ψ_r resulted in very low survival in Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco). There was no effect on the survival of Sitka spruce until Ψ_w fell below -2.0 MPa (Tabbush, 1987). He found that rewetting of desiccated plants for one hour in the dark maintained root moisture content but not Ψ_w and this did not affect survival or growth. Survival was similar with or without rewetting in both species. Exposure of the root and shoot diminished the moisture content of all tissues measured and reduced Ψ_w in Sitka spruce (Coutts, 1981). When the root was exposed, water moved from the shoot into the root system, but this movement did not prevent a substantial reduction in the moisture content (MC) of the root system. The lowest MC occurred in the fine roots.

Photosynthesis and transpiration can be reduced by plant water stress. One-year-old loblolly pine seedlings exposed to moisture-stress conditioning (MSC) continued photosynthesis at much lower needle water potentials (-1.9 MPa) than controls (-1.4 MPa) (Seiler and Johnson, 1985). This response was attributed to the 0.45 MPa osmotic adjustment found in preconditioned seedlings. Although it was not clear whether this adjustment was an active osmotic adjustment, it resulted in preconditioned seedlings maintaining turgor to lower needle water potentials. Transpiration rate was diminished 30% and water-use efficiency increased 67% as a result of MSC and these results suggest significant improvements in loblolly pine drought tolerance (Seiler and Johnson, 1985).

Different plant species can develop both morphological and physiological adaptations to survive and grow in unfavorable conditions (Dickson and Tomlinson, 1995). Morphological adaptations in leaves, stems and roots assist in both drought avoidance and drought tolerance. Physiological adaptations involve control of stomatal conductance, leaf water potential, osmotic adjustment and photosynthetic carbon fixation. Epron and Dreyer (1995) studied the effect of moderate soil drought on the rate of CO_2 assimilation and the amount of soluble and insoluble carbohydrates in leaves of 4-year old saplings of *Quercus petraea* (Matt) Liebl. After 10 days exposure to water stress by withholding irrigation, predawn leaf Ψ_w was decreased to -2.0 MPa, and leaf photosynthesis was reduced by 55 %. At this stage, 47% and 48% decreases were observed in starch and sucrose concentrations, respectively, and a five-fold increase was observed in glucose and fructose concentrations of water-stressed saplings compared with well-watered plants. It was suggested that drought-induced changes in sugar composition contribute to osmotic adjustment in *Q. petraea* (Epron and Dreyer, 1995).

The water potential (Ψ_w) in plants is the sum of turgor potential (Ψ_p), osmotic potential (Ψ_π), matric potential (Ψ_m), and gravitational potential (Ψ_g) as given by the equation: $\Psi_w = \Psi_\pi + \Psi_p + \Psi_m + \Psi_g$ (1) (Hale and Orcutt, 1987; Lassoie and Hinckley, 1991; Kozlowski and Pallardy, 1997). Ψ_π is osmotic or solute potential which arises from dissolved solutes, either molecules or ions, and it is lowered in proportion to the number of particles in solution. Ψ_p is turgor potential created by water molecules pressing against the surfaces of membranes and cell walls, and is necessary for cell elongation and growth (Hale and Orcutt, 1987). Ψ_m (matric potential) and Ψ_g (gravitational potential) are not important components of water potential of vegetative tissues and are negligible for all practical purposes in studying seedling plant water relations (Lange et al., 1982).

Water potential and its components can be measured by many different techniques including vapor pressure methods with thermocouple psychrometers and dew point hygrometers, and the pressure plate apparatus and the pressure chamber. Excellent reviews of these methods can be found in Lassoie and Hinckley (1991) and Boyer (1995).

Tyree and Hammel (1972) showed that determination of the relationships between relative water content (RWC), Ψ_w, Ψ_π, and Ψ_p of plant tissues could be determined by using a pressure chamber combined with an analytical balance. To study plant water relations, use of the pressure chamber has received much attention. Comparisons between the pressure chamber technique and other methods of measuring plant Ψ_w are numerous (Ritchie and Hinckley, 1975; Wilson et al., 1979), and the pressure chamber has sometimes provided better results than other techniques in laboratory research as well as in field studies (Wilson et al., 1979; Cleary and Zaerr, 1980). This technique of Ψ_w measurement is based on the fact that the effect of pressure on water potential is thermodynamically equivalent to that of solutes, matric, and turgor potentials (Lassoie and Hinckley, 1991; Kozlowski and Pallardy, 1997). The pressure chamber can be used to derive the relationship between Ψ_w and expressed water volume or RWC of plant tissues to generate moisture release curves or pressure-volume (PV) curves. First developed by Scholander et al. (1964) and reviewed by Tyree and Hammel (1972), the pressure-volume technique has become one of the most useful methods for analyzing plant water relations parameters such as osmotic potential, symplast and apoplast water and cell elasticity (Neufeld and Teskey, 1985). The PV curve is a graph of a series of Ψ_w measurements with a pressure chamber on initially rehydrated plant material as the material's water is reduced either by bench drying, sap expression or a combination of both (Tyree and Hammel, 1972; Ritchie and Roden, 1984; Parker et al., 1995). Plotting these data as $1/\Psi_w$ vs water lost (Ve) or vs RWC generates a PV curve of the plant sample (Fig. 1). Another alternative transformation of Ψ_w isotherms, Ψ_w vs 1/RWC, has been discussed (Richter, 1978; Tyree and Richer, 1980). However, Tyree and Richter (1980) suggested that the $1/\Psi_w$ vs RWC transformation generally produced the best estimate of osmotic potential at full turgor by linear extrapolation. The relationship between $1/\Psi_w$ and Ve or RWC initially is curvilinear since water loss decreases Ψ_p and Ψ_π, but it becomes linear at the point of turgor loss (incipient plasmolysis) where the osmotic potential of the cell sap has become equal to Ψ_w. Below this Ψ_w, cells in the tissue behave as osmometers. An essential assumption of the PV theory is that the volume of apoplastic water (V_a) which is contained in the cell walls, xylem lumina and intercellular spaces of the sample, remains constant because changes in RWC are assumed to reflect only water loss from the symplast (Lassoie and Hinckley, 1991). Although water in the interfibrillar spaces of cell walls is held very tightly and only can be removed at very high pressure during PV analysis (>15 MPa) (Tyree and Jarvis, 1982), changes in the amount of water held in the xylem and intercellular spaces may occur during PV dehydration (Tyree et al., 1984). Another assumption during PV analysis is that thermodynamic equilibrium exists when Ψ_w and RWC are measured. A lack of equilibrium between symplastic water in the cells and apoplastic water held in xylem during dehydration might result in overestimation of Ψ_w (Lassoie and Hinckley, 1991). By changing the chamber pressure very slowly (<0.01 MPa s^{-1}) and minimizing the transpiration rate of the sample, the experimenter may reduce the error of PV analysis. The curve formed by PV analysis has proved very useful in measurement of several aspects of tissue water relations, including measurement of osmotic potentials at full turgor and zero turgor, incipient plasmolysis, osmotic adjustment, water content at zero turgor, apoplastic or bound water and elastic properties of plant tissues. Theoretical treatments of pressure chamber and pressure volume curves can be found in Tyree and Hammel (1972), Tyree and Jarvis (1982), and Lassoie and Hinckley (1991).

Osmotic adjustment

In the plant, water movement is controlled by the difference of water potential. The decline of water potential caused by water stress or desiccation rapidly affects basic physiological processes such as growth, photosynthesis and respiration (Seiler and Johnson, 1985). Maintenance of turgor inside the cell depends on anatomical, phenological or physiological properties of the plant

(Ludlow, 1989). One of these properties is transport, accumulation or compartmentation of inorganic ions and organic solutes within cells which decreases the osmotic component of Ψ_w and consequently maintains the Ψ_p (Guicherd et al., 1996). This is osmotic adjustment or osmoregulation which occurs in most higher plants and generally involves soluble carbohydrates, amino acids, organic acids or ions (Morgan, 1984). Factors that maintain turgor are: (1) capacity to lower the Ψ_π by accumulating solutes, (2) high cell or tissue elasticity and (3) small cells (Hale and Orcutt, 1987). Within a particular tissue or cell, the osmotic potential is based on the balance between rate of solute accumulation and rate of use by the cells (Hennessey and Dougherty, 1984).

Osmotic adjustment can be measured either at full turgor (Ψ_π^{100}, where $\Psi_w=0$ and $\Psi_p=-\Psi_\pi$), or at zero turgor (Ψ_π^0, where $\Psi_p=0$ and $\Psi_w=\Psi_\pi$) (Ksontini, 1983; Hale and Orcutt, 1987). Quantitative analysis of a number of osmotically active cell constituents in six moss species demonstrated that about one-third of the osmotic potential resulted from accumulation of sugars (Santarius, 1994). It was found that the total free amino acids accounted for about 15-20 % of the osmolality of the cell solution. Pritchard et al. (1996) exposed the roots of maize (*Zea mays* L.) to 400 mol m^{-3} mannitol which corresponded to an osmotic potential of -0.96 MPa for 24 hours and found that turgor pressure fully recovered during root expansion and was almost equal to that of unstressed roots. Vacuolar osmotic potential decreased from an average of -0.69 MPa to -1.61 MPa, and this decrease of 0.93 MPa accounted for "complete" osmotic adjustment in the root of maize (Pritchard et al., 1996).

Over four months after initiation of irrigation treatments in the nursery, Ψ_π at full turgor was 0.405 MPa lower for moderately water-stressed seedlings than for the well-watered seedlings of loblolly pine, suggesting that accumulation of solutes occurred in sufficient quantity to change Ψ_π (Hennessey and Dougherty, 1984).

Many factors influence osmotic adjustment such as the rate of development of stress, the degree of stress, the environmental conditions, the species and cultivars, the tissue or organ exposed, and the age of the plant (Hale and Orcutt, 1987). Since plant growth depends on turgor, a plant's ability to maintain positive cell turgor by increasing and compartmentalizing solutes within the cells, i.e. by osmotic adjustment, is a key factor for plants to combat and to adapt to water stress.

Root growth potential (RGP) and post-lifting practices

For survival and growth in the field, it is critical for bare-root seedlings to rapidly produce new roots and start taking minerals and water from the soil. Root-growth potential (RGP) is the potential of roots to initiate or elongate new roots soon after transplanting or outplanting (Ritchie and Dunlap, 1980). RGP appears to be the most useful indicator of seedling physiological quality and seedling vitality. RGP is affected by species, genotype, nursery environment, lifting date, storage, handling, and desiccation (Ritchie, 1982; Carlson, 1985; Feret et al., 1985; Insley and Buckley, 1985; Burdett, 1987; Hallgren and Tauer, 1989; Deans et al., 1990) and by many cultural practices including undercutting, wrenching and root pruning (Ritchie and Dunlap, 1980), and seedbed density (Carlson, 1986). RGP can be used as a measure of physiological vigor as demonstrated in many studies with many different species. High RGP is often correlated with high survival. Ritchie and Dunlap (1980) cited twenty-six different studies with a variety of forest tree species. Only one study found an inverse relation between RGP and field survival in sycamore (*Platanus occidentalis* L.); two studies showed no relation, and a poor correlation was found only in one study. The other studies demonstrated good, strong relationships between RGP and field survival (Ritchie and Dunlap, 1980). Hallgren and Tauer (1989) reported that survival showed a significant correlation with RGP and the number of primary lateral roots, whereas the presence of terminal buds and secondary needles showed little value in predicting seedling performance in different families of shortleaf pine (*Pinus echinata* Mill.). They found that the number of primary lateral roots was also very strongly correlated with RGP which further supports the idea that it might be a useful measure of seedling quality (Hallgren and Tauer, 1989).

Carlson (1986) noted that in loblolly pine seedlings, hydraulic conductivity was seven times higher after new root growth. He suggested that tissues of the new roots rather than the old roots more closely described the potential for conducting water, probably due to unsuberized roots being more conductive than suberized roots.

Ritchie and Dunlap (1980) pointed out that the time when seedlings demonstrated high RGP closely coincided with the time when they were most tolerant to desiccation and physical damage and more likely to survive rough lifting, handling, storing and outplanting. On droughty sites, Hobbs (1984) suggested that one of the most significant seedling characteristics that will affect first year survival and growth might be RGP. Tinus (1995) stressed container-grown Douglas-fir seedlings to water potentials of -0.2 (unstressed), -2.2 (moderate stress), and -3.8 MPa (severely stressed) and placed half of them in a root mist chamber set at 10, 20, or 28 ^{0}C, (where the other half were potted). It was found that RGP of unstressed trees was higher than that of moderately stressed trees at all temperatures, but in severely stressed trees there was no new root production at any temperature. Survival was 90 % in the unstressed and moderately stressed potted trees and 40 % in severely stressed potted trees. Height growth was reduced by 75% by severe stress treatment compared to the control (Tinus, 1995).

Carefully handled seedlings had high RGP which was positively correlated with plant water potential, root water content, shoot relative growth rate and field survival. It was reported that overall RGP was diminished 59% by desiccation (shoot water potentials of -2 to -3 MPa), 85% by rough handling (after lifting, seedlings were struck on the toe of a boot to remove soil), and 98% by desiccation and rough handling combination (Deans et al., 1990). Storage at -1 ^{0}C for four weeks in April-May reduced RGP significantly in Sitka spruce but there was no relationship between plant size and the ability to tolerate both desiccation and rough handling. The effects of exposure to air drying on RGP were significant and each 15 minutes exposure reduced RGP by about 50% in loblolly pine seedlings (Feret et al., 1985). In non-exposed shortleaf pine seedlings, RGP was reduced in 28 days cold storage for seedlings lifted on December 1, with only 314 chilling hours before lifting, and was increased in storage for December 29 lifted seedlings, with 758 chilling hours (Hallgren and Tauer, 1989).

As summarized by Ritchie and Dunlap (1980), evidence indicates that RGP periodicity is linked to the bud dormancy cycle, although the mechanism is not well understood. The most plausible mechanism is that the elevated RGP in winter corresponds with accumulation of chilling hours and peaks with the fulfillment of the chilling requirement.

Because growth processes are dependent on energy, available metabolic substrates, principally carbohydrates, should be present in the plant for root growth. Sucrose is the most plentiful translocatable carbohydrate in trees and resource carbohydrates are stored primarily as starch in both conifers and hardwoods (Ritchie and Dunlap, 1980). RGP was compared with sugar and starch concentrations of Douglas-fir seedlings lifted during the winter and freezer-stored for 2,6,9 and 12 months. There was no significant correlation between RGP and carbohydrate levels in leaves, stems and roots (Ritchie and Dunlap, 1980).

The period from December through February is the traditional and optimum planting season for many of the southern pines and this range can be extended one to one and a half months earlier (Hallgren, 1991). In the Pacific Northwest as well as in the South, recognition of a relatively narrow midwinter "lifting window" for high quality planting stock and necessity for high-elevation site planting in late spring have required large scale cold storage of forest tree seedlings (Ritchie, 1982). The planting season of loblolly pine in the southern United States after the lifting season is only three months. Seedlings might be lifted early and cold stored until they can be planted to avoid planting stock quality losses caused by lifting flushing seedlings (Dewald and Feret, 1988). Cold storage has different effects on the subsequent survival, and growth and on planting stock quality, depending on lifting date and storage time (Carlson, 1985; Hallgren and Tauer, 1989; Hallgren et al., 1993).

Objectives

Research showed loblolly pine seedlings to be capable of osmotic adjustment when they were exposed to moisture stress conditioning (MSC) in the nursery (Hennessey and Dougherty 1984) or in pots (Seiler and Johnson 1985). Seedlings exposed to MSC appeared to become more drought hardy than non-treated seedlings. On the other hand, it was shown that loblolly pine bare-root seedlings were sensitive to desiccation during handling (Dierauf and Marler 1971, Feret et al. 1985). Seedling quality was seen to decrease rapidly with length of air-drying. The overall goal of the project reported here was to determine whether bare-root loblolly pine seedlings could be conditioned to be more drought hardy by carefully controlled air-drying. Earlier studies of the effects of air-drying correlated seedling quality with length of treatment and not with seedling water status. An important improvement in this project was to describe treatment level by seedling water status after air-drying.

The specific objectives of this project were:

1. determine effects of air-drying (MSC) on water status of seedling roots and shoots
2. determine relation between seedling water status after MSC and root growth potential
3. determine relation between seedling water status after MSC and osmotic adjustment,
4. determine whether MSC can improve the capacity of seedlings to retain vigor during cold storage.

Materials and Methods

Plant material

One-year old loblolly pine (*Pinus taeda* L.) seedlings were obtained from the Weyerhaeuser Company Nursery at Fort Towson, Oklahoma. Seedlings were hand-lifted from three different beds (replicates), quickly placed in kraft-polyethylene bags and transported to cold storage (4 ^{0}C) at Stillwater, Oklahoma during the normal commercial lifting season on February 27, 1996. Approximately four hundred non-damaged seedlings of similar height (20-30 cm) and stem diameter (3-5 mm) were selected from each replicate and the soil was washed off the plants.

Moisture stress conditioning

All plants were then exposed to air-drying on screens (moisture stress conditioning, MSC) in the greenhouse until root and shoot water potentials were reduced to desired levels. There were five treatment levels including the control (Table 1). Control plants were placed on the screens and immediately removed. Water potential of roots and shoots rather than duration was chosen as an indicator of the exposure level for each treatment. For each MSC level, root and shoot water potentials of five randomly selected plants from each replicate (nursery bed) were measured with a pressure chamber (Scholander et al., 1964). Fresh weight and dry weight after 48 h in an oven at 70 C^0 were determined for leaves, stems, and roots. Water content (WC) of each part was determined by the equation:

$$WC=[(\text{fresh weight-dry weight})/\text{dry weight}]*100.$$

One half of the seedlings were kept for measurement of RGP and PV analysis, and one half were placed in kraft-polyethylene bags and stored at 4 C^0. The stored seedlings were subsequently measured for WC, RGP and PV analysis after 42 d storage.

RGP test

Three seedlings were planted into 1 L milk carton pots filled with a 1:1 peat:vermiculite (v:v) mixture and irrigated immediately. Pots were arranged in a randomized complete block design within a controlled environment chamber set for a 16-hour photoperiod and a 25 ^{0}C day/15 ^{0}C night and 40% day/65% night relative humidity. The seedlings were removed from the growth chamber after four weeks and placed in cold storage until the roots could be washed and the new root tips $\geq$ 1 cm counted. RGP counts were completed within 2 days. The experimental unit was a pot of 3 seedlings. There were 10 replicates.

PV analysis

Water relations characteristics of shoots and roots were determined using a PV curve technique based on pressure chamber measurements (PMS Instruments, Corvallis, OR, USA). The free transpiration method was used to generate PV curves from a series of parallel fresh weight and pressure chamber Ψ_t and Ψ_s measurements (Parker and Pillardy, 1988; Lassoie and Hinckley, 1991; Parker and Colombo, 1995). Trees were rehydrated overnight for 15 h at room temperature in the dark by submerging the roots to the root collar and covering the shoots with plastic bags (Parker and Colombo, 1995). An aerator was inserted into the water in order to supply roots with oxygen.

Roots and shoots were separated at the root collar and PV curves of each part were generated. Root surface water was removed delicately with a paper towel to reduce over-estimation of relative water content (RWC) at full turgor. Chamber pressure was increased and at the rate of ≤ 0.01 MPa/s^{-1} (Ritchie and Hinckley, 1975; Parker and Colombo, 1995) until water appeared at the cut surface which indicated the balance point. The pressure in the chamber at the balance point was recorded and the sample was removed from the chamber and allowed to air dry on a bench between consecutive Ψ_w determinations. When approximately 4-6 data points on the linear portion of the PV curves (plot of $1/\Psi_w$ versus RWC) were obtained, samples were placed in an oven at 70 ^{0}C for 48 h and dry weights were measured. The 4-6 data points in the linear portion of the PV curves corresponded to a pressure of -2.0 to -2.5 MPa for the shoots and -3.5 to -4.0 MPa for the roots. Time averaged 12-15 h for the roots and 15-20 h for the shoots to generate PV curves. Sample RWC was calculated as:

RWC=(fresh weight-dry weight/ turgid weight-dry weight)*100.

The plot of $1/\Psi_w$ versus RWC was used (type II plot from Tyree and Richter, 1981; Parker and Colombo, 1995) to identify data points to be included in regression analysis of the linear portion of PV curves.

The fully turgid weight of each sample was estimated as described by Wilson et al. (1979) by extrapolating the linear relation between pressure (up to 1.0 MPa for the shoots and 1.5 MPa for the roots) and sample fresh weight to obtain the estimated sample weight at zero Ψ_w. From the plot of $1/\Psi_w$ against RWC, the parameters of osmotic potential at full turgor (Ψ_π^{100}) and zero turgor (Ψ_π^0), RWC^0 at zero turgor and the proportion of apoplasmic water (V_a) were derived. PV analysis was conducted on non-stored seedlings from the control and stored seedlings from all treatments. PV analysis was conducted on the entire shoot and root system separately. There were 3 replicates (nursery beds), and each replicate consisted of three seedlings.

Statistical analysis

Data were analyzed using the analysis of variance (ANOVA) procedure of SAS (SAS Institute, Inc., Cary, NC, USA). The plot logout was a randomized complete block design with 3 blocks. A least significant difference (LSD) test was performed for comparison of parameter means at the 5 % probability level.

Results

Tissue water content and water potential

MSC significantly reduced WC of leaves, stems and roots (Fig. 2 and Table 2). The lowest WC was found in the root system followed by stem and leaf. Forty-two d of cold storage tended to increase WC in all plant parts ($p= <0.001$ to 0.026). The amount of increase in WC with storage decreased with increasing severity of MSC for the leaf and stem. The increase in WC after storage was significant ($p< 0.05$) in leaves for MSC levels 0, 1, and 2, in the stems for MSC level 0 and in the roots for MSC levels 0 and 3. The most severe MSC level decreased water content by 21% in leaves, 27% in stems, and 34% in roots. The MSC treatments significantly reduced Ψ_r and Ψ_s (Table 1). Storage did not significantly change Ψ_r and Ψ_s (Table 1).

RGP and survival

Seedlings that were not moisture stress conditioned or stored had a RGP of over 100 new roots (Fig. 3). Both MSC and storage caused large reductions in RGP, bud expansion and survival (Figures 3, 4, 5 and Table 3). Storage alone caused a reduction of 80% in RGP, 50% in bud expansion and 30% in survival. The mildest level of MSC caused nearly the same reductions. When MSC and storage were combined the reductions were even greater. The most severe MSC level had nearly nil RGP, bud expansion and survival, whether or not the seedlings were stored.

Water relations parameters

Storage for 42 d had no effect on water relations parameters of roots and shoots of seedlings receiving MSC level 0 (Tables 4, 5, and 6). Ψ_π^{100} decreased by 0.37 MPa in the roots and 0.44 MPa in the shoots as MSC increased from level 0 to 4 (Tables 5, 7, and 8). Ψ_π^{0} decreased by 0.30 MPa in the roots and 0.55 MPa in the shoots as MSC increased from the least to the most severe (Tables 5, 7, and 8). RWC^0 was 61 to 67% in the roots and 75 to 80% in the shoots and largely unaffected by MSC. V_a was -23 to -12% in the roots and 22 to 31% in the shoots of stored seedlings and unaffected by MSC.

Discussion

A major contribution of this study was to show that even very mild MSC (Ψ_s of –0.47 Mpa) was severe enough to significantly decrease RGP. Although seedlings were capable of significant osmotic adjustment when subjected to MSC, they showed a large decrease in RGP whether the seedlings were tested immediately or after 42 d of storage. Further work should be done to determine whether less severe MSC could cause osmotic adjustment or improve seedling performance. The pressure-volume analysis showed large negative values for V_a. An earlier study (Teskey and Neufeld, 1986) found negative V_a in defoliated twigs of loblolly pine. Further work should be done to determine whether the negative values of V_a were due to the lack of new roots or to some aspect of the technique. The lack of new roots may have the same effect of the lack of foliage.

Tissue water content and water potential

MSC significantly reduced WC in leaves, stems, and roots. The root system decreased its WC more rapidly than the stem and shoot (Fig. 2). In addition, the root system had the lowest WC, followed by stem and leaf. Coutts (1981) reported similar results in a study of Sitka spruce where seedlings were exposed to drying in three different ways: 1.) only roots exposed, 2.) only shoots exposed, and 3.) roots and shoots exposed. Plant WC decreased rapidly for 1 h during exposure in the greenhouse, then more slowly through 4.5 h. A plant with both root and shoot exposed lost water more rapidly than plants with only their roots or shoots exposed. The largest WC decrease was in the fine roots (Coutts

1981). The decrease in WC in the fine roots was much greater when only roots were exposed (349% to 97%) than when only shoots were exposed (349% to 177%). He showed that during exposure the amount of water lost from the roots exceeded the amount of water originally present in the entire root system, verifying that water moved from shoot to root. But this movement did not prevent a substantial reduction in the WC of the root system.

The MSC treatments reduced Ψ_r and Ψ_s (Table 1). In the Sitka spruce study (Coutts, 1981), all exposure treatments reduced leaf water potential and a -2.0 MPa leaf Ψ_w was attained after only 0.7 h in root + shoot exposed plants, after 3.3 h in shoot-exposed, and after 4.1 h in root-exposed plants. He concluded that measurements of neither leaf Ψ_w nor whole plant moisture content before planting is likely to give an accurate guide to the quality or condition of planting stock in Sitka spruce.

Sucoff et al. (1985) found that when a seedling was halved at the root collar before exposure, WC and Ψ_w decreased much more rapidly in the root systems than shoots. They reported that after different exposure treatments in different conditions Ψ_s was positively correlated with Ψ_r and at least 94% of the variation in an individual treatment was explained by simple linear regression of Ψ_s against Ψ_r in white spruce and red pine. They also showed, as was shown in the present study, that Ψ_r was always lower than Ψ_s except in the treatment where only shoots were exposed, and the difference increased as the plant dried. They explained the difference between Ψ_r and Ψ_s in terms of xylem resistance to water transport coupled with the more rapid evaporation from roots than from shoots. Since the Ψ_r was sometimes very different from Ψ_s and roots were more likely to be damaged by a given level of desiccation during handling and planting, they suggested that Ψ_r may be a better predictor of postplanting seedling behavior.

Heth (1980) exposed seedlings of *P. radiata* and *P. brutia* grown in the phytotron of Duke University to four different climatic conditions (dry warm, moist warm, dry cool, and moist cool). He found a linear relation between xylem water potential (Ψ_x) and Ψ_r in *P. radiata* and curvilinear relation in *P. brutia*. Ψ_x was lower in *P. brutia* than *P. radiata* when Ψ_r in the soil was near field capacity. For Ψ_r of -1.2 to -1.8 MPa, Ψ_x of *P. brutia* was nearly equal to Ψ_r, but with increasing water stress Ψ_x became much lower than Ψ_r, possibly because of a higher resistance to water transfer in *P. brutia*. He concluded that Ψ_x in drought-tolerant species is lower than in non-tolerant species. He suggested there might be a correlation between drought tolerance and the relation of Ψ_x to Ψ_r. A curvilinear shape may indicate a higher rate of tolerance than a linear one. He reported in all treatments for both species a gradient of decreasing Ψ_w from the soil to the roots and from the roots to the shoots in planted seedlings. However, as seedlings are lifted from the soil, the gradient was reversed and Ψ_r was lower than Ψ_s.

In general, my data showed that during 42 d cold storage WC increased in all plant parts. The difference in WC before and after storage for each MSC level was higher for unstressed than stressed seedlings in all parts. Respirational losses of food reserves which could cause the dry weight to decrease and percent water content (dry weight basis) to increase may explain this increase in WC, at least in part. In addition, it could be argued that the difference in WC was higher in healthy unstressed seedlings than stressed seedlings because the unstressed seedlings respired more than stressed seedlings during storage. For example, WC increased by about 15% in leaves of control and only by 4% in leaves of MSC level 4. Deans et al. (1990) found that total non-structural carbohydrate (TNC) content of plants held in cold storage decreased from 22 January until 24 April, with apparent respiratory losses of TNC averaged 0.3 mg g^{-1} d^{-1} in Sitka spruce planting stock. This amount of TNC decrease may not fully explain the WC increases in the present study, but may be a partial explanation. Ritchie (1982) reported similar results that TNC concentrations decreased rapidly in foliage, stem, and roots during the first 2 months storage at +2 and -1 ^{0}C in Douglas-fir seedlings suggesting that TNC depletion in storage is due to respiratory consumption. Dewald and Feret (1988) reported similar results in cold-stored loblolly pine seedlings and that cold storage decreased dry weights which they attributed to respirational losses during storage.

RGP and survival

The results of this study showed that MSC and cold storage could decrease RGP and survival (Fig. 3 and 5). There was a strong direct correlation between RGP and survival in the growth chamber for 28 d, which is consistent with the common belief that RGP is correlated with survival potential (Ritchie and Dunlap

1980, Feret et al. 1985, Burdett 1987, Hallgren and Tauer 1989). In contrast, Tinus (1995) found a poor correlation between RGP and field survival in Douglas-fir seedlings. RGP was greater in non-stressed seedlings (-0.2 MPa plant Ψ_w) than in trees stressed to -2.2 MPa and there was no new root production in trees stressed to -3.8 MPa. Field survival of the seedlings that were unstressed and stressed to -2.2 MPa was 90%, and survival of severely stressed trees was only 40%. These results suggested the RGP test may be sensitive enough to detect a decrease in seedling vigor even before the decrease is large enough to reduce field performance. This may explain why there is sometimes a poor correlation between RGP and field performance.

Deans et al. (1990) showed significant effects of desiccation (seedlings dried for 1 to 1.5 h to Ψ_s of -2 to -3 MPa) and rough handling on RGP in Sitka spruce seedlings. They reported RGP was reduced 59% by desiccation, 85% by rough handling, and 98% by a combination of desiccation and rough handling. In contrast, they reported that despite the severe handling and drying treatments, more than 90% of the seedlings in all treatments survived the first year after transplanting to the field. A possible explanation for high survival of sitka spruce would be that seedlings had a very mild climate in the field.

In the current study the control seedlings (MSC level 0) had a high RGP and survival (Figures 3, 5) and even the smallest amount of MSC reduced RGP by 80% and survival by 50%. This is consistent with earlier findings that RGP and survival decreased as exposure time increased for outdoor drying and indoor drying in loblolly pine (Feret et al. 1985). They found the half-life of seedlings (as measured by RGP) exposed at a planting site may be as short as 15 minutes, where in the laboratory the half-life was approximately 35 minutes.

Cold storage for 42 d significantly reduced RGP and survival. Storage alone decreased RGP by 80% and survival by over 30%. When seedlings were subjected to the least amount of MSC (shoot water potential of -0.47 MPa), cold storage reduced RGP by almost 99% and survival by 80%. These results are consistent with earlier work showing that storage can reduce RGP in shortleaf pine (Hallgren et al. 1993) and loblolly pine (Carlson 1985).

Water relations parameters

The first 2-3 points determined in the PV analysis of roots of rehydrated seedlings showed large decreases in RWC and at the same time no significant drop of Ψ_w occurred. It is not likely that this was because of over-hydration, but probably because the surface water on the root system was not completely removed in sample preparation. In support of this conclusion is the fact that the shoots did not show this trend in any PV curve. Similar results were reported previously in PV curves for western hemlock roots (Kandiko et al. 1980).

The decrease in Ψ_π^{100} and Ψ_π^0 with increasing MSC in both roots and shoots indicates osmotic adjustment can occur rather rapidly. In contrast, Ritchie and Roden (1985) using the same free transpiration PV curve method reported opposite results in Douglas-fir seedlings. Ψ_π^{100} and Ψ_π^0 increased in shoots and remained constant in roots in seedlings stressed to pre-dawn Ψ_w of -1.76 MPa. They suggested that the free transpiration procedure requires a tissue sample be inserted and removed from the pressure chamber more than ten times during the analysis which may cause disequilibrium in the sample. This could result in an overestimation of Ψ_π^{100} and Ψ_π^0 (Tyree et al. 1978).

In the present study, RWC^0 was unaffected by MSC levels in the roots (61 to 67%) and the shoots (75 to 80%), but RWC^0 was significantly lower in the roots than the shoots. RWC in the roots dropped so rapidly that the points on the PV curves were widely spaced which may cause inaccurate estimation of the turgor loss point and shift it to the right side of the PV curves. Kandiko et al. (1980) also reported that roots lost a larger percentage of water before reaching incipient plasmolysis than the shoots in Western hemlock seedlings. In contrast, they found that shoots had lower Ψ_π^{100} and Ψ_π^0 than the roots. They found that 2-week drought stress (-1 MPa pre-dawn Ψ_w) resulted in a significant change in the water status of the seedlings. Ψ_π^{100} and Ψ_π^0 were lower in the drought-stressed seedlings suggesting an adaptation to drought conditions.

V_a was between 22-31% and unaffected by MSC levels in the shoots. In contrast, negative V_a (-23 to -12%) was found in all treatments in the roots. The reason for this may be rapid water loss during bench drying did not permit thermodynamic equilibrium between symplast and apoplast in roots causing Ψ_w to be higher than the true value for the symplast. This would cause the slope of PV curve to be shallower resulting in negative estimates of V_a. Changes in the amount of water held in intercellular spaces and xylem lumina may occur during PV dehydration (Pallardy et al. 1991). The roots may have lost water directly from xylem lumina and intercellular spaces which are in a larger portion in roots than shoots which have foliage. Shoots or twigs with foliage are supposed to have more proportion of apoplasmic water than foliage or leaves (Nuefeld and Teskey 1986). A fundamental assumption of PV curve analysis is that V_a remains constant. Apoplast water can not be removed until very high pressures (>15 MPa) (Pallardy et al. 1991).

Cavitation may have occurred in the roots during the depressurization of the pressure chamber. If xylem conduits that become embolized during depressurization do not refill during subsequent pressurization, V_a at the next measured Ψ_w will be reduced (Pallardy et al. 1991). Crombie et al. (1985) reported cavitation when Ψ_w dropped below some threshold value, commonly ranging from -0.8 to -2.0 MPa in several species. Tyree et al. (1984) also reported cavitation in the sapwood of northern white cedar shoots during the depressurization of the pressure chamber. Parker and Pallardy (1987) found shallow slope values for the linear portion of PV curve and greater than 100% symplastic water fraction for oak shoots using the free transpiration method. They suggested the reason could be that cavitation occurred during repeated Ψ_w determinations or lack of Ψ_w equilibration between leaf and stem tissues.

Negative V_a has been reported in several other studies (Cortes and Sinclair 1985, Neufeld and Teskey 1986, Pallardy et al. 1991, Parker and Colombo 1995). Neufeld and Teskey (1986) found V_a in loblolly pine to be less than 0 in fully defoliated twigs, 38% in the fascicles and 31% in twigs with no foliage removed. It was expected that in the stem, a large proportion of water would be in the apoplast, while the proportion in the needles would be lower.

They concluded, as suggested by Richter et al. (1980) and Cortes and Sinclair (1985), that determination of V_a using the intercept method may be unreliable.

Another potential source of error in determination of V_a is the presence of "free water" or plateaus at full turgor in the PV curve where a decrease in RWC does not cause a change in Ψ_w. This has been reported for single leaves, shoots, and roots for both the sap expression and free transpiration methods (Pallardy et al. 1991). Water in the intercellular spaces is the most likely source of apoplastic water associated with the presence of plateaus (Parker and Pallardy 1987). In a plant leaf 5 to 40% of its total volume may be occupied by air-spaces (Meidner and Sheriff 1976). Root systems may contain similar air-spaces. Since the trees were rehydrated by submerging the roots into water for 15 h, the spaces in the root system may have completely or partially filled with water as roots reached full turgidity. As reported by Parker and Pallardy (1987) and Pallardy et al. (1991), the water located within these intercellular spaces is very dilute and held to cell walls only weakly by matric forces. Parker and Pallardy (1987) reported that this water will either flow directly to evaporation sites via cell walls and exit the sample which may cause V_e per unit reduction in Ψ_w to be greater, or move into adjacent dehydrating cells which would serve to buffer changes in cell Ψ_w. This can cause RWC to increase and V_a to decrease during PV curve analysis.

The data from PV curves showed 0.37 MPa osmotic adjustment in the roots and 0.44 MPa in the shoots in the most stressed seedlings. However, this adjustment did not maintain vigor or viability in stressed seedlings; more than

90% of the seedlings in MSC levels 3 and 4 died during the RGP test. Hennessey and Dougherty (1984) found a similar osmotic adjustment of 0.41 MPa in moderately water-stressed loblolly pine seedlings grown in the nursery in late summer as compared to well-watered seedlings. Osmotically adjusted seedlings showed greater turgor maintenance over a range of water potentials, and greater root regeneration in February suggesting that seedlings may have undergone osmotic adjustment which may increase their ability to tolerate water stress in droughty sites. Parker and Pallardy (1988) found 0.30 and 0.42 MPa osmotic adjustments using free transpiration and sap expression methods, respectively, in drought stressed leaves of black locust. Sieler and Johnson (1985) reported 0.45 MPa osmotic adjustment in MSC seedlings planted in 2-liter plastic pots and watered only when pre-dawn needle Ψ_w fell below -1.4 MPa as compared to well-watered seedlings in loblolly pine. MSC seedlings maintained the same turgor potential as well-watered seedlings and had slightly higher photosynthesis than well-watered seedlings, but osmotic potential was 0.45 MPa lower in MSC seedlings which was assumed to be osmotic adjustment. They suggested that osmotically adjusted seedlings can tolerate and maintain growth under more water stress than others. Osmotic adjustment has been demonstrated in many other species, in cotton (*Gossypium hirsutum* L.) roots and leaves (Oosterhuis 1987), in Q. petraea (Epron and Dreyer 1995), in maize roots (Pritchard et al. 1996), in *Fraxinus exelsior* L. leaves (Guicherd et al. 1997), and in White ash (*Fraxinus americana* L.) (Premachandra et al. 1997).

Since osmotic adjustment has been shown to occur in the presence of slowly developing stress (Seiler and Johnson 1985), seedlings exposed to extreme drying conditions for very short time during lifting, handling and planting may not be able to osmotically adjust in such a short time. The results of the study reported here (Table 6) indicate osmotic adjustment can occur even during rapid drying. Since most of the seedlings in MSC levels 3 and 4 died after RGP test in the growth chamber, seedlings in these levels may have been overstressed and despite showing significant osmotic adjustment they died after 28 d in the growth chamber. The reason may be subsequent stress in the growth chamber.

Further work needs to be done to directly measure respirational losses in storage to determine whether these explain the apparent MC increases. Further work needs to be done to determine whether less severe air-drying could improve seedlings capacity to retain vigor during storage. The severity of air-drying can be reduced by conducting the treatment slowly at low temperature and high humidity. Further research should investigate the disequilibrium inside a seedling caused by rapid water loss and whether this may cause V_a to be negative or underestimated. Neufeld and Teskey (1986) reported negative V_a in fully defoliated twigs but not in whole shoots. It would be interesting to learn whether the lack of new roots on the seedlings tested in the current study resulted in negative V_a values just as the lack of foliage did for twigs in the Neufeld and Teskey study. In addition, it would be very interesting to make an independent measure of V_a by morphometric methods and determine whether this approach could support results of PV analysis.

Conclusions

The results lead to the following conclusions concerning bare-root loblolly pine seedlings:

1. When the seedlings were exposed to MSC, the roots decreased Ψ and MC much faster than the shoots.
2. The seedlings showed significant osmotic adjustment after brief MSC and 42 d cold storage. The amount of osmotic adjustment increased with severity of MSC.
3. Seedling vigor was reduced by MSC; RGP and survival decreased as the severity of MSC increased.
4. Storage for 42 days did not alter seedlings Ψ_π^{100}, Ψ_π^0, RWC^0, and V_a of seedlings receiving no MSC.
5. Pressure-volume analysis of seedlings without new roots showed negative V_a.
6. MSC did not improve capacity of the seedlings to retain vigor during storage.

References

Baker, J.B. & Langdon, O.G. (1990). Silvics of north America. USDA Forest Service. Volume 1. Conifers. Washington, DC. 497-512.

Boyer, J. S. (1995). Measuring the Water Status of Plants and Soils. Academic press, San Diego, CA.

Brown, M.J. & McWilliams, W.H. (1990). Pine stands across the south - trends and projections. In: Proceedings of Southern Plantation Wood Quality Workshop, Southern Forest Experiment Station, Asheville, NC.

Burdett, A.N. (1987). Understanding root growth capacity: theoretical considerations in assessing planting stock quality by means of root growth tests. Can. J. For. Res. **17**: 768-775.

Carlson, W.C. (1985). Effects of natural chilling and cold storage on bud break and root growth potential of loblolly pine (*Pinus taeda* L.). Can. J. For. Res. **15**: 651-656.

Carlson, W.C. (1986). Root system considerations in the quality of loblolly pine seedlings. S. J. App. For. **10**: 87-92.

Cleary, B.D. & Zaerr, J.B. (1980). Pressure chamber techniques for monitoring and evaluating seedling water status. N. Z. J. For. Sci. **10**(1): 133-141.

Cortes, P.M. & Sinclair, T.R. (1985). Extraction of apoplastic water during pressure-volume dehydrations. Agron. J. **77**: 798-802.

Coutts, M.P. (1981). Effects of root or shoot exposure before planting on the water relations, growth, and survival of Sitka spruce. Can. J. For. Res. **11**: 703-709.

Crombie, D.S., Milburn, J.A., & Hipkins, M.F. (1985). Maximum sustainable xylem sap tensions in *Rhododendron* and other species. Planta **163**: 27-32.

Deans, J.D., Lundberg, C., Tabbush, P.M., Cannell, M.G.R., Sheppard, L.J., & Murray, M.B. (1990). The influence of desiccation, rough handling and cold storage on the quality and establishment of Sitka spruce planting stock. Forestry **63**(2): 129-142.

Dewald, L.E. & Feret, P.P. (1988). Changes in loblolly pine seedling root growth potential, dry weight, and dormancy during cold storage. For. Sci. **34**(1): 41-54.

Dickson, R.E. & Tomlinson, P.T. (1996). Oak growth, development and carbon metabolism in response to water stress. Ann. Sci. For. **53**: 181-196.

Dierauf, T.A. & Marler, R.L. (1971). Exposure, clay treatment and storage of loblolly pine seedlings. Virginia Div. For. Occas. Rep. 34. 10 p.

Edwards, M.B. (1987). Natural Regeneration of Loblolly Pine. USDA Forest Service, General Technical Report SE-47.

Epron, D. & Dreyer, E. (1995). Starch and soluble carbohydrates in leaves of water-stressed oak saplings. Ann. Sci. For. **53**: 263-268.

Feret, P.P., Kreh, R.E., & Mulligan, C. (1985). Effects of air drying on survival, height, and root growth potential of loblolly pine seedlings. Southern J. App. For. **9**: 125-128.

Fitter, A.H. & Hay, R.K.M. (1987). Environmental Physiology of Plants. Academic Press, San Diego, CA.

Forward, P.W., Moulton, R.J., & Snellgrove, J.D. (1990). 1989 U.S. Forest Planting Report. USDA Forest Service. Washington, DC. 15 p.

Guicherd, P., Peltier, J.P., Gout, E., & Bligny, R. (1997). Osmotic adjustment in *Fraxinus excelsior* L.: malate and mannitol accumulation in leaves under drought conditions. Trees **11**: 155-161.

Hale, M.G. & Orcutt, D.M. (1987). The Physiology of Plants Under Stress. John Wiley & Sons, Blacksburg, VI.

Hallgren, S.W. (1991). The impact of lift date and storage practices on field performance of shortleaf pine seedlings. Professional paper No. PP-3628 of the Oklahoma Agricultural Experiment Station. Stillwater, OK.

Hallgren, S.W. & Tauer, C.G. (1989). Root growth potential, first-year survival, and growth of shortleaf pine seedlings show effects of lift date, storage, and family. South. J. App. For. **13**(4): 163-169.

Hallgren, S.W., Tauer, C.G., & Weeks, D.L. (1993). Cultural, environmental, and genetic factors interact to affect performance of planted shortleaf pine. For. Sci. **39**(3): 478-498.

Hennessey T.C. & Dougherty, P.M. (1984). Characterization of the internal relations of loblolly pine seedlings in response to nursery cultural treatments: implications for reforestation success. Journal Article p-1590 of the Agricultural Experiment Station, Oklahoma State University, OK.

Heth, D. (1980). Root and shoot water potentials in stressed pine seedlings. N. Z. J. For. Sci. **10**: 142-147.

Insley, H. & Buckley, G.P. (1985). The influence of desiccation and root pruning on the survival and growth of broadleaved seedlings. Journal of Hort. Sci. **60**: 377-387.

Kandiko, R.A., Timmis, R., & Worrall, J. (1980). Pressure-volume curves of shoots and roots of normal and drought conditioned western hemlock seedlings. Can. J. For. Res. **10**: 10-16.

Kozlowski, T.T. & Pallardy, S.G. (1997). Physiology of Woody Plants. 2. Edition. Academic Press, San Diego, CA.

Ksontini, M. (1983). The physiological effect of nursery water management on the drought tolerance of loblolly pine. Master Thesis, Oklahoma State University, Stillwater, OK.

Lange, O.L., Nobel, P.S., Osmond, C.B., & Ziegler, H. (1982). Physiological Plant Ecology II, Water Relations and Carbon Assimilation. Springer-Verlag Berlin Heidelberg, New York.

Lassoie, J.P. & Hinckley, T.M. (1991). Techniques and Approaches in Forest Tree Ecophysiology. CRC Press, Boca Raton, FL.

Larcher W. (1975). Physiological Plant Ecology. Springer-Verlag Berlin Heidelberg, New York.

Ludlow, M.M. (1989). Strategies of response to water stress: In: Kreeb K.H., Richter H., & Hinckley, T.M. Eds. Structural and functional responses to environmental stresses. SPB Academic, The Hague, The Netherlands, p 269-281.

Meidner, H. & Sheriff, D.W. (1976). Water and Plants. John Wiley & Sons, New York.

Morgan, J.M. (1984). Osmoregulation and water stress in higher plants. Ann. Rev. Plant Physiol. **35**: 299-319.

Neufeld, H.S. & Teskey, R.O. (1986). Variation in the amount of foliage on woody shoots and its effects on water relations parameters derived from pressure-volume curves. Can. J. For. Res. **16**: 239-243.

Nilsen, E.T. & Orcutt, D.M. (1996). The Physiology of Plants Under Stress, Abiotic Factors. John Wiley & Sons, New York, N.Y.

Nobel, P.S. (1991). Physiochemical and Environmental Plant Physiology. Academic Press, San Diego, CA.

Oosterhuis, D.M. (1987). A technique to measure the components of root water potential using screen-caged thermocouple psyhrometers. Plant Soil **103**: 285-288.

Pallardy, S.G., Pereira, J.S., & Parker, W.C. (1991). Measuring the state of water in tree systems. In: Techniques and Approaches in Forest Tree Ecophysiology. Lassoie, J.P. & Hinckley, T.M. Eds. pp 27-76. CRC Press, Boca Raton, FL.

Parker, W.C. & Colombo, S.J. (1995). A critical re-examination of pressure-volume analysis of conifer shoots: comparison of three procedures for generating pv curves on shoots of *pinus resinosa* Ait. Seedlings. J. Exp. Bot. **46**(292): 1701-1709.

Parker, W.C. & Colombo, S.J. (1996). Cut-shoot vs root rehydration: effects on pressure-volume analysis of shoots and fascicles of *Pinus resinosa* Ait. seedlings. Physiol. Plant. **97**: 651-658.

Parker, W.C. & Pallardy, S.G. (1987). The influence of resaturation method and tissue type on pressure-volume analysis of *Quercus alba* L. seedlings. J. Exp. Bot. **38**(188): 535-549.

Parker, W.C. & Pallardy, S.G. (1988). Pressure-volume analysis of leaves of *Robinia pseudoacacia* L. with the sap expression and free transpiration methods. Can. J. For. Res. **18**: 1211-1213.

Powell, D.S., Faulkner, J.L., Darr, D.R., Zhu, Z., & MacCleery, D.W. (1992). Forest resources of the United States. USDA Forest Service, General Technical Report RM-234.

Premachandra, G.S., Chaney, W.R., & Holt, H.A. Gas exchange and water relations of *Fraxinus americana* affected by flurprimidol. Tree Physiol. **17**: 97-103.

Pritchard, J., Fricke, W., & Tomos, D. (1996). Turgor-regulation during extension growth and osmotic stress of maize roots. An example of single-cell mapping. Plant Soil **187**: 11-21.

Richter, H. (1978). A diagram for the description of water relations of plant cells and organs. J. Exp. Bot. **29**: 1197-1202.

Richter, H., Duhme, F., Glatzel, G., Hinckley, T.M., & Karlic, H. (1980). Some limitation and application of the pressure-volume curve technique in ecophysiological research. In: Plants and their atmospheric environment. Symp. Br. Ecol. Soc., 21st.

Ritchie, G.A. (1982). Carbohydrate reserves and root growth potential in Douglas-fir seedlings before and after cold storage. Can. J. For. Res. **12**: 905-912.

Ritchie, G.A. & Dunlap, J.R. (1980). Root growth potential: its development and expression in forest tree seedlings. N. Z. J. For. Sci. **10**(1): 218-248.

Ritchie, G.A. & Hinckley, T.M. (1975). The pressure chamber as an instrument for ecological research. Adv. Ecol. Res. **9**: 165-254.

Ritchie, G.A. & Roden, J.R. (1985). Comparison between two methods of generating pressure-volume curves. Plant Cell Environ. **8**:49-53.

SAS. (1988). SAS procedures guide, SAS Institute Inc., NC., USA.

Scholander, P.F., Hammel, H.T., Hemmingsen, E.A., & Bradstreet, E.D. (1964). Hydrostatic pressure and osmotic potentials in leaves of mangroves and some other plants. Proc. Natl. Acad. Sci. **51**: 119-125.

Seiler, J.R. & Johnson, J.D. (1985). Photosynthesis and transpiration of loblolly pine seedlings as influenced by moisture-stress conditioning. For. Sci. **31**(3): 742-749.

Sucoff, E., Buschena, C., & Tamte, P. (1985). Desiccation and water potentials in the roots, leaves, and shoots of bare-root red pine and white spruce. Can. J. For. Res. **15**: 989-992.

Tabbush, P.M. (1987). Effect of desiccation on water status and forest performance of bare-rooted Sitka spruce and douglas-fir transplants. Forestry **60**(1): 31-43.

Tinus, R. (1996). Root growth potential as an indicator of drought stress history. Tree Physiol. **16**: 795-799.

Tyree, M.T., Dixon, M.A., & Thompson, R.G. (1984). Ultrasonic acoustic emmissions from the sap-wood of *Thuja occidentalis* measured inside a pressure bomb. Plant Physiol. **74**: 1046-1052.

Tyree, M.T. & Hammel, H.T. (1972). The measurement of the turgor pressure and the water relations of plants by the pressure-bomb technique. J. Exp. Bot. **23**(74): 267-282.

Tyree, M.T. & Jarvis, P.G. (1982). Water in tissues and cells, in Encyclopedia of Plant Physiology (New Series), Vol. 12B, Lange, O.L., Nobel, P.S., Osmond, C.B., and Zeigler, H., Eds., Springer-Verlag, Berlin. P. 35.

Tyree, M.T., MacGregor, M.E., Petrov, A., & Upenieks, M.I. (1978). A comparison of systematic errors between the Richards and Hammel methods of measuring tissue-water relations parameters. Can. J. Exp. Bot. **56**: 2153-2161.

Tyree, M.T. & Richter, H. (1981). Alternative methods of analyzing water potential isotherms: some cautions and clarifications. J. Exp. Bot. **32**(128): 643-653.

U.S. Department of Agriculture, Forest Service. (1989). A guide to the care and planting of southern pine seedlings. Atlanta, GA.

Weatherley, P.E. (1970). Some aspects of water relations. Adv. Bot. Res. **3**: 171-206.

Wiebe, H.H. (1972). The role of water potential and its components in physiological processes in plants. In: Psychrometry in water relations research (R.W. Brown and B.P. Van Haveren, eds.), Logan: Utah State Univ. Press. p 194-197.

Wilson, J.R., Fisher, M.J., Schulze, E.D., & Dolby, G.R. (1979). Comparison between pressure-volume and dewpoint-hygrometry techniques for determining the water relations characteristics of grass and legume leaves. Oecologia **41**: 77-88.

Table 1. Effect of MSC and 42 d cold storage on Ψ_r and Ψ_s. There were five levels of MSC determined by shoot water potential (Ψ_s). Values are means from five individual samples. Means within columns followed by the same letters are not significantly different at the 5% level.

Treatment	Root Ψ (MPa)		Shoot Ψ (MPa)	
MSC Level	Not Stored	Stored	Not Stored	Stored
0	-0.17^{a}	-0.17^{a}	-0.14^{a}	-0.14^{a}
1	-0.56^{b}	-0.56^{b}	-0.47^{b}	-0.56^{b}
2	-1.19^{c}	-1.52^{c}	-1.12^{c}	-1.32^{c}
3	-1.67^{d}	-1.90^{c}	-1.48^{d}	-1.59^{d}
4	-2.40^{e}	-2.71^{d}	-2.03^{e}	-2.10^{e}

Table 2. The results of the analysis of variance to test for the effects of storage and moisture stress conditioning (MSC) on leaf, stem, and root water contents and root and shoot water potentials.

Source of variation	DF	Leaf WC		Stem WC		Root WC		Ψ_r		Ψ_s	
		F Value	P>F	F Value	P>F	F Value	P>F	F Value	P>F	F Value	P>F
Replicate	2	13.72	0.002	6.32	0.008	8.11	0.003	2.86	0.083	1.94	0.171
Storage (S)	1	63.72	<0.001	5.87	0.026	17.56	<0.001	4.52	0.047	4.99	0.038
MSC (M)	4	90.05	<0.001	144.91	<0.001	43.03	<0.001	107.52	<0.001	265.14	<0.001
S*M	4	4.37	0.012	6.81	0.001	0.47	0.759	0.82	0.527	0.53	0.716
Error	18	MS=29.793		MS=16.982		MS=47.483		MS=0.051		MS=0.014	

Table 3. The results of the analysis of variance to test for the effects of storage and MSC on RGP, bud expansion, and survival.

Source of variation	DF	RGP		Bud expansion		Survival	
		F Value	P>F	F Value	P>F	F Value	P>F
Replicate	2	0.55	0.584	0.25	0.779	2.01	0.163
Storage (S)	1	29.08	<0.001	17.50	<0.001	31.83	<0.001
MSC (M)	4	36.42	<0.001	50.26	<0.001	70.07	<0.001
S*M	4	15.21	<0.001	5.87	0.003	4.86	0.008
Error	18	MS=117.321		MS=0.005		MS=0.008	

Table 4. The results of the analysis of variance to test for the effects of storage on PV curve parameters for the roots of control seedlings (MSC=0).

Source of variation	DF	Ψ_{π}^{100}		Ψ_{π}^{0}		RWC^{0}		V_a	
		F Value	P>F	F Value	P>F	F Value	P>F	F Value	P>F
Replicate	2	10.93	0.084	4.40	0.185	5.59	0.152	32.41	0.029
Storage	1	1.70	0.322	0.87	0.449	4.53	0.167	0.55	0.534
Error	2	MS=0.038		MS=0.083		MS=1.795		MS=35.612	

Table 5. The results of the analysis of variance to test for the effects of storage on PV curve parameters for the shoots of control seedlings (MSC=0).

Source of variation	DF	Ψ_{π}^{100}		Ψ_{π}^{0}		RWC^{0}		V_a	
		F Value	P>F	F Value	P>F	F Value	P>F	F Value	P>F
Replicate	2	0.80	0.557	0.37	0.731	2.64	0.275	1.18	0.458
Storage	1	0.47	0.564	4.95	0.156	5.45	0.145	12.17	0.073
Error	2	MS=0.003		MS=0.007		MS=1.259		MS=19.417	

Table 6. Effects of moisture stress conditioning (MSC) on water potential components and apoplasmic water fraction of roots and shoots. Only roots and shoots of MSC level 0 seedlings were measured before storage. The asterix indicates significant difference ($p<0.05$) between root and shoot for each parameter within each column. Means within columns after storage for each part followed by the same letter are not significantly different. n=3.

Part		MSC	Water relation parameters			
		levels	ψ_{π}^{100} (MPa)	ψ_{π}^{0} (MPa)	RWC^{0} (%)	V$_{a}$ (%)
	Before storage	0	-2.04	-2.89	66	-13
	After storage	0	-1.83^{a}	-2.67^{a}	63^{ab*}	-16^{a}
Root		1	-2.00^{b*}	-2.91^{ab*}	63^{ab*}	-12^{a}
		2	-2.06^{bd}	-3.03^{b*}	61^{b*}	-19^{a}
		3	-2.20^{c}	-3.02^{b*}	67^{a*}	-20^{a}
		4	-2.14^{cd}	-2.97^{b}	64^{ab}	-23^{a}
	Before storage	0	-1.17	-1.85	77	37
	After storage	0	-1.13^{a}	-1.70^{a}	75^{b*}	25^{a}
Shoot		1	-1.20^{a*}	-1.72^{a*}	79^{ac*}	31^{a}
		2	-1.36^{b}	-1.85^{a*}	80^{a*}	28^{a}
		3	-1.48^{bc}	-2.08^{b*}	78^{ac*}	26^{a}
		4	-1.57^{c}	-2.25^{b}	76^{bc}	22^{a}

Table 7. The results of the analysis of variance to test for the effects of MSC on PV curve parameters for the roots.

Source of variation	DF	Ψ_{π}^{100}		Ψ_{π}^{0}		RWC^{0}		V_a	
		F Value	P>F	F Value	P>F	F Value	P>F	F Value	P>F
Replicate	2	133.33	<0.001	27.43	<0.001	0.58	0.582	36.76	<0.001
MSC	4	13.78	0.001	3.67	0.055	1.73	0.235	0.92	0.497
Error	8	MS=0.004		MS=0.018		MS=7.693		MS=57.039	

Table 8. The results of the analysis of variance to test for the effects of MSC on PV curve parameters for the shoots.

Source of variation	DF	Ψ_π^{100} F Value	Ψ_π^{100} P>F	Ψ_π^{0} F Value	Ψ_π^{0} P>F	RWC^0 F Value	RWC^0 P>F	V_a F Value	V_a P>F
Replicate	2	1.44	0.293	0.17	0.848	0.83	0.472	5.20	0.035
MSC	4	15.29	<0.001	20.15	<0.001	3.73	0.053	1.31	0.345
Error	8	MS=0.006		MS=0.008		MS=3.197		MS=26.542	

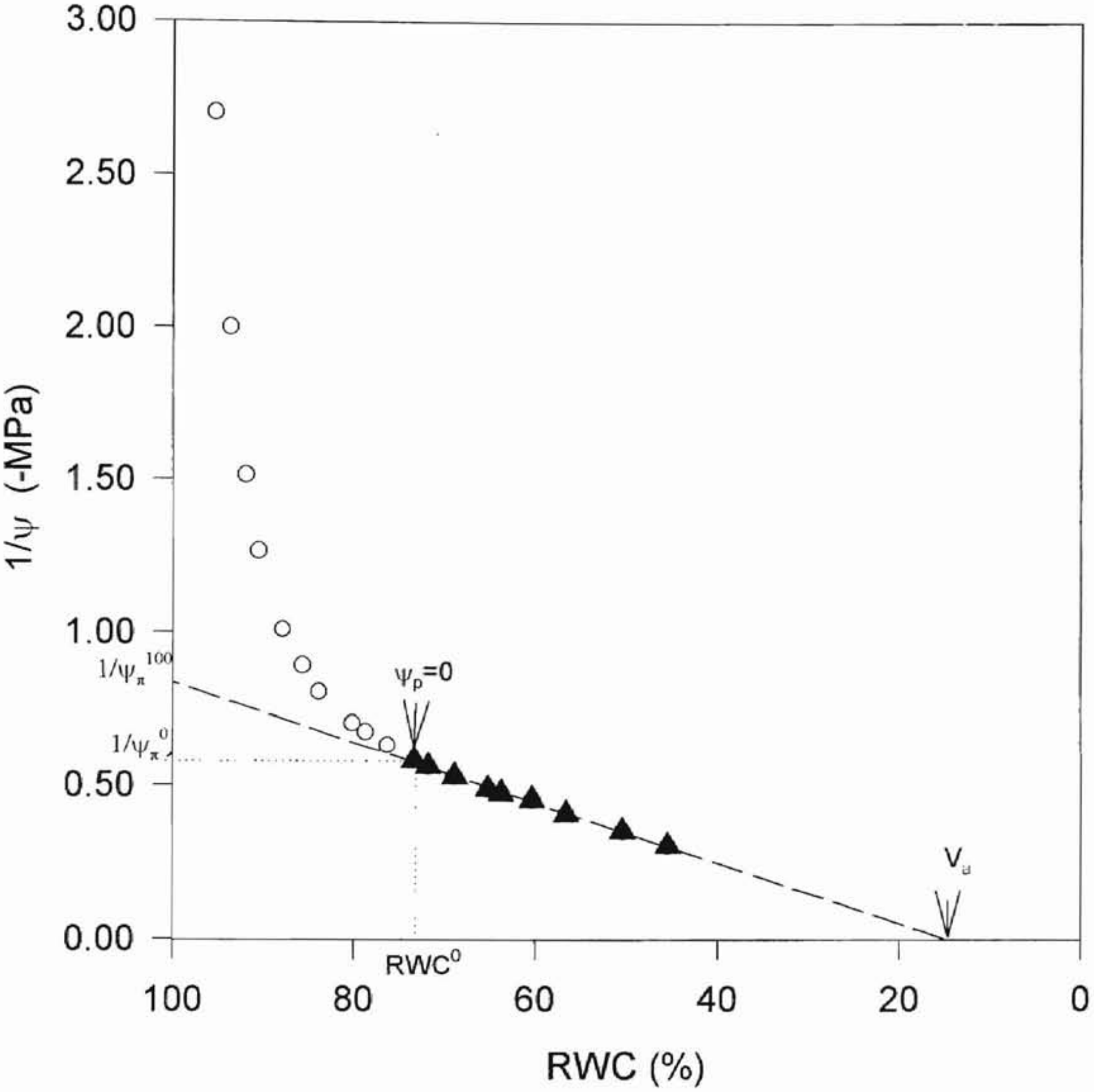

Fig. 1. A pressure-volume curve of a loblolly pine shoot shows the derivation of parameters from the relation between RWC in percent and reciprocal of total water potential ($1/\psi$). The osmotic potential at full turgor ($1/\psi_\pi^{100}$) and apoplasmic water fraction (Va) can be estimated by extrapolation of the linear portion of the curve. Incipient plasmolysis ($1/\psi_\pi^{0}$) is the point where $1/\psi_W$ becomes a linear function of further water loss. At this point ψ_π^{0} and RWC^0 (at zero turgor) can be obtained.

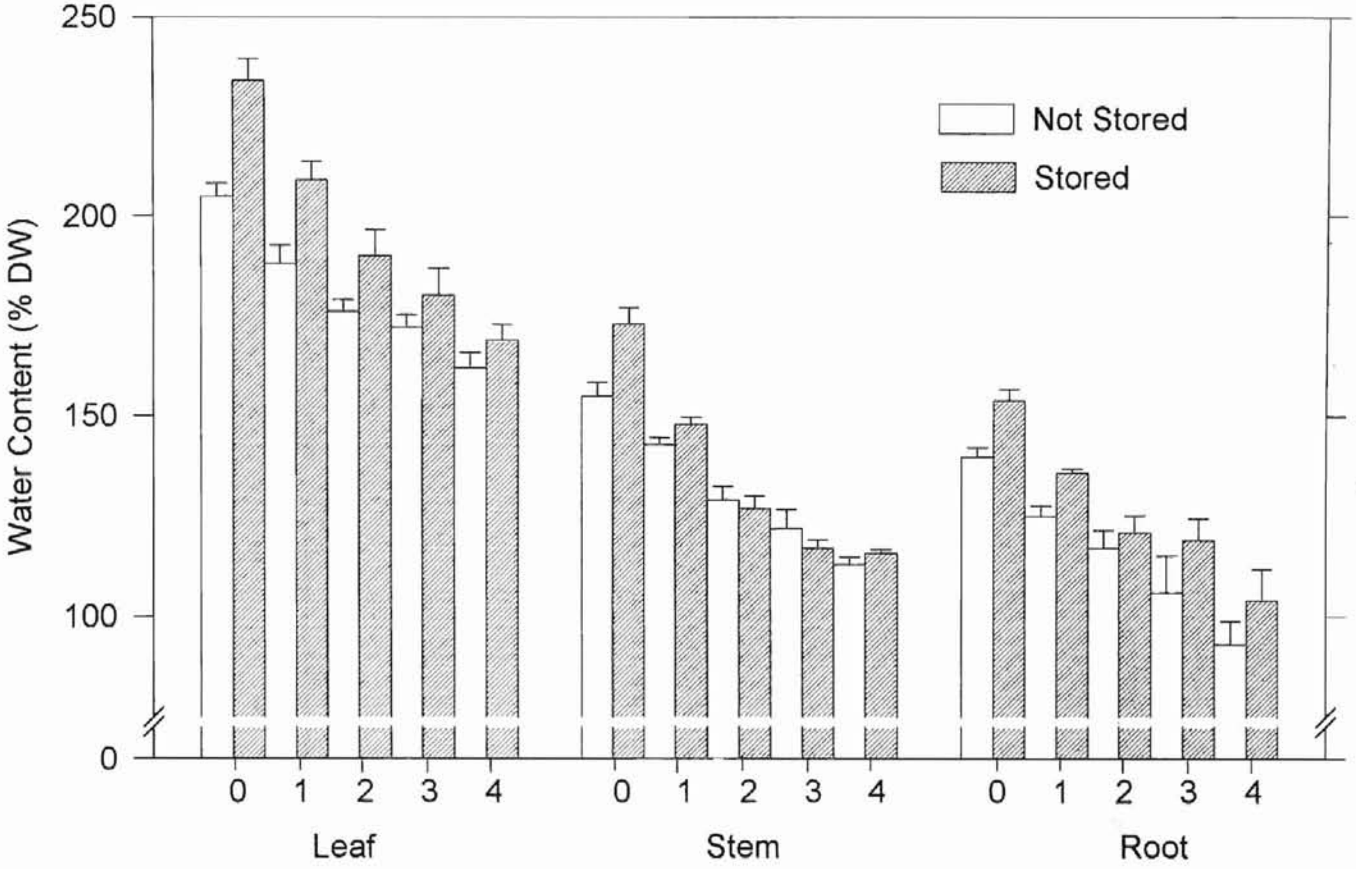

Fig. 2. Water content (WC) of leaf, stem and root (% dw) of loblolly pine seedlings after MSC. There were five levels of MSC determined by shoot water potential (ψ_s) immediately after MSC: (0) no MSC, ψ_s=–0.14, (1) ψ_s=–0.47, (2) ψ_s=–1.12, (3) ψ_s=–1.48, and (4) ψ_s=–2.03 MPa. WC was measured immediately after MSC and again after 42 d cold storage. n=3 and bars= standard error of the mean.

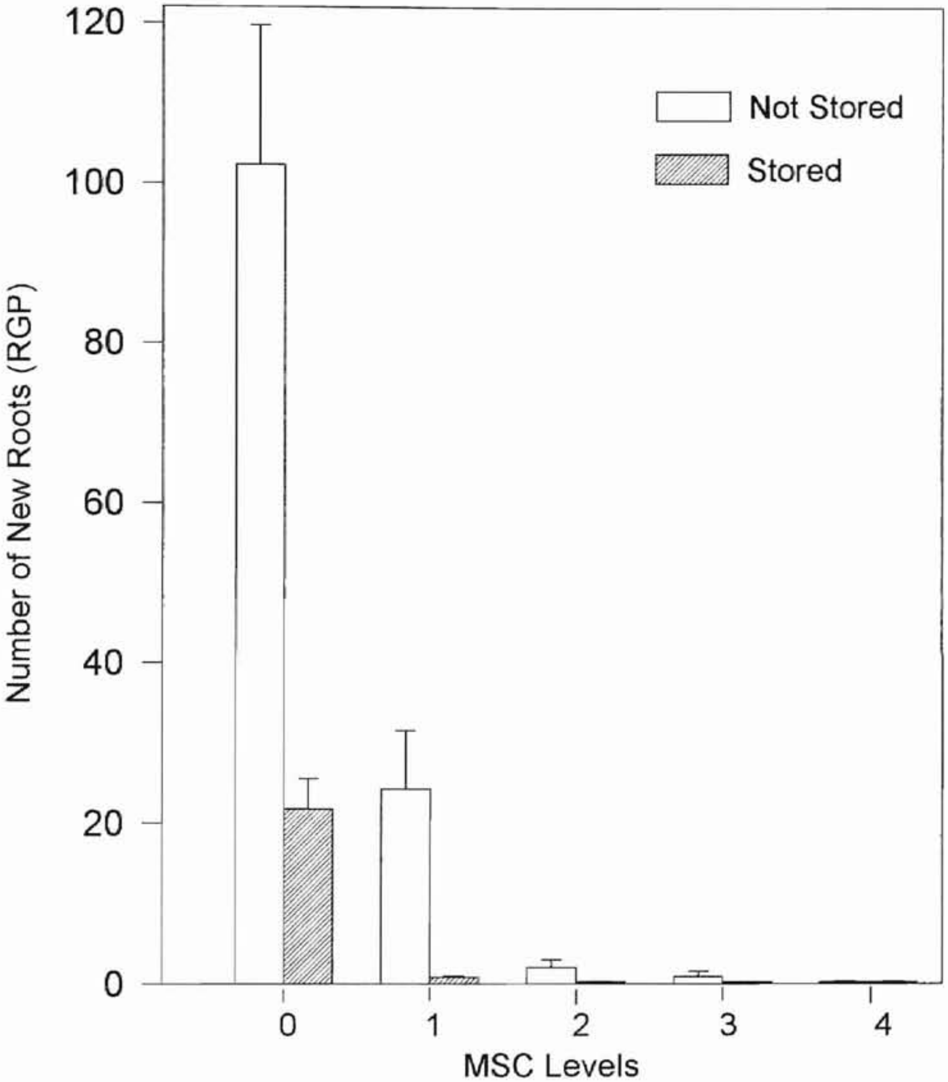

Fig. 3. Effect of MSC on RGP. There were five levels of MSC determined by shoot water potential (ψ_S) immediately after MSC: (0) no MSC, ψ_S=-0.14, (1) ψ_S=-0.47, (2) ψ_S=-1.12, (3) ψ_S=-1.48, and (4) ψ_S=-2.03 MPa. RGP was measured immediately after MSC and again after 42 d cold storage. RGP values are the mean of three replicates and 30 seedlings per replicate. Bars represent standard error of the mean.

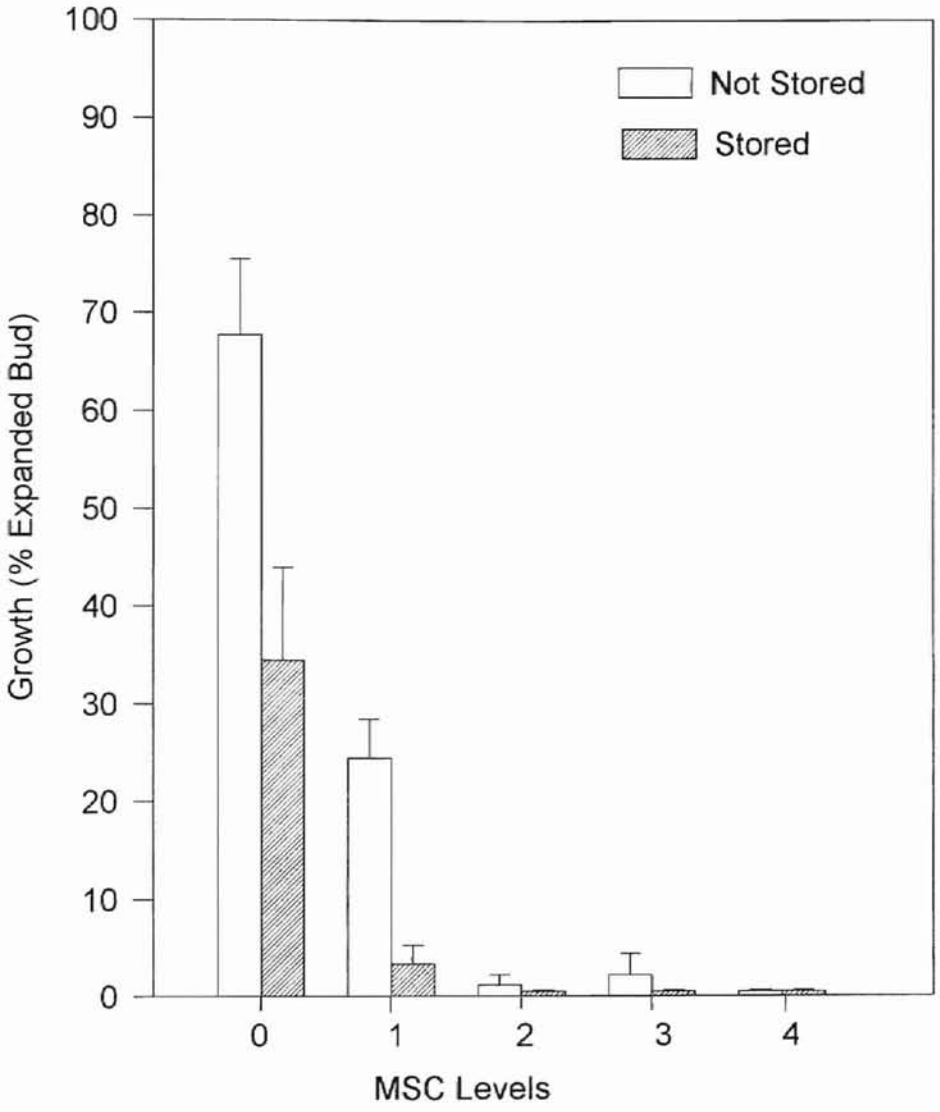

Fig. 4. Effect of MSC on bud expansion. There were five levels of MSC determined by shoot water potential (ψ_S) immediately after MSC: (0) no MSC, ψ_S=–0.14, (1) ψ_S=–0.47, (2) ψ_S=–1.12, (3) ψ_S=–1.48, and (4) ψ_S=–2.03 MPa. Bud expansion was measured immediately after MSC and again after 42 d cold storage. Values are the mean of three replicates and 30 seedlings per replicate. Bars represent standard error of the mean.

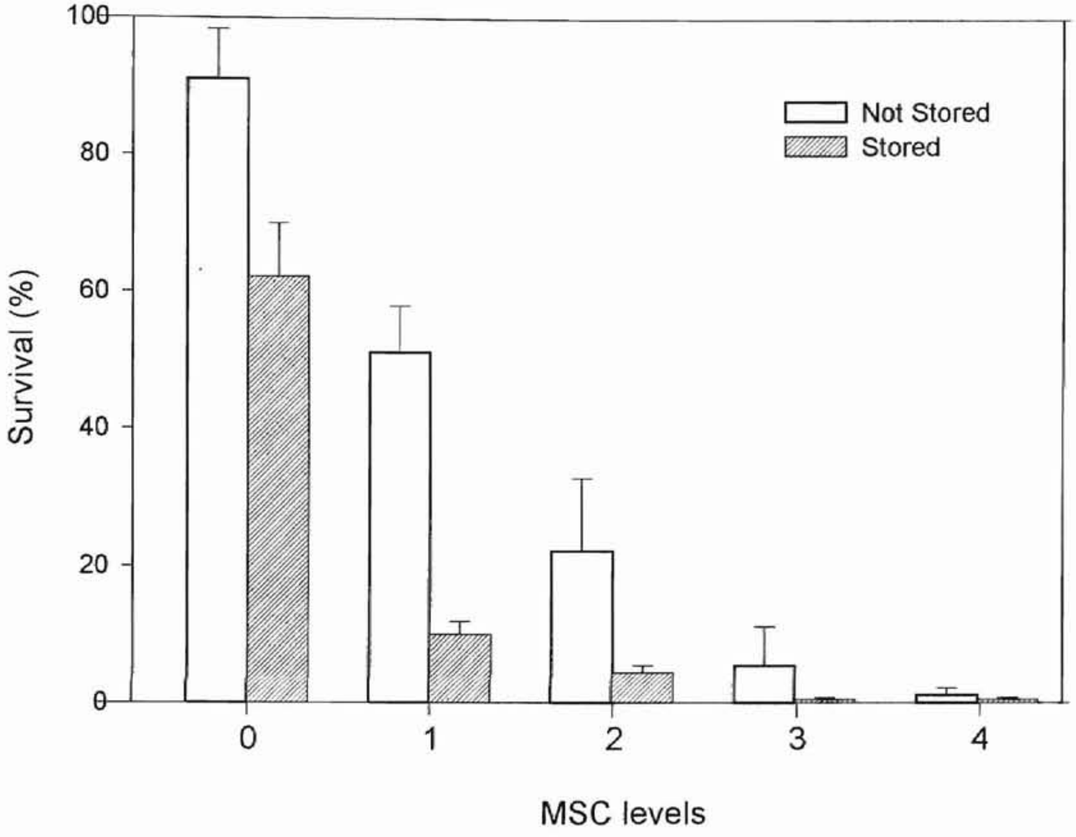

Fig. 5. Effect of MSC on survival. There were five levels of MSC determined by shoot water potential (ψ_S) immediately after MSC: (0) no MSC, ψ_S=–0.14, (1) ψ_S=–0.47, (2) ψ_S=–1.12, (3) ψ_S=–1.48, and (4) ψ_S=–2.03 MPa. Survival was measured immediately after MSC and again after 42 d cold storage. Values are the mean of three replicates and 30 seedlings per replicate. Bars represent standard error of the mean.

Chapter II

Effects of New Roots on Pressure-Volume Curve Parameters of Loblolly Pine Seedlings.

Introduction

Pressure-volume (PV) curve analysis is one of the most useful methods to determine water relations parameters of plants. The pressure chamber technique has been used for single leaves, shoots, and roots of many species to collect data for PV curve analysis (Tyree and Hammel, 1972; Neufeld and Teskey, 1985; Anderson et al., 1991; Parker and Colombo, 1996). Sometimes PV curve analysis has led to the identification of possible sources of error and deviations from theoretical assumptions. Cortes and Sinclair (1985) reported the symplastic water fraction to be >1 in many cases in drought-stressed soybean leaves. A relatively large symplastic water fraction, and correspondingly low apoplastic water fraction (V_a), has been reported in shoots compared to single leaves of white oak using the free transpiration method of PV curve analysis. This result is opposite to expectations, as woody parts are thought to have greater V_a (Parker and Pallardy, 1987). They attributed this apparent contradiction to the presence of "free water" filling air spaces in the sample near full turgor. The result was the presence of plateaus in the PV curve where relative water content (RWC) decreased without a corresponding decrease in

Ψ_w. Parker and Colombo (1995) found the symplastic water fraction values estimated from composite PV curves exceeded theoretically possible values (>1) in three out of five cases in shoots of red pine seedlings. They suggested that the apoplastic fraction may change during dehydration and the intercept method used to analyze PV curves may be inappropriate for estimation of this parameter. Neufeld and Teskey (1986) reported that loblolly pine twigs with high relative foliage weight had a larger estimated V_a, a result that was also opposite to expected. They found that V_a was mostly negative in fully defoliated twigs. Parker and Pallardy (1987) reported similar results in white oak shoots. Negative V_a was also found in root PV curves of loblolly pine in the first part of this thesis (Chapter I).

The objective of this study was to determine whether the presence of new roots affected the value of V_a estimated by PV curve analysis. Since Neufeld and Teskey (1986) found negative V_a in fully defoliated twigs, it was hypothesized that negative V_a was found in the roots of the first study (Chapter I) because the seedlings did not have any new roots. This hypothesis was tested by two independent approaches. First, V_a was compared between roots of seedlings lifted from the nursery in mid-winter with no new roots and roots of similar seedlings after prolific growth of new roots in a mist chamber. Second, V_a was compared among seedlings with prolific new roots where varying amount of new roots (0 to 100%) were removed. The distribution of water in plant parts was also studied. The effect of new root growth on PV curve parameters has not yet been reported.

Materials and Methods

One-year old loblolly pine (*Pinus taeda* L.) seedlings were obtained from Oklahoma State Forest Regeneration Center at Washington, Oklahoma, and transported to Stillwater, Oklahoma in kraft-polyethylene bags during the normal commercial lifting season on February 10, 1997. Three hundred undamaged, similar size seedlings were selected and washed free of soil. Six holders of fifty seedlings each were set on a root mist chamber (Rietveld and Tinus, 1987). The root mist chamber was disinfected and rinsed with water before the holders were placed on the chamber. The root mist chamber was placed in a growth room under a 1000-W high pressure sodium lamp at Oklahoma State University, Stillwater, OK. The lamp was on for the 16 h day and off for the 8 h night. Light intensity measured with quantum sensor was 150-200 μmol m^{-2} s^{-1} PPFD at seedling level. The seedlings were misted every 2.5 minutes for 20 seconds.

The PV curve analysis procedures used in this study were the same as in the first experiment (Chapter I) except that: 1) the roots were kept in separate plastic bags during bench-drying between consecutive Ψ_w determination to control and slow the rate of water loss of the samples, and 2) the weights of the samples were measured before and immediately after each pressure was measured and the mean of the two weights was used to determine water lost between each consecutive Ψ_w measurement. The PV curve analysis was used to estimate Ψ_π^{100}, Ψ_π^0, RWC^0, and V_a in two separate experiments.

The first experiment compared PV curve parameters on roots before and after new root growth. PV curve analysis was conducted on seedling roots after

2 (4 seedlings) and 17 d (6 seedlings) in the mist chamber. There were no new roots after 2 d and new roots were abundant after 17 d.

The second experiment compared PV curve parameters among seedlings with varying amount of new root removed. After 45 d new root growth, three treatments were applied to three randomly selected seedlings: 1.) new roots intact, 2.) 50% of new roots removed, and 3.) 100% of new roots removed. The root removal was carried out on a wetted table surface by detaching the new roots with a razor where they originated on the old ones. The combined length of new roots in the first treatment was measured, and half and all of this length was removed in treatments 2 and 3, respectively. There were 8 replicates of each treatment arranged in a randomized complete block design.

Ten seedlings were randomly chosen from the mist chamber after 35 d new root growth and rehydrated overnight (12 h). The next morning, the water content (g of water) was determined for each plant part: needles, stems, bark, new shoots, dry needles, buds, new roots, fine roots, tap root stems and bark. The portion of total plant water in each part was determined by dividing the weight of the water in each part by the total weight of water in the plant.

Data were analyzed using the General Linear Model (GLM) procedure of SAS (SAS Institute, Inc, Cary, NC, USA). A least significant difference (LSD) test was performed for comparison of parameter means at the 5% probability level.

Results

The Ψ_{π}^{100} and Ψ_{π}^{0} of seedling roots significantly increased after 17 d of new root growth in the mist chamber (Tables 1 and 2). RWC^0 and V_a did not change during 17 d in the mist chamber.

Removal of new roots had significant effects on all PV curve parameters measured in the roots (Table 3 and 4). Ψ_{π}^{100} and Ψ_{π}^{0} significantly decreased after 50% new root removal and did not change from 50% to 100% removal. RWC^0 and V_a did not change with the 50% removal and increased significantly with the 100% removal.

The distribution of water within the seedlings is shown in Figure 1. The majority of water was in the shoot (71%) and more than half of this water was in the needles. The root system had 29% of the total water present in the seedlings and almost half of this water was in the new roots.

Discussion

New root growth appeared to have a large effect on water relations parameters obtained from PV curves of roots of loblolly pine (Tables 1, 2, 3, and 4). Seedlings that had recently been lifted from the nursery bed with no new roots had water relations parameters similar to results from previous studies. Ritchie and Roden (1985) reported that Ψ_π^{100} was -1.07 MPa and Ψ_π^{0} was -2.28 MPa in the roots of non-stressed Douglas-fir seedlings. In contrast, when loblolly pine seedlings had 17 d new root growth in the mist chamber, Ψ_π^{100} significantly increased by 0.68 MPa and Ψ_π^{0} increased by 1.31 MPa in the roots. The increases may have resulted from: 1.) the seedlings being under stress at the beginning, 2.) new roots at 17 d. Since the growth process depends on turgor and high Ψ_w, it is reasonable to expect roots after 17 d in the mist chamber to increase osmotic potentials at both full and zero turgor. Earlier studies (Cheung et al. 1975; Ritchie and Shula 1984) reported that osmotic potentials are relatively high during plant growth. The low osmotic potentials in roots before new root growth may reflect the high root sugar concentrations in the roots. During root elongation the concentration of sugars may decline causing the osmotic potential to increase as reported by Ritchie and Shula (1984) in shoots and roots of Douglas-fir seedlings. They reported that the value of Ψ_π^{100} in the shoots was the lowest (~ –2.5 MPa) during midwinter and midsummer and the highest (~ –0.9 MPa) during April and May when new shoots were vigorously elongating. The value of Ψ_π^{0} showed a similar trend. It was the lowest (~ –3.8 MPa) during midwinter and the highest (~ –2.0 MPa) during shoot elongation in

April and May. Ψ_{π}^{100} and Ψ_{π}^{0} varied by ca. 0.5 and 1.0 MPa in the roots throughout the year, respectively. The smaller variation in root Ψ_{π}^{100} and Ψ_{π}^{0} may result from smaller variation in root sugar concentrations. In addition, roots did not attain significant winter cold hardiness in Douglas-fir seedlings.

The result of the current study showed V_a to be 21% in the seedlings which were recently lifted from the nursery and in the seedlings grown 17 d in the mist chamber. This result is consistent with earlier finding that V_a obtained from PV curves of barley (*Hordeum vulgare* L.) leaves was about 15% (Anderson et al., 1991). Parker and Colombo (1996) found 30% V_a in red pine shoots using the same PV and rehydration methods by submerging the roots into water. Santarius (1994) estimated V_a to be 20% in six moss species by comparison of the osmotic potentials of fully turgid living tissues and killed shoots. Similar V_a's have been reported for jack pine (*Pinus banksiana* Lamb.) and red pine using the free transpiration method of PV curves (Parker and Colombo, 1996).

V_a was negative in the roots in all treatments in the experiment described in Chapter I. In this study the same PV curve and rehydration methods and species were used as in the first study but V_a was positive. The only difference between the first and this study was that roots were kept in plastic bags separately during bench drying between each consecutive Ψ_w measurement to control and reduce the rate of water loss. Rapid rate of water loss can cause lack of equilibrium between apoplastic water held in xylem and symplastic water in the cells during bench drying which could lead to measured values of Ψ_w

being higher than the true values (Pallardy et al. 1991). In addition, rapid water loss may lead to cavitations in the xylem.

Neufeld and Teskey (1986) found negative V_a in fully defoliated twigs and it was hypothesized in this study that negative V_a's in roots resulted from seedlings not having any new roots. This hypothesis must be rejected according to the results (Tables 1 and 3). Removal of 50% of the new roots did not significantly change V_a and RWC^0 in the roots, but removal of 100% new roots significantly increased V_a and RWC^0, a result opposite to the result necessary to support the hypothesis.

Unsuberized new roots were assumed to have relatively larger V_a in the cortex cells than old brown roots; however, removal of all new roots increased V_a (Table 3). Apoplastic water held in cell walls and in intercellular spaces may have been lost during PV curve analysis. Almost half the water present in the whole root system was in the new roots (Fig. 1). V_a in the 100% removal treatment was almost two times more than that in the 0% removal indicating that new roots had a very small V_a. Therefore, new roots may have decreased the average V_a in the whole root system. Ψ_π^{100} and Ψ_π^{0} significantly decreased in 50% removal and did not change from 50% to 100% removal. In contrast, Neufeld and Teskey (1986) reported no significant differences in Ψ_π^{100} and Ψ_π^{0} between fascicles and 90% defoliated twigs of loblolly pine. However, they found defoliated twigs had values of Ψ_π^{100} and Ψ_π^{0} much lower than in fascicles.

Further work should be done to test the effect of different drying rate during bench drying on PV curve parameters and especially on V_a. More detailed studies should investigate the disequilibration resulting from fast water loss, cavitation inside seedlings, and the associated effects on PV curve parameters. It would still be very interesting to make an independent measure of V_a by morphometric methods and compare the results with estimation of V_a from PV curve analysis.

Conclusions

The results of this study lead to the following conclusions concerning bare-root loblolly pine seedlings:

1- New roots growth significantly affected PV curve parameters but not V_a in the roots.

2- Removal of new roots significantly lowered Ψ_π^{100} and Ψ_π^{0} in the roots and increased V_a.

3- The negative V_a observed in roots is not caused by the lack of new roots. Evidence suggests the negative V_a's may result from a lack of equilibrium between water in the symplasm and apoplasm due to rapid water loss on the bench between measurements.

4- More than half of the water present in the shoot system was in the needles and almost half of the water present in the root system was in new roots.

References

Anderson, M.N., Jensen, C.R., & Lösch, R. (1991). Derivation of pressure-volume curves by a non-linear regression procedure and determination of apoplastic water. J. Exp. Bot. **42**(235): 159-165.

Cheung, Y.N.S., Tyree, M.T., & Dainty, J. (1975). Water relations parameters on single leaves obtained in a pressure bomb and some ecological interpretations. Can. J. Bot. **53**: 1342-1346.

Cortes, P.M. & Sinclair, T.R. (1985). Extraction of apoplastic water during pressure-volume dehydrations. Agron. J. **77**: 798-802.

Neufeld, H.S. & Teskey, R.O. (1986). Variation in the amount of foliage on woody shoots and its effects on water relations parameters derived from pressure-volume curves. Can. J. For. Res. **16**:239-243.

Pallardy, S.G., Pereira, J.S., & Parker, W.C. (1991). Measuring the state of water in tree systems. In: Techniques and Approaches in Forest Tree Ecophysiology. Lassoie, J.P. & Hinckley, T.M., Eds. pp 27-76. CRC Press, Boca Raton, FL.

Parker, W.C. & Colombo, S.J. (1995). A critical re-examination of pressure-volume analysis of conifer shoots: comparison of three procedures for generating pv curves on shoots of *Pinus resinosa* Ait. Seedlings. J. Exp. Bot. **46**(292):1701-1709.

Parker, W.C. & Colombo, S.J. (1996). Cut-shoot vs root rehydration: effects on pressure-volume analysis of shoots and fascicles of *Pinus resinosa* Ait. seedlings. Physiol. Plant. **97**: 651-658.

Parker, W.C. & Pallardy, S.G. (1987). The influence of resaturation method and tissue type on pressure-volume analysis of *Quercus alba* L. seedlings. J. Exp. Bot. **38**(188): 535-549.

Rietveld, W.J. & Tinus R.W. (1987). Root misting chamber. Proj. 796. Missoula, MT: USDA Forest Service Equipment Development Center. 5 p.

Ritchie, G.A. & Shula, R.G. (1984). Seasonal changes of tissue-water relations in shoot and root system of Douglas-fir seedlings. For. Sci. **30**:538-548.

Ritchie, G.A. & Roden, J.R. (1985). Comparison between two methods of generating pressure-volume curves. Plant Cell and Environ. **8**:49-53.

Santarius, K.A. (1994). Apoplasmic water fraction and osmotic potentials at full turgidity of some Bryidae. Planta **193**: 32-37.

SAS. (1988). SAS procedures guide, SAS Indtitute Inc., NC., USA.

Tyree, M.T. & Hammel, H.T. (1972). The measurement of the turgor pressure and the water relations of plants by the pressure-bomb technique. J. Exp. Bot. **23**(74):267-282.

Tyree, M.T. & Jarvis, P.G. (1982). Water in tissues and cells, in Encyclopedia of Plant Physiology (New Series), Vol. 12B, Lange, O.L., Nobel, P.S., Osmond, C.B., and Zeigler, H., Eds., Springer-Verlag, Berlin.

Tyree, M.T., MacGregor, M.E., Petrov, A., & Upenieks, M.I. (1978). A comparison of systematic errors between the Richards and Hammel methods of measuring tissue-water relations parameters. Can. J. Exp. Bot. **56**: 2153-2161.

Tyree, M.T. & Richter, H. (1981). Alternative methods of analyzing water potential isotherms: some cautions and clarifications. J. Exp. Bot. **32**(128): 643-653.

Table 1. The results of the analysis of variance to test for the effects of new root production after 2 and 17 days on PV curve parameters of the roots.

Source of Variation		Ψ_π^{100}		Ψ_π^{0}		RWC^0		V_a	
	DF	F	F>P	F	F>P	F	F>P	F	F>P
Time	1	56.71	<0.001	160.84	<0.001	5.00	0.056	0.00	0.999
Error	8	MS=1.890		MS=2.659		MS=21.568		MS=83.785	

Table 2. PV curve parameters of roots of the seedlings placed in a mist chamber to grow new white roots for 2 and 17 days. Means within columns followed by the same letter are not significantly different ($\propto=0.05$). n= sample size.

Time (d)	Ψ_π^{100} (MPa)	Ψ_π^{0} (MPa)	RWC^0 (%)	V_a (%)	n
2	-1.27^b	-2.27^b	65^a	21^a	4
17	-0.59^a	-0.94^a	71^a	21^a	6

Table 3. The results of the analysis of variance to test for the effects of new root removal on PV curve parameters of the roots.

Source of Variation	DF	Ψ_π^{100}		Ψ_π^{0}		RWC^0		V_a	
		F	F>P	F	F>P	F	F>P	F	F>P
Replicate	7	0.88	0.548	2.58	0.062	1.12	0.406	1.07	0.430
Treatment	2	7.68	0.005	16.16	<0.001	5.93	0.013	15.54	<0.001
Error	14	MS=0.427		MS=0.708		MS=6.573		MS=26.612	

Table 4. PV curve parameters of roots of the seedlings grown in a mist chamber for 40 days. Three treatments were applied to the roots: 1) 0% of new roots removed (control), 2) 50% of new roots removed, and 3) 100% of new roots removed. Means within columns followed by the same letter are not significantly different at the 5% level. n= sample size.

Treatment	Ψ_π^{100} (MPa)	Ψ_π^{0} (MPa)	RWC^0 (%)	V_a (%)	n
0% removed	-0.68^a	-1.03^a	71^b	17^b	8
50% removed	-0.77^b	-1.23^b	71^b	22^b	8
100% removed	-0.80^b	-1.24^b	75^a	31^a	8

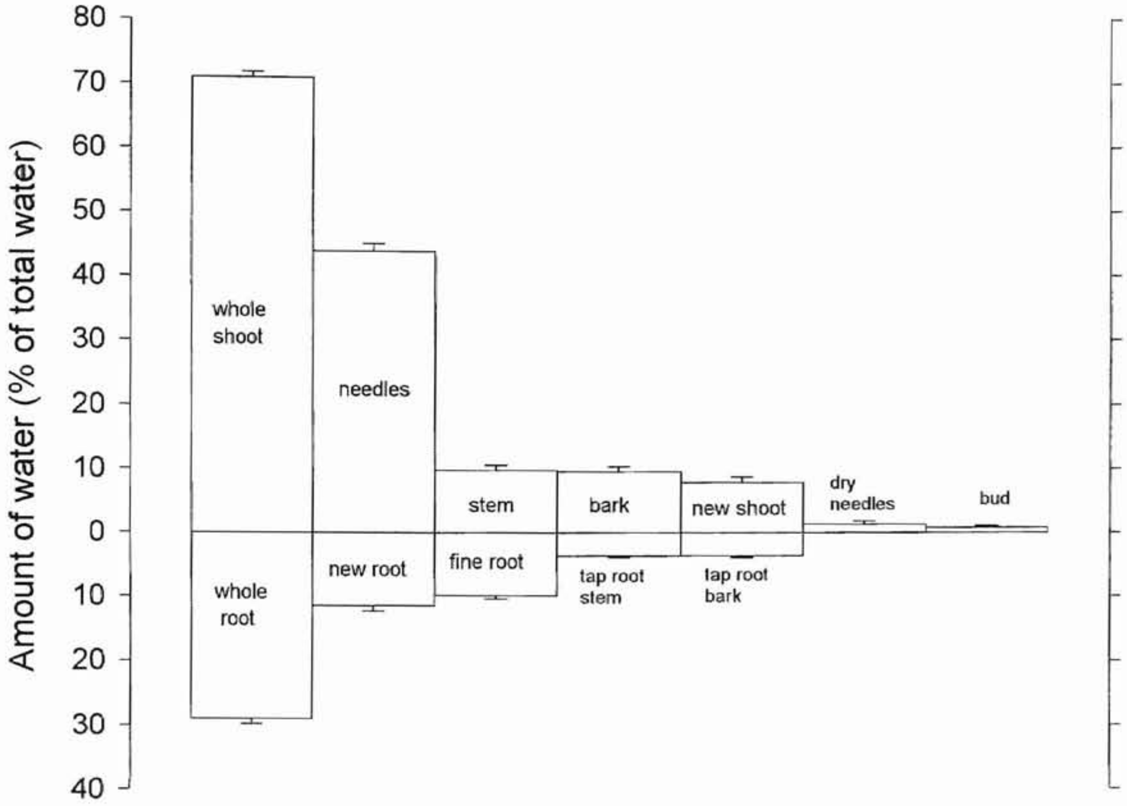

Figure 1. Distribution of water in parts of loblolly pine seedlings as a percent of total water present in the whole seedlings. Bars represent the standart error of the mean. n=4 for bud, 8 for dry needles, and 10 for other parts.

VITA

Ferit Kocacinar

Candidate for the Degree of

Master of Science

Thesis: EFFECTS OF MOISTURE STRESS CONDITIONING AND THE PRESENCE OF NEW ROOTS ON THE WATER RELATIONS PARAMETERS DERIVED FROM PRESSURE VOLUME CURVES

Major Field: Forest Resources

Minor Field: Silviculture

Biographical:

Personal Data: Born in Antakya, Turkey, February 6, 1971, the son of Mehmet and Turkiye Kocacinar.

Education: Graduated from TSG High School, Mersin, Turkey, in July 1988; attended University of Istanbul Faculty of Forestry, Istanbul, Turkey, 1989-1993; received Bachelor of Science degree in Forestry from University of Istanbul in 1993; attended Oklahoma State University, United States of America, 1995-97; completed requirements for Master of Science degree at Oklahoma State University in December, 1997.