EFFECTS OF ROOT GROWTH AND PHYSIOLOGY ON DROUGHT RESISTANCE IN DOUGLAS-FIR, LODGEPOLE PINE, AND WHITE SPRUCE SEEDLINGS

by

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ABSTRACT

Two aspects of drought resistance were investigated on wet and dry ecotypes of three conifer species: 1) the relative importance of drought avoidance and drought tolerance mechanisms in resisting drought stress was assessed on Douglas-fir (*Pseudotsuga menzieseii*) and lodgepole pine (*Pinus contorta*) seedlings, and 2) the effects of drought on root hydraulic conductance and low temperature on root water flow rates were assessed on first-year seedlings of Douglas-fir, lodgepole pine and white spruce (*Picea glauca*).

To study drought avoidance, Douglas-fir and lodgepole pine seedlings were grown in sealed containers in wet (522% water content) or dry (318% water content) peat/vermiculite soil in a factorial treatment design. Dry weights, water use, and root length were determined for seedlings at each of five harvests and stomatal conductance and shoot water potentials were measured during the last 12 weeks of the experiment. Lodgepole pine seedlings had greater dry matter production, water use, stomatal conductance and new root length than Douglas-fir seedlings. New root weight of lodgepole pine seedlings exceeded that of Douglas-fir seedlings during the last five weeks of the experiment, and specific root length of new roots was higher for lodgepole pine seedlings throughout the experiment. Douglas-fir seedlings showed higher water use efficiency (WUE) than lodgepole pine seedlings, although water uptake rates per unit of root dry weight showed little difference between species. Soil water treatment influenced specific root length of new roots, water uptake per unit of new root length, and WUE in Douglas-fir seedlings more than in lodgepole pine seedlings.

To study drought tolerance, Douglas-fir and lodgepole pine seedlings were grown under drought and well-watered conditions. At each of three harvests a pressure-volume curve was produced for each seedling. Douglas-fir maintained a lower osmotic potential at full saturation ($\psi_{\pi sat}$) and lower turgor loss point ($\psi_{\pi tlp}$) than lodgepole pine under both watering regimes. Both species had lower $\psi_{\pi sat}$ and $\psi_{\pi tlp}$ when drought-stressed.

Douglas-fir appears to be a more conservative species, maintaining low stomatal conductance and tolerating drought conditions, whereas lodgepole pine avoids drought by producing large amounts of roots to exploit the soil resource.

To study root hydraulic conductance (L_{proot}) and water flow rates through roots (WFRR), water flow was measured through de-topped roots of Douglas-fir, lodgepole pine, and white spruce seedlings in a pressure chamber. In a drought experiment, seedlings were grown in sandy soil in a greenhouse under drought and well-watered conditions during their first growing season and, in a low temperature experiment, seedlings were grown in sandy soil in growth chambers at 25/20^{o}C (day/night) and 15/10^{o}C.

In the drought experiment, water flow through roots was measured at three pressures. No differences in L_{proot} were found for Douglas-fir and white spruce seedlings grown under the two watering regimes, however, lodgepole pine seedlings had reduced L_{proot} when grown under drought conditions. Well-watered seedlings of lodgepole pine and white spruce had higher L_{proot} in 1989 than in 1990 whereas Douglas-fir seedlings had the same L_{proot} in both years.

In the low temperature experiment, WFRR was measured at 1.0 MPa and temperatures of 20^{o}C for 24 hours or 20^{o}, 12^{o}, and 4^{o}C for 18, 15, and 15 hours respectively. At 20^{o}C, white spruce seedlings had higher WFRR than the other two species. Lodgepole pine and white spruce seedlings grown in the 15^{o}/10^{o}C

growth chamber had higher WFRR than seedlings grown in the 25°/20°C growth chamber. Water flow rate decreased with temperature in all three species. After correcting for viscosity, all seedlings had lower WFRR with reduced temperature, except for Douglas-fir and white spruce seedlings grown at 15°/10°C which had the same WFRR at 20°C and 12°C. Therefore, Douglas-fir and white spruce seedlings were found to become less sensitive to low temperature (chilling) stress when pre-conditioned at low temperatures.

In the drought and low temperature studies, dry weight biomass of white spruce was lowest but white spruce had a greater specific root length than lodgepole pine and Douglas-fir. In the drought study, biomass production in seedlings from wet ecotypes of each species was more reduced when drought-stressed than seedlings from dry ecotypes.

Examiners:

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS AND ABBREVIATIONS

ABA	Abscisic acid
EC	ecotype
ERWC	estimated relative water content
GC	growth chamber temperature
HT	high temperature
HV	harvest date
J	flow rate ($g\ s^{-1}$ or $m^3\ s^{-1}$)
J_c	corrected flow rate ($g\ s^{-1}$)
J_m	measured flow rate ($g\ s^{-1}$)
J_v	flux density ($m^3\ m^{-2}\ s^{-1}$)
l	length (m)
L_r	hydraulic conductivity ($m^2\ s^{-1}\ MPa^{-1}$)
L_p	hydraulic conductance ($m\ s^{-1}\ MPa^{-1}$)
L_{proot}	root hydraulic conductance
LT	low temperature
NRL	new root length (m)
PC	pressure chamber temperature
PV	pressure-volume
r	radius (m)
R_p	resistance ($s\ MPa\ m^{-1}$)
RLE	root length efficiency ($g\ m^{-1}$)
RWC	relative water content
RWE	root weight efficiency ($g\ g^{-1}$)
SDW	shoot dry weight (g)
SM	soil moisture
SP	species
SPAC	soil-plant-atmosphere continuum
SRL	specific root length ($m\ g^{-1}$)
SW	soil water treatment
SYMFR	symplastic fraction
TLP	turgor loss point
WFRR	water flow rate through roots ($g\ s^{-1}$)
WR	watering regime
WU	water use (g or kg)
WUE	water use efficiency (g dry matter g^{-1} H_2O used)
YR	year
E	bulk elastic modulus (MPa)
E_{max}	bulk elastic modulus near full saturation (MPa)
η	viscosity (MPa s)
P	hydrostatic or turgor pressure (MPa)
σ	reflection coefficient of solutes
ψ	water potential (MPa)
ψ_π	osmostic potential (MPa)
$\psi_{\pi sat}$	osmotic potential at full saturation (MPa)
$\psi_{\pi tlp}$	osmotic potential at turgor loss point (MPa)

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Chapter 1.
GENERAL INTRODUCTION

Planting of conifer seedlings is an important component of reforestation in British Columbia. Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), lodgepole pine (*Pinus contorta* Dougl.) and white spruce (*Picea glauca* (Moench) Voss) are conifer species commonly planted in the Pacific northwest. To select species, provenances, or families for optimal survival and growth in different environments, it is desirable to understand how conifer seedlings adjust morphologically and physiologically to environmental conditions, such as drought and low temperature.

As seedlings become drought-stressed, they develop plant water deficits which can lead to injury and, eventually, death. To reduce the detrimental effects of drought, seedlings may initiate drought-avoidance and/or drought-tolerance mechanisms. Plant species may use one or both of these mechanisms to withstand drought, but little is known about the relative importance of these mechanisms in determining survival and growth of different conifer seedlings. By growing conifer seedlings under wet and dry watering regimes and recording root growth, water uptake, transpiration rates, water loss, and osmotic adjustment, an understanding of how drought resistance mechanisms vary between and within species can be obtained.

Water movement through seedlings is driven by water potential (ψ) gradients in the soil-plant-atmosphere continuum (SPAC). Although the resistance of the stomata to water movement through plants has been much studied, the effect of drought on water movement through roots has received less attention. Since roots supply water to shoots, changes in water conductance through roots can have a direct effect on shoot physiology. Under well-watered conditions, when stomata

are open and soil to root contact is good, the suberized endodermal cells in the roots may provide the greatest resistance to water movement in seedlings. Using a pressure chamber to measure hydraulic conductance in de-topped roots, the importance of root resistance to seedling water flow, and the effect of environmental factors, such as drought and low temperature, on root resistance can be studied.

Chilling temperatures can simulate drought, since increased water viscosity reduces water uptake and movement through seedlings. Chilling can also cause changes in membrane permeability, leading to secondary biochemical and physiological effects. Chilling effects on water flow through roots can be studied by controlling root temperature in a pressure chamber. Since conifer seedlings are often planted into soils with low temperatures, the effect of direct and pre-conditioning temperatures on water flow through roots can have practical applications.

This thesis deals with interrelated aspects of drought resistance in conifer seedlings. Drought avoidance and drought tolerance of wet and dry ecotypes of Douglas-fir and lodgepole pine seedlings are reported in Chapters 3 and 4. The general objective of this work was to assess the relative importance of drought avoidance and drought tolerance for protecting typical conifer seedlings from drought stress. Effects of drought stress and temperature on water flow through wet and dry ecotypes of Douglas-fir, lodgepole pine, and white spruce root systems are reported in Chapters 4 and 5. The intention was to find out what part root responses might play in determining drought resistance of conifer planting stock.

Chapter 2
LITERATURE REVIEW

2.1 WATER POTENTIAL

Water potential (ψ) is a thermodynamic variable which quantifies the amount of work water can do (Slatyer, 1967) and is used to relate water status at various positions in the soil-plant-atmosphere continuum (SPAC). The two components of ψ are hydrostatic or turgor pressure (P), which is the force exerted by water pressure differing from ambient, and osmotic potential (ψ_π), which is the change in chemical potential of water when solutes are present. Matric potential and gravitation potential components of ψ have been excluded since the former can be incorporated into the P term and the latter is insignificant when studying seedlings and roots (Jones, 1992). Therefore, in this study,

$$\psi = P + \psi_\pi \qquad (1)$$

where ψ (MPa) is negative, P (MPa) is positive, except when a system is under tension (negative pressure), and ψ_π (MPa) is negative due to solutes reducing the chemical potential of water. The chemical potential of pure water has arbitrarily been assigned a value of zero.

The driving force for water movement in SPAC is along a gradient of decreasing ψ. Generally, water flow can be described using an analogy to Ohm's law,

$$J_v = L_p \times (\psi_{soil} - \psi_{air}) = (\psi_{soil} - \psi_{air}) / R_p \qquad (2)$$

where J_v = flux density ($m^3\ m^{-2}\ s^{-1}$), L_p = hydraulic conductance ($m\ s^{-1}\ MPa^{-1}$), R_p = resistance = $1/L_p$ (Jones, 1992). Under well-watered conditions, water moves through the soil and along the apoplastic pathway to the endodermis with little resistance. At the endodermis, water must cross the cell membrane into the

endodermal cells, since the suberized endodermal cell walls are impermeable to water. After crossing the endodermis, water moves into the air with little resistance if the stomata are open. Therefore, under well-watered conditions the largest resistance in the SPAC is in the roots.

2.2 DROUGHT RESISTANCE

Under drought conditions, drought being defined as the environmental condition where restricted water supply reduces plant productivity, water movement in SPAC is reduced due to decreased conductances (or increased resistances) in the plant, soil, and/or across the soil:root interface (Passioura, 1980) and, therefore, root resistance may no longer be the major resistance to water movement. Plants that are drought-stressed build up water deficits due to water loss by transpiration being greater than water uptake by roots (Kozlowski *et al.*, 1991; Hinckley *et al.*, 1991). Although plant morphology, physiology, and biochemistry are affected by drought, cellular growth is the most sensitive process to drought-stress (Hsaio *et al.*, 1976; Passioura, 1982).

Conifer seedlings grown under drought conditions have reduced seedling dry weight (Kaufmann, 1968; Joly *et al.*, 1989; van den Driessche, 1991), photosynthesis rates (Grieu *et al.*, 1988), transpiration rates (Lopushinsky & Klock, 1974; Roberts & Dumbroff, 1986; Livingston & Black, 1987) and stomatal conductance (Squire *et al.*, 1988; Grossnickle & Russell, 1991). Drought conditions are also known to increase water use efficiency (WUE) (Seiler & Johnson, 1988); WUE is the amount of seedling dry matter produced per unit water used. Within the same conifer species, seedlings from dry ecotypes are more drought resistant than seedlings from wet ecotypes (Ferrell & Woodard, 1966; Pharis & Ferrell, 1966; Zavitkovski & Ferrell, 1968). A recent study by Joly *et al.* (1989) found one-year-

old Douglas-fir seedlings from inland sites to have greater root to shoot ratios than seedlings from coastal sites.

To reduce the detrimental effects of drought, drought-stressed seedlings are able to initiate drought avoidance (or desiccation postponement) and/or drought tolerance (or desiccation tolerance) mechanisms (Levitt, 1980). Drought avoidance mechanisms allow seedlings to maintain high cellular water potentials under drought conditions by reducing plant water loss and/or increasing plant water uptake. Drought tolerance mechanisms allow seedlings to maintain cellular growth at low cellular water potentials by actively increasing cellular solute concentrations.

2.2.1 Drought Avoidance

Plant water loss is reduced by decreasing transpiration rates by closing stomata (short term) and decreasing leaf area (long term). The evaporation rate of water from the mesophyll cells and movement of the water vapour to the ambient air is a function of environmental factors, such as vapour pressure difference between intercellular spaces in needles and the relative humidity of the air and photon flux density, and internal plant variables, such as internal CO_2 concentration and leaf P (Schulze, 1986; Kaiser, 1987).

The causes of stomatal closure in drought-stressed plants have recently received considerable attention. Although leaf turgor pressure was previously perceived as a major factor determining stomatal conductance, since leaf guard cells must be turgid for stomata to open, studies on wheat and sunflower by Gollan *et al.* (1986) and Schurr *et al.* (1992) have shown that stomata will close under low soil moisture conditions even when leaf cells are kept turgid. Therefore, it appears that guard cells may react to drought-stress differently than other leaf cells.

Furthermore, since stomata respond to soil moisture, there appears to be communication between roots and shoots using a chemical messenger, probably abscisic acid (ABA), which causes stomata to close when soil moisture is low (Davies *et al.*, 1986; Davies & Zhang, 1991). Taiz & Zeiger (1991) have suggested that ABA from the roots provides an early warning signal and that ABA from the mesophyll cells is later released into the apoplast and guard cells causing stomatal closure. High ABA concentrations have been found in foliage of drought-stressed Monterey pine seedlings (Roberts & Dumbroff, 1986; Squire *et al.*, 1988). More recent preliminary work on conifer species by Livingston (pers. comm.) has indicated that in some conifers, stomatal closure is controlled by hydraulic signals. More work is needed to show which species rely on hydraulic and which species on chemical signals for stomatal control.

However the stomata close, stomatal closure can provide the greatest resistance to water movement through SPAC. Two detrimental effects of stomatal closure are reduced carbon uptake and increased leaf temperature. Since the gradient for carbon uptake by plants is less than the gradient for water loss, as stomata close, WUE increases until the soil becomes very dry (Taiz & Zeiger, 1991). Measurements of photosynthesis and transpiration rates can be used to measure instantaneous WUE and carbon isotope discrimination can be used to determine WUE over time (Jones, 1992).

When stomata close, leaf temperature increases because transpiration has a cooling effect. As leaf temperature rises, cuticular transpiration will increase without a concomitment uptake of carbon due to saturation vapour pressure increasing approximately exponentially with temperature. Therefore, in the long term, reducing leaf area is a better mechanism for reducing water loss than stomatal closure (Passioura, 1982).

Plants can increase water uptake by increasing biomass allocation to root growth, thereby increasing root surface area. Increasing root length permits exploration of new water resources and increases new, white root length. Since new, white roots take up four times more water than older, suberized, brown roots (Kramer & Bullock, 1966; Chung & Kramer, 1975), root growth would greatly increase water uptake. However, brown roots are important for water uptake since they comprise the majority of the root after the first growing season and are the only root type present in the spring when water is required for new root growth (van den Driessche, 1987).

2.2.2 Drought Tolerance

Osmotic adjustment is a drought tolerance mechanism sometimes found in seedlings resistant to drought conditions. During osmotic adjustment cells use energy to produce or take up solutes, resulting in decreased ψ_π and decreased ψ, with little change in P. Consequently, seedling roots can take up water from soils with lower ψ than would be possible without osmotic adjustment. It is not clear whether solutes are produced or taken up by the cell, however, the solutes suspected of decreasing cellular ψ_π are sugars, organic acids, and ions (i.e. K^+) (Taiz & Zeiger, 1991). These solutes are found in the tonoplast since in the cytosol they will inhibit enzyme function. Solutes such as proline, sugar alcohols, sorbitol, and glycine betaine are known as compatible solutes. Since compatible solutes do not affect enzyme function, they are present in the cytosol and act to neutralize the charge of solutes in the tonoplast (Taiz & Zeiger, 1991). The active increase of solutes for osmotic adjustment should not be confused with the passive increase in solute concentration that occurs when seedlings become drought-

stressed and lose cellular water. Passive solute increase results in decreased ψ and ψ_π, and also decreased P.

Cell turgor maintenance is an important result of osmotic adjustment; however, maintenance of turgor is also dependent on cell water content and bulk elastic modulus of the cell wall (Zimmerman, 1978; Morgan, 1984). Perfect osmotic adjustment would occur if the decrease in ψ_π was equal to the decrease in ψ such that P was maintained, however, P tends to be reduced when plants are drought-stressed and the amount of reduction is a function of cell wall characteristics. Bulk elastic modulus (E) is a measure of cell wall elasticity, which is dependent on cell wall physiology and biochemistry, shape of the cell, and neighbouring cells. In a tissue,

$$E = (\Delta P/\Delta RWC) \times RWC \qquad (3)$$

where E (MPa), ΔP = change in cell P (MPa) and ΔRWC = change in cellular relative water content (m^{-3}). Cells with rigid cell walls have high E, since there is a relatively large drop in P with a small decrease in relative water content.

A common technique used to study osmotic adjustment involves calculating osmotic potential at full saturation ($\psi_{\pi sat}$), osmotic potential at the turgor loss point ($\psi_{\pi tlp}$), and E near full turgor (E_{max}) from graphs of pressure-volume curves. Development of the pressure bomb was instrumental in the measurement of ψ_π and P in cells (Hinckley *et al.*, 1991). Dixon (1914) built the first pressure chamber to verify his 'cohesion theory', which suggested that movement of water up the xylem was due to the tensile strength of water. Although Dixon's pressure chambers blew up, Scholander *et al.* (1964; 1965) later built a pressure chamber which provided evidence for Dixon's theory. The pressure bomb determines the tension in xylem of a plant before harvesting by measuring the back-pressure required to push xylem sap back to the cut end of the stem. By repeatedly

measuring xylem ψ for a drying shoot (or leaf), a pressure-volume (PV) curve can be drawn.

PV curves are usually plotted with relative water content (RWC) as a function of the inverse of ψ. The data points of a drying shoot show a PV curve with a curving region and a linear region. Based on Boyle-Van't Hoff's law,

$$\psi_\pi \times \mathrm{RWC} = \text{constant}, \quad (4)$$

Tyree and Hammel (1972) showed mathematically that the ψ for the curving region is the sum of P and ψ_π, whereas the ψ for the linear region equals ψ_π, since cells have plasmolyzed (P=0).

Exponential functions (Sinclair & Venables, 1983; Schulte & Hinckley, 1985) and power functions (Joly & Zaerr, 1987; Livingston & De Jong, 1991; Livingston *et al.*, 1992) have been used to fit curve data for conifer material. Values of $\psi_{\pi sat}$ and $\psi_{\pi tlp}$ can be extrapolated from the curve and ε_{max} can be calculated as the slope of the curve when RWC approaches full saturation. Complications arise in determining the turgor loss point and calculating ε_{max} since, best-fit curves drawn by eye can be biased and best-fit curves calculated numerically are affected by the type of equation used (Schulte & Hinckley, 1985). Although values of $\psi_{\pi sat}$ and $\psi_{\pi tlp}$ are resilient to the curve function (Schulte & Hinckley, 1985), values of calculated ε_{max} are not resilient (Clayton-Greene, 1983; Schulte & Hinckley, 1985).

There are two methods for collecting pressure-volume data for shoots: the 'Scholander' or 'sap expression' method (Scholander *et al.*, 1964; 1965) involves over-pressurizing the shoot and measuring the xylem sap expressed, and the 'Richter' or 'free transpiration' method (Talbot *et al.*, 1975; Richter, 1978) involves removing shoots from the pressure chamber and leaving them to transpire freely between measurements. Both methods have disadvantages (Turner, 1988; Pallardy

et al., 1991) and, although Ritchie and Roden (1985) found the 'Scholander' method to be more accurate, Parker and Pallardy (1988) found agreement between the two methods. The 'Richter' method has the added advantage of permitting measurement of large sample sizes in a short time period.

In conifer seedlings, not all species can maintain P under drought conditions (Abrams, 1988). Kandiko *et al.* (1980) found eastern hemlock to osmotically adjust to drought conditions since seedlings had lower $\psi_{\pi sat}$ and $\psi_{\pi tlp}$ under drought. Using polyethylene glycol, drought caused cell wall relaxation (decreased $\psi_{\pi tlp}$) in black spruce (Blake *et al.*, 1991). Buxton *et al.* (1985) determined that white spruce is more tolerant to drought than jack pine since jack pine wilted (lost turgor) under milder stress than white spruce. Results for Douglas-fir are ambiguous. Livingston and Black (1987) found drought-stressed Douglas-fir seedlings in the field maintained P longer than drought-stressed Pacific silver fir and western hemlock. Ritchie and Roden (1985) found Douglas-fir to osmotically adjust when ψ was measured by the 'sap expression' method but not by the 'free transpiration' method. Joly and Zaerr (1987) found no osmotic adjustment in their Douglas-fir seedlings, however, ε_{max} decreased when seedlings were drought-stressed, suggesting cell wall relaxation.

Studies of osmotic adjustment in conifers at different times during the year have indicated that values of $\psi_{\pi sat}$ and $\psi_{\pi tlp}$ change during the year, having low values in winter (van den Driessche, 1989), increasing in the spring and decreasing in the summer (Ritchie & Shula, 1984; Colombo, 1987; Grossnickle, 1989), increasing in late summer (Grossnickle, 1989), and decreasing again during the fall (van den Driessche, 1989). Consequently time of year is important in interpreting osmotic adjustment in conifers.

2.3 HYDRAULIC CONDUCTANCE

Water movement through plants is often determined from measurements of transpiration rates and differences between leaf and soil ψ (using equation 2), since it is assumed that flow rate is conserved throughout the plant and that stomatal conductance controls water flow (Ewers & Cruiziat, 1991). However, to better understand water movement through plants, major resistances in the soil, root, and xylem must be further investigated (Passioura, 1982). Resistances in the soil may increase under low soil moisture conditions due to increased tension in soil water as air pockets enlarge (Taiz & Zeiger, 1991). Also, reduced contact between soil and roots from root shrinkage may have an influence (Faiz & Weatherley, 1977; 1978; 1982). Resistance in the xylem may increase under low soil moisture conditions due to the formation of embolisms in tracheids (Sperry *et al.*, 1988; Tyree & Ewers, 1991). The major resistance in the root is at the endodermis, although under drought conditions early suberization of cortical tissue can also reduce water flow (Kozlowski *et al.*, 1991).

In roots, radial movement of water can occur through the symplast or apoplast, however, it is generally accepted that water flows through the pathway of least resistance, the apoplast, until it reaches the endodermis (Passioura, 1982). Since radially and transversely suberized endodermal cell walls, known as the Casparian strip, are impermeable to water (and solutes) (Esau, 1977; Johnson-Flanagan, 1984), water must cross the cell membrane to reach the vascular system. Water movement across a membrane occurs by osmosis, a slow process relative to water movement by bulk flow which occurs in the apoplast. Once across the endodermis, water returns to the apoplast at or before reaching the xylem. Water movement through the xylem is subject to little resistance, unless cavitation in tracheids limits flow rate. Relative to angiosperms, water flow rate through conifer xylem is slow

since the short, narrow tracheids in conifers have greater resistance to flow than the long, wide vessels connected by perforated plates in angiosperms (Esau, 1977).

Alterations to Scholander *et al.*'s (1964) pressure chamber has provided a technique to measure root hydraulic conductance. This technique involves suspending de-topped roots into a container of water inside a pressure chamber and applying pressure to push water through the roots and out of the cut end, above the base of the roots.

Early measurements of water flow rates through roots (WFRR) over a range of applied pressures showed that, although water flow is linearly related to pressure at high pressures (>0.3 MPa), the relationship at low pressures is non-linear (Mees & Weatherley, 1957). Fiscus (1975) and Dalton *et al.*, (1975) independently developed a theory, based on equation 2, which describes the relative importance of the components of ψ, such that,

$$J_v = L_p \times (\Delta P - \sigma \Delta \psi_\pi) \qquad (5)$$

where σ=reflection coefficient of solute(s). Their theory suggested that when the applied pressure is low $\Delta\psi_\pi$ across the radial pathway in the root has a significant effect on WFRR, whereas, under high pressures, $\Delta\psi_\pi$ is negligible. Therefore, at high pressures equation 5 can be simplified to

$$J_v = L_p \times \Delta P. \qquad (6)$$

Newman (1976) was the first to note that the value for ψ_π, calculated as the x-intercept on the water flow-pressure curve (see Newman, 1976; Passioura, 1984), was lower than the ψ_π of the external solution. To correct for this anomoly, Newman (1976) modified the Fiscus-Dalton theory, suggesting that solutes must cross two membranes, passing into and out of endodermal cells. However, studies on barley and lupin showed that neither theory could explain the differences between ψ_π of the external solution and ψ_π calculated from the flow rate-pressure

curves (Passioura & Munns, 1984; Munns & Passioura, 1984). Furthermore, if solute buildup at membranes was responsible for the non-linearity, increased solute buildup would be expected as pressure increased (Passioura, 1984); however, the curve is linear at high pressures.

There are problems inherent to measuring L_p on de-topped root systems, such as movement of water through unnatural pathways, loss of continued supply of photosynthate, and high pressure effects on cells (Passioura, 1988; Markhart & Smit, 1990). Since anaerobic conditions have been found to reduce WFRR in herbaceous plants and trees (Smit & Stachowiak, 1988; Everard & Drew, 1989; Swietlik, 1989), adequate oxygen must be supplied to the system. Markhart & Smit (1990) provide a comprehensive review on techniques and associated problems of measuring hydraulic conductance in de-topped root systems.

There is some confusion in the literature regarding the terminology in resistance/conductance research. Since flux density, or flow rate per surface area, (J_v) is difficult to calculate because root surface area is difficult to measure, flow rate (J_m) is commonly measured on de-topped roots and L_p is calculated using equation 6. Hydraulic conductance must then be normalized by some measure of root surface area, such as root length or root weight, or, if intact seedlings are used, L_p can be normalized by leaf surface area. Confusion arises when hydraulic conductivity is described as hydraulic conductance per root length since, mathematically,

$$L = L_p \times \Delta l \qquad (7)$$

where L=hydraulic conductivity ($m^2\ s^{-1}\ MPa^{-1}$) and l=root length (m). In this study, hydraulic conductance is calculated using equation 6, although J_m ($g\ s^{-1}$) is measured instead of J_v, and L_p is normalized by root length.

Although hydraulic conductance of roots in conifers has been investigated to determine the effects of root growth (Colombo & Asselstine, 1989; Grossnickle & Russell, 1990) and mycorrhizal infection (Sands *et al.*, 1982; Coleman *et al.*, 1990), no studies have addressed the effect of drought on hydraulic conductance in conifer roots. In angiosperms, de-topped roots have been used to investigate root growth (Anderson *et al.*, 1988; Moreshet *et al.*, 1990b), mycorrhizal associations (Levy & Syvertsen, 1983; Anderson *et al.*, 1988), and the relative importance of root resistance to whole plant resistance (Black, 1979; Blizzard & Boyer, 1980) in trees and crops. Studies of the effect of drought stress on hydraulic conductance in roots indicate that woody and herbaceous plants show decreased hydraulic conductance under drought conditions (Blizzard & Boyer, 1980; Levy & Syvertsen, 1983; Cruz *et al.*, 1992; Huang & Nobel, 1992; Saliendra & Meinzer, 1992). Alternate techniques to measure hydraulic conductance in roots include pressure bomb measurements (Steudle & Jeschke, 1983), isopiestic method (Blizzard & Boyer, 1980), and heat pulse measurements (Moreshet *et al.*, 1990a). Reduction in hydraulic conductance under drought conditions may be due to the suberization of the root epidermis (Cruz *et al.*, 1992), root abscission (Huang & Nobel, 1992), and/or xylem embolism (Saliendra & Meinzer, 1992).

Chilling temperatures can act as a form of drought and cause wilting in plants due to increased viscosity of water (Lopushinsky & Kaufmann, 1984). According to the Hagen-Poiseuille law for movement of water in cylinders, such as in xylem and between microfibrils in the cell wall,

$$J_v = (r^2/8\eta l) \times \Delta P \qquad (8)$$

where r=radius (m) and η=viscosity (MPa s). Therefore, flow rate is inversely proportional to viscosity.

Below a critical temperature, factors other than viscosity reduce water flow through plants (Lyons, 1973). When plants are chilled below a critical temperature, water flow decreases more than expected from increased viscosity. Critical temperatures are species-dependent, based on their sensitivity to chilling, i.e. chilling-sensitive species have higher critical temperatures than chilling-tolerant species (Markhart, 1986). Studies have shown that plants react to chilling stress by adjusting their physiological and biochemical activity, such as altering the state of lipids in membranes, increasing production of ethylene, increasing rate of respiration, and increasing required energy of activation (Lyons, 1973; Wang, 1982; McWilliam, 1983; Markhart, 1986).

Species sensitivity to chilling temperatures is often determined using Arrhenius plots, which relate the rate of a plant process to temperature. Studies have shown that plant resistance increases with decreased temperature in conifers (Kramer, 1942; Running & Reid, 1980; Teskey *et al.*, 1984; Grossnickle & Blake, 1985; Grossnickle, 1988), however, the study by Smit-Spinks *et al.* (1984) is the only investigation of root hydraulic conductance at different temperatures in conifers. Smit-Spinks *et al.* (1984) found that water flow through Scotch pine (*Pinus sylvestris* L.) seedling roots had lower water flow rates at a lower pressure chamber temperature. Surprisingly, they also found that seedlings pre-conditioned at a low temperature had lower water flow rates through roots than seedlings grown at a high temperature. De-topped root systems have also been used to determine critical temperatures of economically important crop and citrus species (Clarkson, 1976; Markhart *et al.*, 1979; Ramos & Kaufmann, 1979).

Chapter 3

DROUGHT AVOIDANCE OF WET AND DRY ECOTYPES OF DOUGLAS-FIR AND LODGEPOLE PINE SEEDLINGS

3.1 INTRODUCTION

Water supply, which is the major factor limiting tree growth during summer in the Pacific Northwest (Waring and Franklin, 1979), can limit plantation growth even in relatively moist maritime climates, such as those of Scotland (Jarvis and Mullins, 1987) and Vancouver Island (Spittlehouse, 1985). It also affects seedling survival (Livingston and Black, 1987). Initial survival of planted tree seedlings is influenced by their drought resistance (Nambiar *et al.*, 1979, Grossnickle and Blake, 1985, Zwiazek and Blake, 1989, Kaushal and Aussenec, 1989, Ni and Pallardy, 1991, van den Driessche, 1991), but plant attributes conferring drought resistance may reduce growth rates (Turner, 1986). For example, nursery treatments that increased drought resistance in three species of conifer seedlings were negatively correlated with seedling size, and in some instances, with growth after planting (van den Driessche, 1991). In the longer term, high water use efficiency (WUE), resulting in the ability to grow despite drought, may be an important determinant of both survival and plantation productivity.

Water use efficiency of trees has been determined over short periods using gas exchange measurements (Sheriff *et al.*, 1986, Grieu *et al.*, 1988, Bassman and Zwier, 1991, Ni and Pallardy, 1991). Such measurements may not be good predictors of long term WUE because dark respiration and developmental changes are unaccounted for in the calculation (Fischer and Turner, 1978). Water use efficiency is often determined on the basis of dry matter production per unit of water used over an extended period of time in agricultural experiments

(Landsberg, 1986), but this has seldom been done with forest tree species (Bradbury and Malcolm, 1977, Livingston and Black, 1988). In the study reported here WUE of one-year-old conifer seedlings was measured in this way. The objective was to test the hyposthesis that lodgepole pine (*Pinus contorta* Dougl.), from the drier interior regions of the Pacific Northwest, would show higher WUE than Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), from the coastal regions. Root growth of these conifer species was also examined in relation to WUE.

3.2 MATERIAL AND METHODS

3.2.1 Plant Material

Two provenances of Douglas-fir from wet (Blue Mountain, lat. 49^{o} 00', long. 122^{o} 00', elev. 305 m) and dry (Devine, lat. 50^{o} 32', long. 122^{o} 28', elev. 385 m) coastal ecotypes, and two provenances of lodgepole pine from wet (Gavin Lake, lat. 52^{o} 30', long. 121^{o} 47', elev. 1,010 m) and dry (Strauss Lake, lat. 52^{o} 30', long. 122^{o} 46', elev. 1,144 m) interior ecotypes, in British Columbia, were used. Long term precipitation averages, obtained from Environment Canada, show that Mission, close to Blue Mountain, receives 1772 mm per annum, and Pemberton, adjacent to Devine, receives 1187 mm per annum. Gavin Lake has an average of 686 mm precipitation per annum, and Strauss lake is in a region receiving between 453 and 564 mm per annum (Spittlehouse, personal communication). Seedlings were grown in styroblock containers (cavity volume 60 mL) during 1990 at a British Columbia Ministry of Forests nursery using standard cultural methods (Matthews, 1983). They were cold-stored from January until 18 April, 1990, and then planted in 100-cm deep containers of 10 cm diameter (7.8 L capacity). The containers were made from lengths of PVC tube cut longitudinally and taped back together so that they could be slit apart with a knife to facilitate undamaged root

recovery later. Half of the 96 containers were filled with growth medium (3 peat:1 vermiculite with 200 g 16-10-10 slow release fertilizer and 3 kg dolomitic limestone m-3) that was saturated (wet soil) and contained 522% water, expressed as a % of dry soil. The remaining 48 containers were filled with growth medium that was 318% water saturated (dry soil). The bottoms of the containers were sealed, and the tops of the containers were sealed around seedling root collars with polyethylene, so that water loss could only occur through the seedling. Each container was covered with aluminium foil to reduce radiant heat exchange.

3.2.2 Experimental Design

The experiment was designed so that three replicates could be harvested on May 24, June 12, July 12 and August 15, 1990 to provide combinations of species x ecotype x soil water regime (i.e. 24 seedlings). A sample of unplanted seedlings was also taken on April 18. All treatment combinations were fully randomised in a block on the ground under a clear polyethylene plastic shelter. At the start, and again immediately before harvest, each sealed container and seedling was weighed so that water use could be determined. For the four weeks before a group of seedlings were to be harvested in June, July and August their stomatal conductance was measured (Li-Cor 1600), at approximately weekly intervals, during a four hour period starting at 11:00 a.m. A section of shoot was marked with a felt pen, repeatedly measured, and then separated at harvest for projected area measurement (Delta-T). At harvest, 24 containers with seedlings were brought into the laboratory in the morning, and shoots were cut from roots and shoot water potentials were measured with a pressure chamber. The containers were slit open and soil samples, representing 20 cm depth profiles, were removed to determine soil water content. The root system was washed free of medium, and

the new roots were cut from the old roots which were well defined by the nursery container "plug". Lengths (Tennant, 1975) and dry weights of old and new roots, and shoot dry weights were determined. Specific root length was calculated as root length per unit of root dry weight.

Water use was calculated from:

$$WU = C_o - C_n, \quad (9)$$

where C is the weight of container plus seedling, o is the start of the experiment, and n is harvest number. No correction for increase in seedling weight, caused by growth, was made. Water use was used to estimate root length efficiency (RLE, water use per unit of new root length), root weight efficiency (RWE, water use per unit of new root dry weight), and also water use efficiency (WUE, seedling dry weight increment per weight of water used during the same time period).

3.2.3 Statistical Analysis

Dry weights and root lengths were transformed to ln(variable + 1) to remove heteroscedasticity. Dry weight, root length, water use, stomatal conductance, and shoot water potentials were treated by analysis of variance (Sokal and Rohlf, 1981) using the general linear models procedure of Statistical Analysis System (SAS Institute, Cary, NC). Regressions of new root length over new root dry weight, water use over new root length, water use over new root dry weight, and total dry weight over water use were carried out with individual container observations obtained at each harvest. Regression slopes of the three-way interaction, species x ecotype x soil moisture treatment (Table 1), were tested for heterogeneity (Freund *et al.*, 1986), using the model:

$$D = I\ SP*EC*SM\ I*SP*EC*SM, \quad (10)$$

where D = dependent variable (e.g. increase in seedling dry weight), I = independent variable (e.g. water use), SP = species, EC = ecotype, and SM = soil moisture. If heterogeneity was found, main effects and two-way interactions were tested.

3.3 RESULTS

Lodgepole pine seedlings grew faster than Douglas-fir seedlings over the 17-week experiment. At the start, mean dry weight of Douglas-fir seedlings was twice that of lodgepole pine seedlings but after 17 weeks mean dry weight of lodgepole pine seedlings from wet and dry soil treatments was slightly greater than Douglas-fir seedlings, leading to a significant species x time interaction (Fig. 1; Appendix, Table 1.1). Seedlings grown on wet soil had greater dry weight than seedlings grown on dry soil by the end of the experiment (Fig. 1). Lodgepole pine used more water than Douglas-fir during the experiment, except during the first interval between harvests (Fig. 1). Average water use over the whole experiment for lodgepole pine was 18 g day^{-1}, and for Douglas-fir was 12 g day^{-1} ($p < 0.001$).

New root length of lodgepole pine seedlings was greater than new root length of Douglas-fir seedlings, becoming more than three times as long by the last harvest whether seedlings were grown under wet or dry soil moisture treatments (Fig. 1). New root length was also greater in wet soil than in dry soil for both species.

Comparison of regression slopes of the three-way interaction (species x ecotype x soil moisture treatment) showed that slopes were significantly different for specific root length (SRL), RLE, RWE, and WUE (Table 1). Results from analyses of two-way interaction effects for SRL, RLE and RWE, and WUE that produced significant results are shown in Tables 2, 3, and 4 respectively.

Figure 1. Dry weight, new root length and water use for Douglas-fir and lodgepole pine seedlings grown in wet and dry soil water treatments, averaged for wet and dry ecotypes. Means of dry weight and root length are backtransformed from ln(variable+1) and bars are 95% confidence intervals. Water use bars are one standard error. The interaction, species x harvest date and soil moisture x harvest date was significant ($p < 0.001$) for all three parameters.

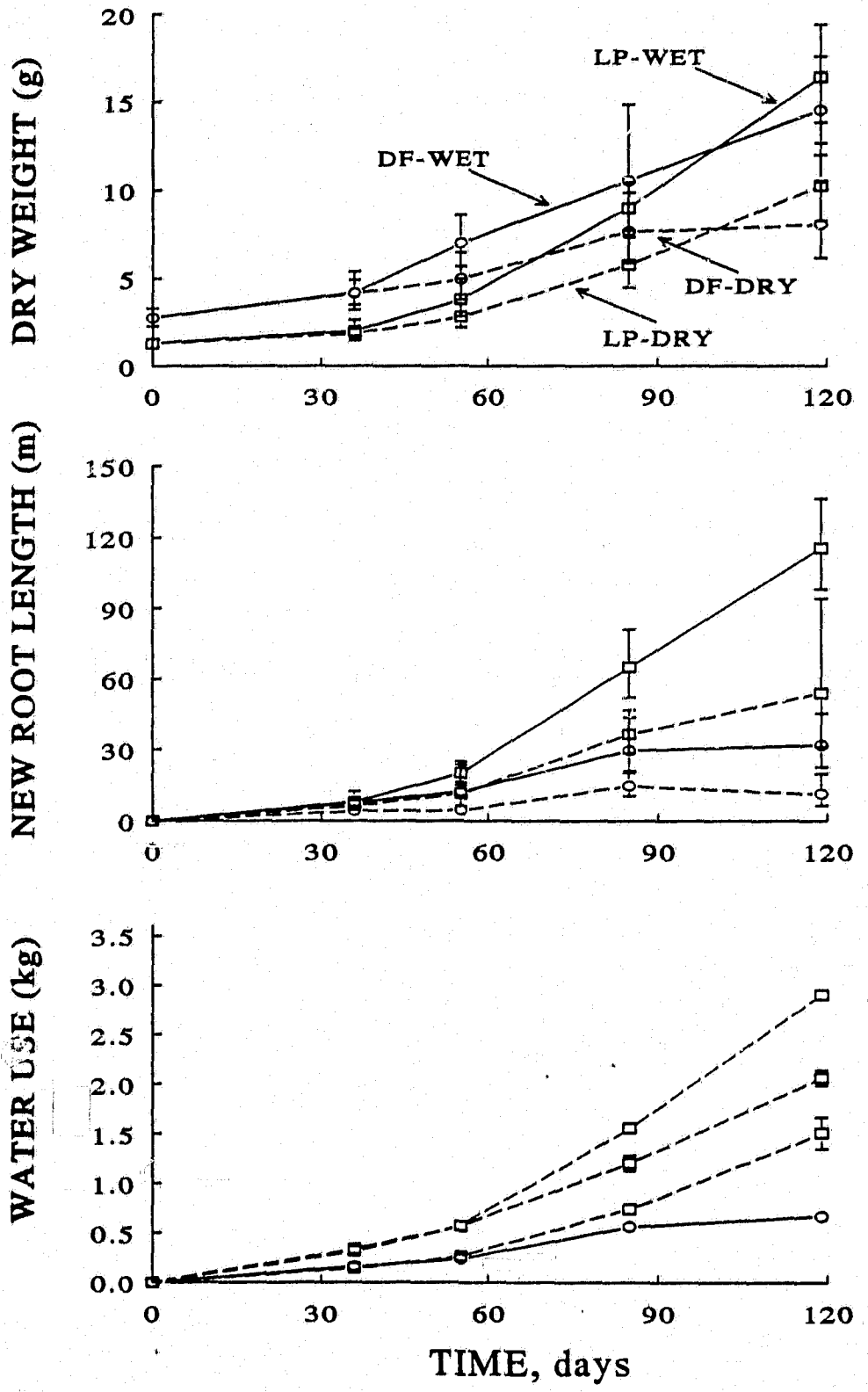

DRY WEIGHT (g)
20
15
10
5
0
LP-WET
DF-WET
DF-DRY
LP-DRY
0
30
60
90
120
NEW ROOT LENGTH (m)
150
120
90
60
30
0
0
30
60
90
120
WATER USE (kg)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
30
60
90
120
TIME, days

Table 1. Probability of significant differences between regression slopes of main effects and interactions for specific root length (SRL), root length efficiency (RLE), root weight efficiency (RWE), and water use efficiency (WUE), calculated for Douglas-fir and lodgepole pine.

Source	Probability for F ratio			
	SRL	RLE	RWE	WUE
Species (Sp)	0.0001	0.0001	0.9576	0.0485
Ecotype (Ec)	0.0259	0.3223	0.9576	0.8194
Soil water (W)	0.9483	0.2764	0.0919	0.5579
Sp x Ec	0.0001	0.0001	0.3477	0.0017
Sp x W	0.0001	0.0001	0.0047	0.0018
Ec x W	0.1637	0.4536	0.0604	0.8057
Sp x Ec x W	0.0001	0.0001	0.0068	0.0001

Regression of new root length over new root dry weight showed that lodgepole pine (21.6 m g^{-1}) had significantly greater specific new root length than Douglas-fir (10.3 m g^{-1}). However, species interacted significantly with both soil moisture and ecotype. The interaction with ecotype was small, with dry ecotypes of both species having higher specific new root lengths than wet ecotypes (Table 2). Douglas-fir specific new root length was greater in wet soil than in dry soil, whereas lodgepole pine showed little difference (Table 2). At the final harvest, after 120 days growth, new root dry weight of Douglas-fir varied from 50.2% (dry-soil treatment) to 65.3% (wet-soil treatment) of total root weight. Corresponding values for lodgepole pine were 72.2% and 77.6% respectively.

Regression showed that RLE was twice as great in Douglas-fir (0.46 g H_2O cm^{-1}) as in lodgepole pine (0.23 g H_2O cm^{-1}). Both RLE and RWE showed strong species by soil water interactions (Table 1, 3). Douglas-fir took up more

water per unit root under wet soil conditions than under dry soil conditions, whereas lodgepole pine took up much the same amount of water per unit root under both soil water conditions.

Table 2. Specific root length (m g^{-1}) determined from regression slopes of new root length over new root dry weight for Douglas-fir and lodgepole pine seedlings. Values in parentheses are standard errors.

	Species	
	Douglas-fir	Lodgepole pine
Ecotype[1]		
Dry	11.2 (1.0)	23.1 (0.9)
Wet	9.7 (0.8)	20.2 (0.8)
Soil Moisture Treatment[1]		
Dry	7.7 (0.5)	22.3 (1.1)
Wet	10.3 (0.8)	21.2 (0.9)

[1]Significance of the above means are show in test provided in Table 1

Table 3. Root length efficiency (g H_2O cm^{-1}) and root weight efficiency (g H_2O g^{-1}) determined from regression slopes of water use over new root length and new root dry weight for Douglas-fir and lodgepole pine seedlings. Values in parentheses are standard errors.

Soil water treatment[1]	Species			
	Root length efficiency		Root weight efficiency	
	Douglas-fir	Lodgepole pine	Douglas-fir	Lodgepole pine
Dry	0.36 (0.03)	0.21 (0.01)	292 (26)	495 (23)
Wet	0.46 (0.04)	0.23 (0.01)	528 (41)	497 (25)

[1]Significance of the above means are shown in test provided in Table 1.

Regression of seedling dry weight over water use showed that WUE was significantly (Table 1) higher in Douglas-fir (0.0061 g g^{-1}) than in lodgepole pine (0.0054 g g^{-1}), but species interacted with both ecotype and soil water. Douglas-fir from the dry ecotype showed higher WUE than Douglas-fir from the wet ecotype, but there was little difference between ecotypes for lodgepole pine (Table 4).

Table 4. Water use efficiency (g dry matter g^{-1} H_2O) determined from regression slopes of seedling dry weight over water use. Values in parentheses are standard errors.

	Species	
Ecotype[1]	Douglas-fir	Lodgepole pine
Dry	0.0069 (0.0004)	0.0052 (0.0002)
Wet	0.0053 (0.0004)	0.0056 (0.0003)
Soil water treatment[1]		
Dry	0.0087 (0.0009)	0.0061 (0.0004)
Wet	0.0062 (0.0003)	0.0054 (0.0002)

[1]Significance of the above means are shown in test provided in Table 1.

Both species showed higher WUE in the dry-soil treatment than in the wet-soil treatment, but the difference was greater in Douglas-fir.

Average shoot water potentials decreased significantly ($p < 0.001$) with harvest from -1.07 MPa to -1.56 MPa, and there was a significant interaction between soil water treatment and species ($p < 0.002$). Douglas-fir water potentials were lower (-1.14 MPa in wet and -1.50 MPa in dry soil) than those of lodgepole pine (-1.04 MPa in wet and -1.09 MPa in dry soil). Stomatal conductance of Douglas-fir (Fig.

2) and lodgepole pine (Fig. 3) seedlings decreased with harvest date due to decreased soil water, except for Douglas-fir seedlings from the wet ecotype that were grown under well-watered conditions. Lodgepole pine had stomatal conductance more than twice that of Douglas-fir in the early part of the experiment, but approached the same values as Douglas-fir towards the end of the experiment (Fig. 2 & 3). Significant differences of interaction effects containing ecotype x harvest date (Appendix, Table 1.2) were due to the mortality of three Douglas-fir seedlings from the dry ecotype, grown under the low soil moisture treatment before final harvest.

Both species reduced soil water similarly to about 70% in the upper 20 cm of the containers by the end of the experiment, but below 20 cm lodgepole pine reduced soil water more than Douglas-fir. Below 80 cm, at the end of the experiment, lodgepole pine reduced soil water more than Douglas-fir, with lodgepole pine reducing soil water to 70% in the wet-soil treatment and 196% in the dry-soil treatment, whereas corresponding soil moisture values at the bottom of the Douglas-fir containers were 323% in the wet-soil treatment and 339% in the dry-soil treatment. Consequently there was a significant species x harvest date interaction ($p<0.001$) and soil water treatment x harvest interaction ($p<0.001$) (Appendix, Table 1.3).

Figure 2. Mean stomatal conductance for three harvest intervals for Douglas-fir seedlings, from wet ecotypes (WE) and dry ecotypes (DE), grown in wet soil (WS) and dry soil (DS) moisture treatments. Bars are one standard error and arrows indicate harvest dates.

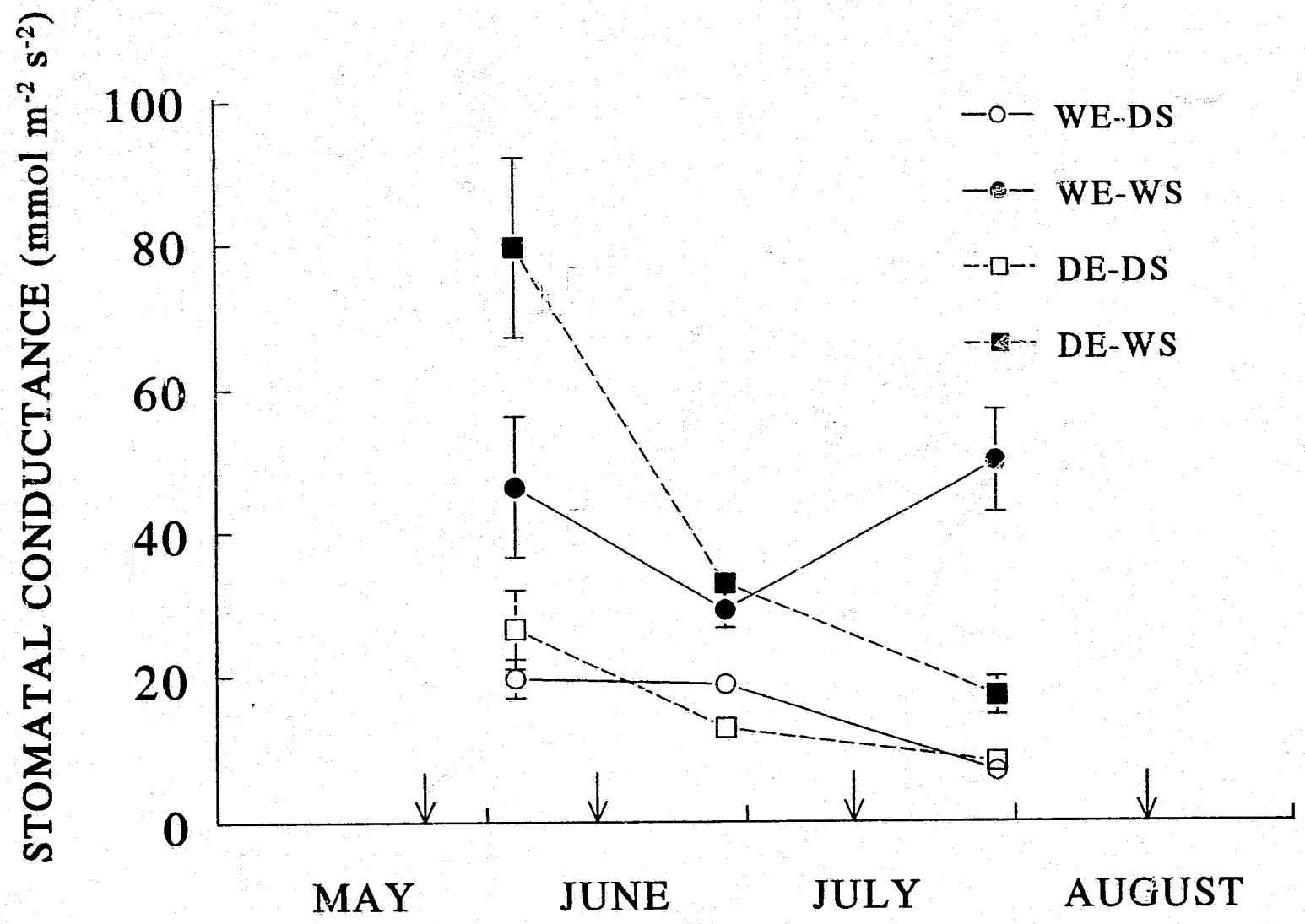
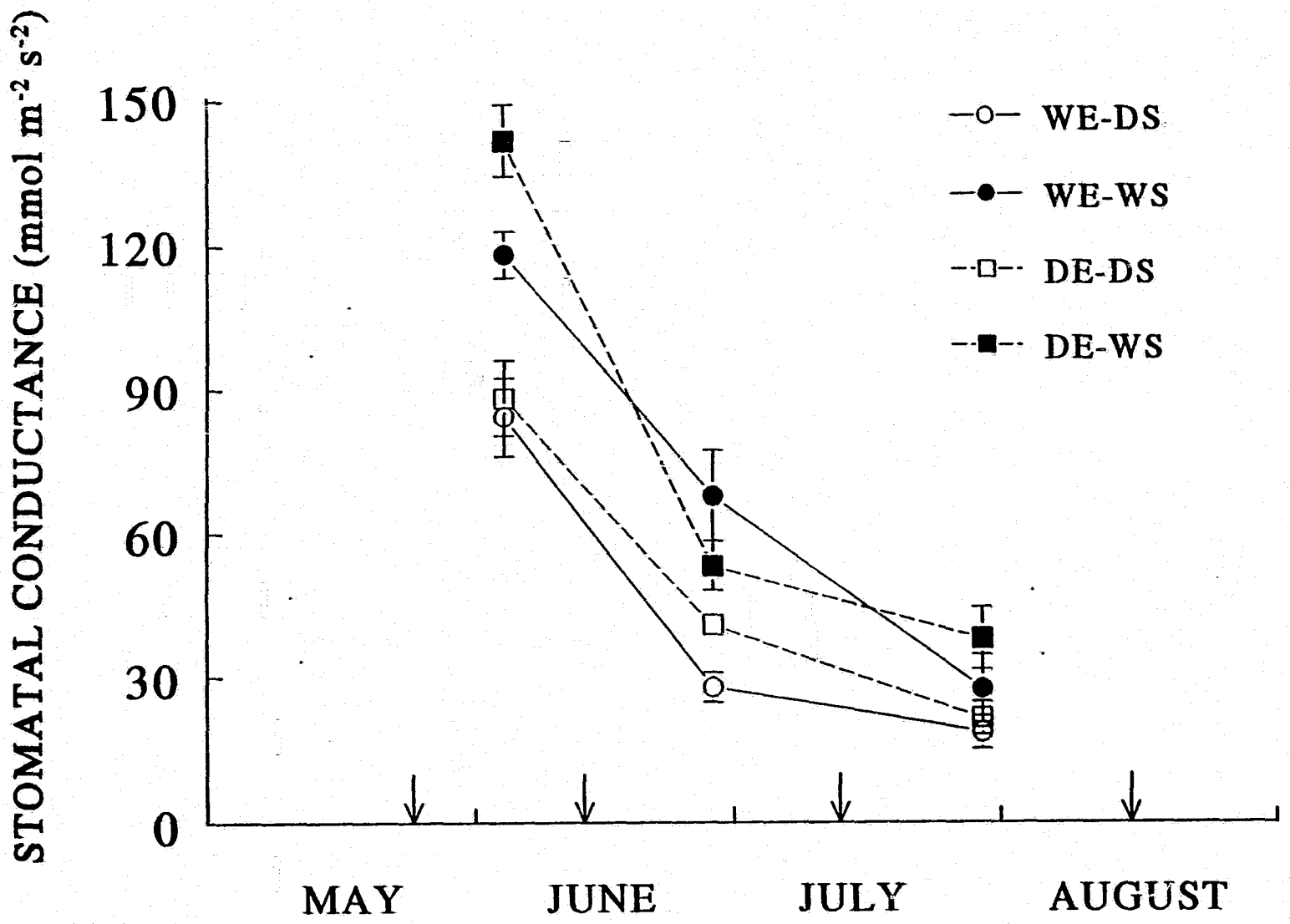

STOMATAL CONDUCTANCE (mmol m-2 s-2)
100
80
60
40
20
0
MAY
JUNE
JULY
AUGUST
WE-DS
WE-WS
DE-DS
DE-WS

Figure 3. Mean stomatal conductance for three harvest intervals for lodgepole pine seedlings, from wet ecotypes (WE) and dry ecotypes (DE), grown in wet soil (WS) and dry soil (DS) moisture treatments. Bars are one standard error and arrows indicate harvest dates.


STOMATAL CONDUCTANCE (mmol m-2 s-2)
150
120
90
60
30
0
MAY
JUNE
JULY
AUGUST
WE-DS
WE-WS
DE-DS
DE-WS

3.4 DISCUSSION

The higher WUE of coastal Douglas-fir was unexpected in view of its maritime habitat, compared with the drier inland habitat of lodgepole pine. Water uptake responses of Douglas-fir were affected by both ecotype and soil water content, but lodgepole pine was less responsive.

Lodgepole pine may have been unresponsive because wet and dry ecotypes were not sufficiently different, or because the dry soil treatment was not dry enough to influence this species. The dry matter productivity of lodgepole pine was greater than that of Douglas-fir, so that the lower WUE of lodgepole pine was due to its considerably greater water use. The greater water use of lodgepole pine, compared with Douglas-fir, seems to have been related to the greater root length developed by lodgepole pine following planting. A unit amount of dry matter invested in lodgepole pine roots produced two to three times as much root length as the same investment in Douglas-fir. This allowed lodgepole pine to make use of water from greater soil depths than Douglas-fir and to exploit the available water resources in the containers more completely. Reduced soil water in the containers increased WUE in both species, but Douglas-fir showed a 40% increase, whereas lodgepole pine showed only a 24% increase. These WUE differences developed under the particular ambient water vapour pressure conditions that prevailed in the uncontrolled atmospheric environment. Atmospheric water vapour pressure influences WUE in conifers (Jarvis, 1986, Sandford and Jarvis, 1986), as well as angiosperms, and WUE relationships between the two species might be different under other water vapour pressure conditions.

Measurement of WUE, as dry matter increment per unit of water transpired, did not give a complete view of tree response to soil water conditions. Lodgepole pine produced more dry matter than Douglas-fir, with a lower WUE, because it

utilised the water in the root environment more completely. Root exploitation of the soil water resource was therefore the key factor in determining the productivity difference between the two species, not WUE.

The rate at which new roots of the two species absorbed water varied according to whether length or dry weight was used as the root measurement. Douglas-fir absorbed more water than lodgepole pine per unit of new root length. Absorption per unit of new root dry weight by Douglas-fir varied according to soil water treatment, and this was partly because low soil water supply decreased specific new root length of Douglas-fir.

The generally higher stomatal conductance shown by lodgepole pine was accompanied by slightly higher shoot water potentials, suggesting that the distribution of lodgepole pine root dry matter in long thin roots was effective in reducing resistance to water uptake between soil and shoot. The lower flux of water per unit of the thinner lodgepole pine new root length, would presumably result in an even lower flux per unit of root surface. Lodgepole pine could therefore absorb water to satisfy transpiration demands with smaller potential differences than Douglas-fir, assuming that resistance per unit surface of root was similar in both species.

The part played by the old root system, developed in the nursery during the previous year, in absorbing water has been ignored in this experiment. It seems likely, however, that the old root system contributed to water absorption immediately after planting, before new root growth commenced (Chung and Kramer, 1975). Intercepts of the regressions of water use over new root length or weight showed small, positive water use values, with the exception of the Douglas-fir seedlings from the dry ecotype grown under drought conditions which had a small, negative intercept.

Water use efficiency of the seedlings was generally similar to that reported previously by methods based on increase in dry weight. Water use efficiency of two- and three-year-old Douglas-fir planted in a clearcut varied between 1.9 to 2.9 mg g^{-1}, according to whether they were irrigated or not (Livingston and Black, 1988), and were therefore slightly lower than values calculated here. Larger values (5.0 to 6.6 mg g^{-1}), were reported for Sitka spruce (Bradbury and Malcolm, 1977). Because these values did not include root growth, WUE on a whole seedling basis would have been higher. Water use efficiency, determined from mol CO_2 fixed per mol H_2O transpired, for several species of three-year old conifers were shown to vary between about 0.0017 to 0.01 (Sandford and Jarvis, 1986). Similar measurements showed WUE of plantation grown Monterey pine to be about 0.002 (Sheriff *et al.*, 1986). Assuming 1.82 g CO_2 are contained in 1 g dry matter (Ledig and Botkin, 1974), mean values in this experiment ranged from about 0.003 to 0.008 mol CO_2 per mol H_2O.

Water use efficiency is not necessarily positively related to productivity, because more dry matter was produced by the less water use efficient lodgepole pine during the course of the experiment. These results suggest that, under conditions of drought stress, productivity is dependent on the ability of roots to exploit available water resources, rather than on WUE, measured as dry matter increment per unit of water transpired.

Chapter 4

DROUGHT TOLERANCE OF WET AND DRY ECOTYPES OF DOUGLAS-FIR AND LODGEPOLE PINE SEEDLINGS

4.1 INTRODUCTION

Maintenance of cell turgor pressure (P) is necessary for plant survival and growth. Studies have shown that cell growth is the most sensitive process to drought (Hsiao *et al*, 1976; Jones, 1992; Nobel, 1991). Osmotic adjustment is a drought tolerance mechanism involving reduction of osmotic potential (ψ_π), by active solute accumulation, to maintain soil water uptake and, therefore, P. Plant dehydration causes decreased ψ_π and P simultaneously, and therefore is not a component of osmotic adjustment.

Maintenance of P is also a function of cell wall elasticity, cell shape, and surrounding cell shapes and pressures, which are collectively measured as bulk elastic modulus (E) (equation 3). For P to be maintained under drought conditions, elastic cell walls, or low E, permit smaller reductions in P with decreased cellular volume than high E. Therefore low E may be viewed as a form of drought tolerance, especially if no osmotic adjustment occurs (Blake et al., 1991).

Although the benefits of osmotic adjustment in higher plants have been questioned (Munns, 1988), numerous studies have found increased solute concentration in plant cells when drought-stressed (Morgan, 1984). Osmotic adjustment and/or changes in cell wall elasticity have been found in various conifer species (Kandiko et al., 1980; Joly & Zaerr, 1987; Blake et al, 1991).

Pressure-volume curves are a common method of studying the effects of cellular water loss on the components of water potential (ψ) (Pallardy et al., 1991).

From these water release curves, values of osmotic potential at full saturation ($\psi_{\pi sat}$) and osmotic potential at the turgor loss point ($\psi_{\pi tlp}$), which measure osmotic adjustment, and bulk elastic modulus, which measures turgor regulation, can be calculated.

In this work, the 'free transpiration' method (Richter, 1978) was used to produce pressure-volume curves for wet and dry ecotypes of Douglas-fir (*Pseudotsuga menziesii* (Mirb. Franco) and lodgepole pine (*Pinus contorta* Dougl.). Seedlings were grown under two watering regimes with the objective of determining the relative importance of osmotic adjustment and turgor regulation in drought tolerance of the two species. In this case the hypothesis was that Douglas-fir would show little adjustment compared with lodgepole pine, based on the contradictory evidence from the literature.

4.2 MATERIAL AND METHODS

4.2.1 Plant Material

Douglas-fir and lodgepole pine seedlings, from wet and dry ecotypes, were grown under B.C. Ministry of Forest nursery regimes (Mathews, 1983) for their first year. The two Douglas-fir families were crosses from wet sites Tahsis Inlet (49°43',126°37') X Head Bay (49°48',126°29'), seedlot 201 X 638, and dry sites Chemainus (48°55',123°45') X Cassidy (49°03',123°53'), seedlot 582 X 40, and the two lodgepole pine provenances were single tree seed collections from Indian Point (53°29',121°34') and Stump Lake (50°23',120°10'), wet and dry sites respectively. The one year-old seedlings were transplanted singly from styroblock containers into 3.2 L pots containing 3:1 peat:vermiculite mix, 8.25 kg m^{-3} slow-releasing fertilizer (16-10-10 (N-P-K) Nutricote), 3.02 kg m^{-3} dolomitic lime, and 7.42 X 10^{-2} kg m^3 trace elements fertilizer (Peters Soluble Trace Element Mix) on

May 19, 1988. Thirty-six pots (nine seedlings per family) were placed on each of two wooden supports 20 cm above the floor of the greenhouse to allow air circulation underneath.

4.2.2 Experimental Design

All seedlings were watered twice during their nine day establishment period. Once the drought-stress treatment began (May 28), seedlings in the control group continued to be watered twice per week and seedlings in the drought-stressed group were watered once every second to third week. A preliminary harvest of three seedlings from each family was performed at time of transplanting to determine pressure-volume curves before the drought-stress treatment was initiated. On June 27-30 and August 3-6 harvests were completed in which three replicates of each family were sampled from each watering regime, except for the August harvest in which only two drought-stressed replicates from each lodgepole pine site ecotype were sampled, due to high mortalities. There were two 2-3 week drought cycles between each harvest. Although a third harvest was begun, 60% of the drought-stressed seedlings had died and insufficient data could be collected for the remaining drought-stressed seedlings to produce pressure-volume curves.

Six seedlings were measured each day of the harvest. Pots were saturated with water, and shoots placed in dark plastic bags overnight to rehydrate all seedlings. The following morning a shoot, or sometimes a branch, was cut from each seedling with a sharp razor, bark was removed from the cut end, the shoot was placed into a pre-weighed rubber stopper, and this assembly was weighed to obtain an initial saturated weight. Within one minute from being cut, the shoot was placed into a Scholander pressure bomb (PMS Corvallis, Oregon) and pressure was increased until xylem sap was just visible at the cut surface of the stem with a dissecting

microscope. The pressure was recorded and the shoot and rubber stopper assembly were immediately weighed. Shoots were allowed to air-dry on the work bench between measurements, following Richter (1978). Pressure and weight measurements were taken at increasing time intervals for the first seven to eight hours (i.e., the first six measurements were taken at 5-20 minute intervals and the following six measurements were taken at 45-60 minute intervals). Four to six hours later and again the following morning, 11-13 hours after previous measurements, final measurements for all seedlings were taken. Morning measurements were recorded for seedlings requiring pressures less than 5.0 MPa only, for safety reasons. Shoots were then oven-dried at 80^{o}C for 24 hours for dry weight determination.

4.2.3 Pressure-Volume Curves

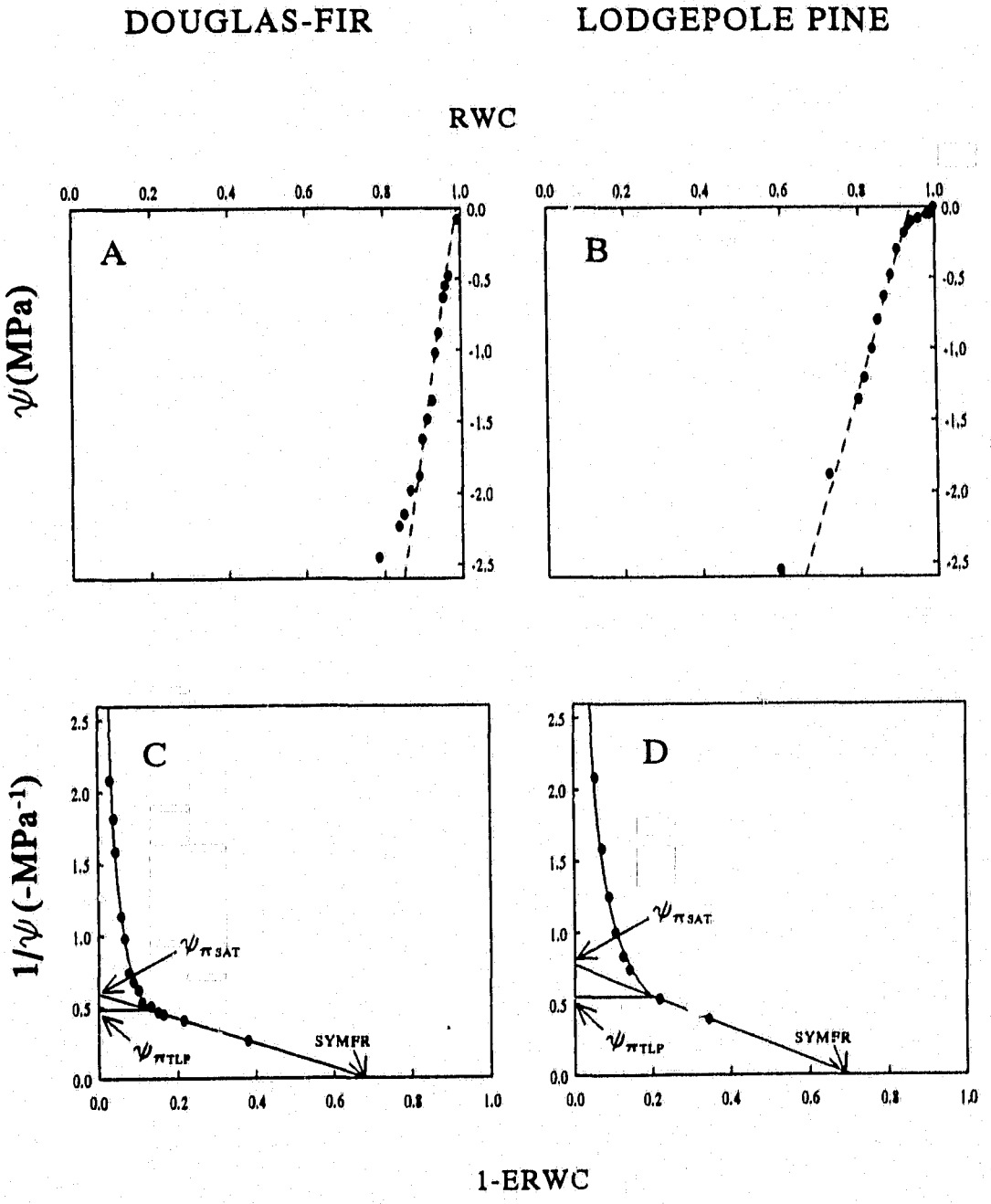
Using the sets of pressure and weight measurements taken on each shoot, graphs of water potential vs relative water content (RWC) were produced (Fig. 4A&B), then later pressure-volume curves were constructed (Fig. 4C&D). Weight measurements were used to calculate shoot relative water content (RWC):

$$RWC = [x - dry]/[sat - dry] \quad (11)$$

where x = weight of shoot after each pressure chamber measurement, dry = dry weight of shoot, and sat = saturated weight of shoot. Inverse shoot water potentials were calculated by inverting the value of pressure required to return shoot xylem sap to the cut surface of the stem. Over time, as the shoot dried out, increased pressure was required to push the xylem sap toward the cut end, (i.e., ψ became more negative) and RWC decreased.

Plateau regions were found near full water saturation in 57% and 96% of the Douglas-fir and lodgepole pine seedlings respectively. Plateau regions result from

Figure 4. Relationship between water potential (ψ) and relative water content (RWC) and $1/\psi$ and 1-estimated RWC (ERWC) for typical Douglas-fir and lodgepole pine seedlings. Linear curves were fitted to data points from near full saturation to the turgor loss points (TLP) on ψ-RWC graphs (1A&1B) and x-intercepts were used to correct RWC values for 'plateau' regions, giving ERWC values. Pressure-volume curves (1C&1D) were produced using ERWC values and were used to determine $\psi_{\pi sat}$, $\psi_{\pi tlp}$, symplastic fraction (SYMFR), and E_{max}. E_{max} values required differentiation of the power function equation used to fit data points above TLP on the pressure-volume curve.


DOUGLAS-FIR
LODGEPOLE PINE
RWC
ψ(MPa)
A
B
C
D
1/ψ(-MPa⁻¹)
ψπSAT
ψπTLP
SYMFR
1-ERWC

over-saturation of the shoots such that water is present in the fully turgid cells and in intercellular spaces (Pallardy *et al.*, 1991). Consequently, when shoots begin to dehydrate and RWC decresases, water leaves the intercellular spaces and moves into the cell, maintaining cell ψ. Plateau regions can be identified on ψ-RWC graphs (Fig. 4A&B) as regions where RWC decreases without a concomitant decrease in ψ. To correct for intercellular water content, linear functions of ψ vs RWC were determined for points between full saturation of the symplast and the turgor loss point (data in plateau region were excluded). All RWC values were adjusted to give estimated RWC (ERWC) based on the difference between measured and estimated RWC at full saturation.

Pressure-volume curves were plotted for each seedling shoot using volume expressed (1-ERWC) and negative inverse ψ (positive values) for x and y axes respectively. The pressure-volume curve is composed of a non-linear region, where ψ_π and P influence ψ, and a linear region, where only ψ_π influences ψ, since cells have plasmolyzed. The program developed by Schulte & Hinckley (1985) used exponential functions to describe the non-linear region but this was unsatisfactory for these data which were better described by the power function.

Equations best fitting the non-linear and linear regions of each shoot's pressure-volume curve were found using a graphics program (Slidewrite Plus, version 4.0, Sunnyvale, California). Data for three lodgepole pine seedlings were removed from the analysis because one drought-stressed seedling from the dry site had a pressure-volume curve made up of non-linear and linear functions which did not intersect and data for two well-watered seedlings from wet and dry ecotypes produced pressure-volume curves that lacked linear regions. Of the remaining 25 lodgepole pine shoots measured, 14 shoots produced curves where only the final two points could be used to calculate the linear portion of the curve (Fig. 4B).

Only two Douglas-fir shoots required that the linear equation be based on two points.

For each seedling, $\psi_{\pi tlp}$ was calculated as the inverse of water potential (negative inverse of y coordinate) at the point of intersection between the non-linear and linear regions, $\psi_{\pi sat}$ was calculated as the inverse of the y-intercept of the linear equation, and symplastic fraction (SYMFR) was calculated as the x-intercept of the linear equation (Fig. 4C&D). Bulk elastic modulus of each shoot was calculated by differentiating the power equation at the estimated RWC of 99.5%, such that, from equation 3,

$$E = (-a_1 ERWC)/(a_2 \times (1-ERWC)^{1+a_1}) \quad (12)$$

where a_1 and a_2 are constants.

4.2.4 Statistical Analysis

Values of $\psi_{\pi tlp}$, $\psi_{\pi sat}$, SYMFR, and E for each shoot were analyzed using the general linear model of SAS (SAS Institute, Cary, NC). These variables were analyzed for species, ecotype, watering regime, and harvest date as main and interaction effects in a split plot design, with harvest as the split plot. Since no main or interaction effects for ecotypes were found to be significant for any of the variables, except for SYMFR where $p=0.0497$ for ecotype, (Appendix, Table 2.1), the ecotype effect was removed from all analyses. The six seedlings (three replicates per ecotype) measured for each species during the preliminary harvest (before drought treatment was begun) were randomly assigned to the two watering regimes to balance cell sizes for analyses.

4.3 RESULTS

For all shoots, power functions of the form $y=ax^c$ gave the best fit for the non-linear regions; all shoots had $r^2>0.91$ and 85% had $r^2>0.98$. Linear functions of the form $y=mx+b$ gave the best fit for linear regions with all shoots having $r^2>0.88$.

Douglas-fir seedlings had lower $\psi_{\pi sat}$ (Fig. 5) and $\psi_{\pi tlp}$ (Fig. 6), and higher symplastic fraction than lodgepole pine seedlings throughout the experiment. A separate analysis using only those seedlings having linear functions based on more than two points (11 lodgepole pine shoots) showed the same results as the analysis using all shoots. These results indicate that Douglas-fir is a more drought tolerant species than lodgepole pine.

Both species adjusted osmotically to drought conditions, as indicated by lower $\psi_{\pi sat}$ (Fig. 5) and $\psi_{\pi tlp}$ (Fig. 6) for drought-stressed seedlings at final harvest. Osmotic adjustment in Douglas-fir drought-stressed seedlings occurred after only two drought cycles and was maintained during the final two drought cycles, whereas in lodgepole pine, osmotic adjustment in drought-stressed seedlings was not evident until after three to four drought cycles (Fig. 6). Under well-watered conditions, $\psi_{\pi sat}$ and $\psi_{\pi tlp}$ for Douglas-fir seedling shoots decreased over time, whereas for lodgepole pine seedling shoots, $\psi_{\pi sat}$ and $\psi_{\pi tlp}$ remained constant throughout the experiment.

Douglas-fir seedlings had more rigid cell walls than lodgepole pine seedlings throughout the experiment (Fig. 7). This difference became more pronounced over time as cell walls of Douglas-fir seedlings became more rigid and cell walls of lodgepole pine seedlings maintained a constant and lower rigidity (Fig. 7). Neither species showed any change in cell wall elasticity with drought (Appendix, Table 2.2).

Figure 5. Comparison of osmotic potential at full saturation ($\psi_{\pi sat}$) for Douglas-fir (DF) and lodgepole pine (LP) seedlings at the preliminary harvest (May 25-26) and after the initiation of drought. DRT = drought soil moisture conditions and CTL = well-watered soil moisture conditions. Douglas-fir had significantly lower $\psi_{\pi sat}$ than lodgepole pine ($p<0.001$) and both species had lower $\psi_{\pi sat}$ under the drought watering regime ($p<0.02$). There was a significant effect of harvest date on $\psi_{\pi sat}$ ($p<0.001$). Bars are one standard error and arrows indicate dates of harvest.

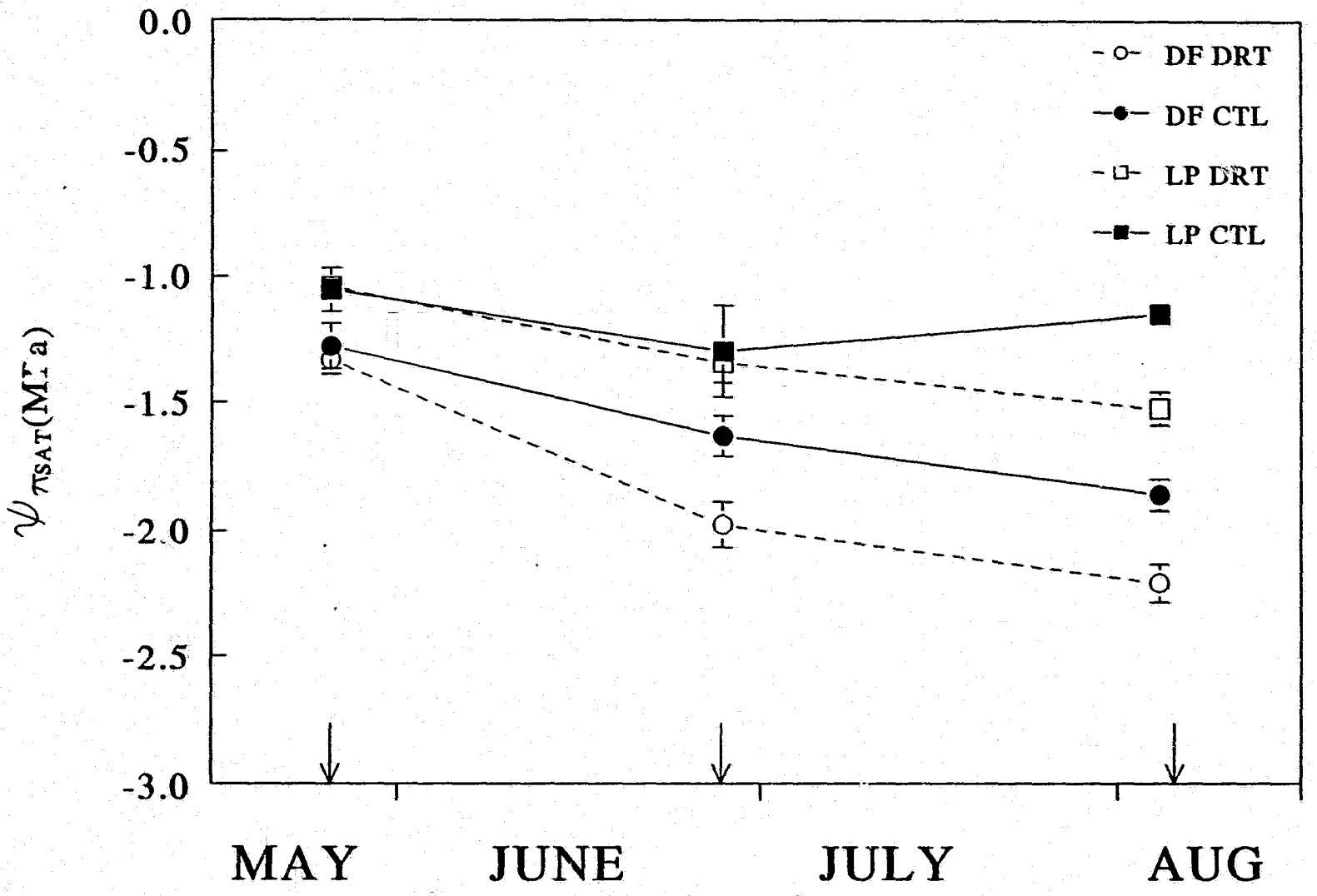

0.0
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
$\psi_{\pi SAT}$(MPa)
DF DRT
DF CTL
LP DRT
LP CTL
MAY
JUNE
JULY
AUG

Figure 6. Comparison of osmotic potential at the turgor loss point ($\psi_{\pi tlp}$) for Douglas-fir (DF) and lodgepole pine (LP) seedlings at the preliminary harvest (May 25-26) and after the initiation of drought. DRT = drought soil moisture conditions and CTL = well-watered soil moisture conditions. Douglas-fir had significantly lower $\psi_{\pi tlp}$ than lodgepole pine ($p<0.02$) and both species had lower $\psi_{\pi tlp}$ under the drought watering regime ($p<0.05$). There was a significant effect of harvest date on $\psi_{\pi sat}$ ($p<0.001$). Bars are one standard error and arrows indicate dates of harvest.

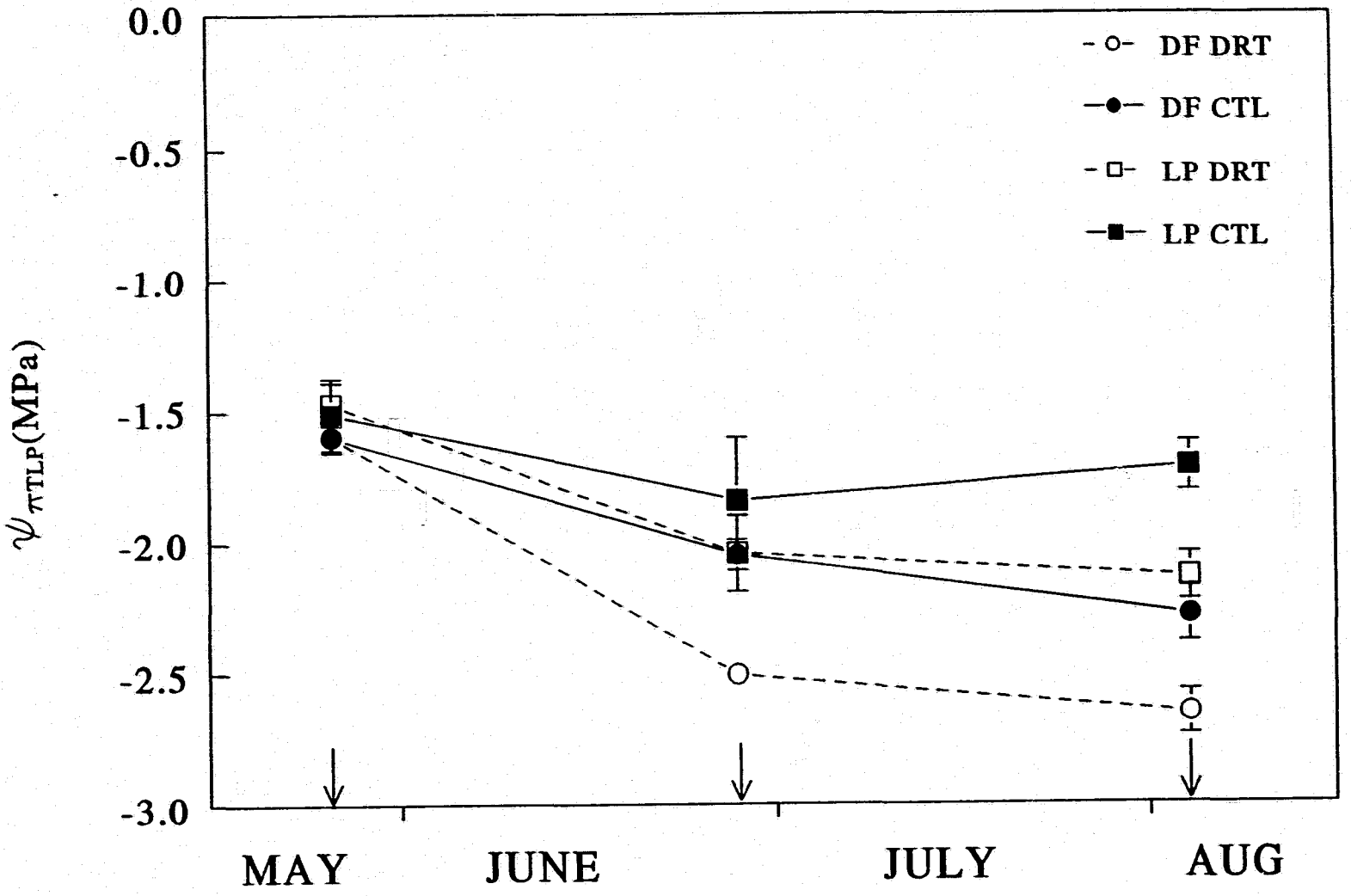
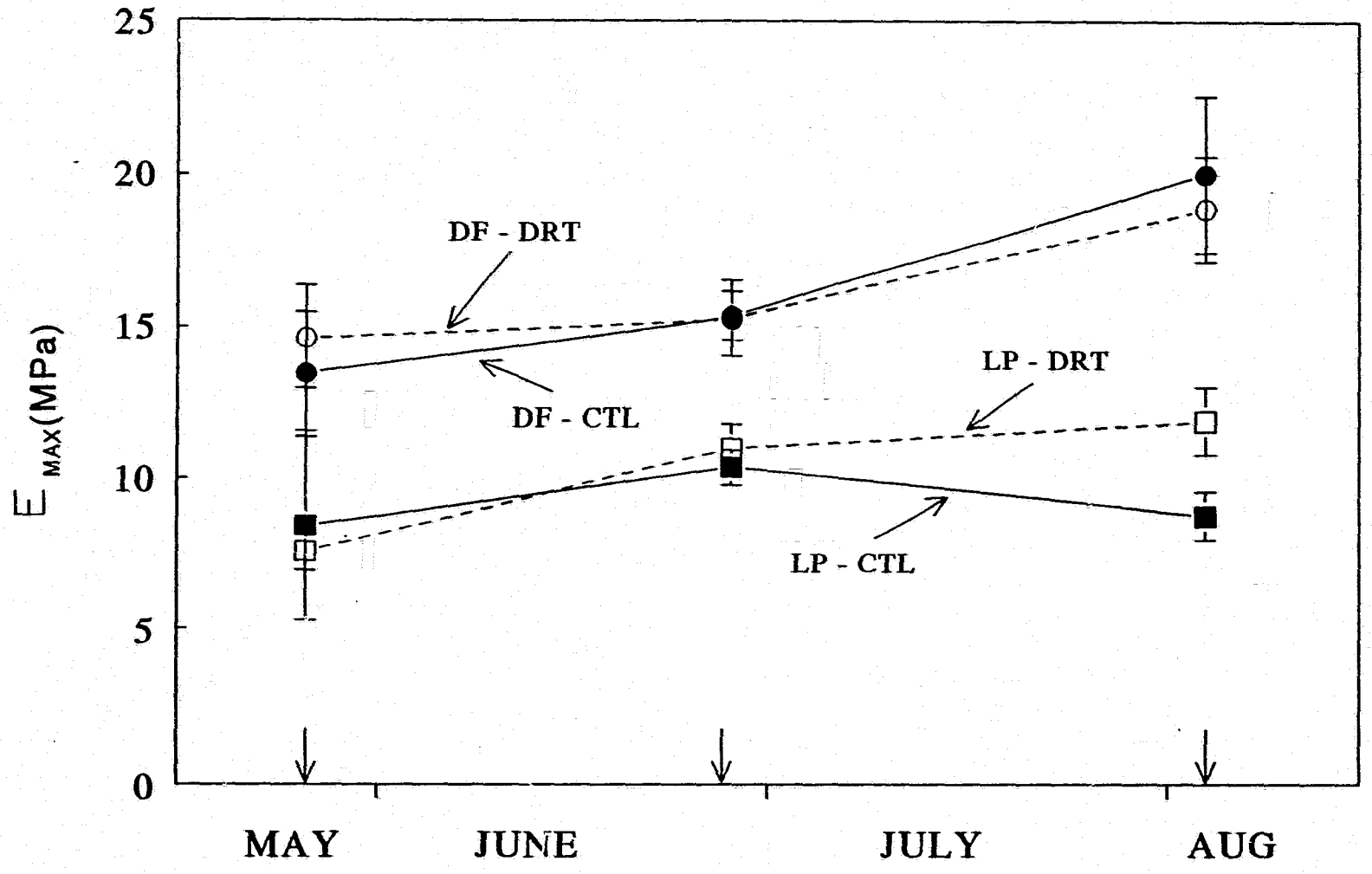

0.0
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
$\psi_{\pi TLP}$(MPa)
DF DRT
DF CTL
LP DRT
LP CTL
MAY
JUNE
JULY
AUG

Figure 7. Comparison of bulk elastic modulus near full saturation (E_{max}) for Douglas-fir (DF) and lodgepole pine (LP) seedlings at the preliminary harvest (May 25-26) and after the initation of drought. DRT = drought soil moisture conditions and CTL = well-watered soil moisture conditions. Douglas-fir had significantly higher E_{max} than lodgepole pine ($p < 0.001$) and there was a significant decrease in E_{max} between harvest dates ($p < 0.01$). Bars are one standard error and arrows indicate dates of harvest.


25
20
15
10
5
0
E_{MAX}(MPa)
DF - DRT
DF - CTL
LP - DRT
LP - CTL
MAY
JUNE
JULY
AUG

4.4 DISCUSSION

PV curves for many lodgepole pine and Douglas-fir shoots demonstrated 'plateaus' at RWC's near full turgor. Plateau regions have been found in Douglas-fir in other studies (Ritchie & Shula; 1984; Kubiske & Abrams, 1991) and in other tree species (Kandiko et al., 1980; Parker & Pallardy, 1987). It has been suggested that water in the interstices of the cell wall microfibrils moves into the cell when shoots begin to dehydrate, preventing loss of full turgor and buffering changes in ψ with decreased RWC (Pallardy et al., 1991). To provide more accurate values of $\psi_{\pi sat}$, $\psi_{\pi tlp}$, and SYMFR, and to calculate E_{max}, it was necessary to adjust full turgor to a value of RWC less than 100% (Pallardy & Parker, 1987; Kubiske & Abrams, 1991). Values of E_{max} should always be interpreted cautiously since they are dependent on the type of curve used to fit the data, i.e. exponential, power, or linear functions (Schulte & Hinckley, 1985; Clayton-Greene, 1983).

Studies have shown that Douglas-fir is a drought tolerant species, although there is ambiguity as to whether turgor pressure is maintained due to osmotic adjustment or cell wall relaxation. Joly & Zaerr (1987) found drought-stressed Douglas-fir seedlings to tolerate drought by cell wall relaxation, since E_{max} was lower in drought-stressed seedlings and there was no difference in $\psi_{\pi sat}$ and $\psi_{\pi tlp}$ between drought-stressed and well-watered seedlings. In contrast, Livingston & Black (1987) found lower pre-dawn ψ_{π} in drought-stressed Douglas-fir seedlings than in well-watered seedlings in the field and Ritchie and Roden (1985) found lower $\psi_{\pi sat}$ and $\psi_{\pi tlp}$ in drought-stressed Douglas-fir seedlings grown in pots, when they used the 'sap expression' method of data collection. These results agree with the latter two studies. However, since there is seasonal variation in $\psi_{\pi sat}$ and $\psi_{\pi tlp}$ in Douglas-fir, with both variables decreasing during the growing season

(Ritchie & Shula, 1984; van den Driessche, 1989), decreases in osmotic potential due to osmotic adjustment found in this study, and others, may be over-estimated.

Drought tolerance has been found in other conifer species. Osmotic adjustment maintained P in western hemlock (Kandiko et al., 1980) and cell wall relaxation maintained P in black spruce and white spruce (Grossnickle, 1989; Blake et al., 1991). Relative to black and white spruce, jack pine was found to have low drought tolerance, since it had higher mortality than the spruce species (Buxton et al., 1985). No other studies have been done on drought tolerance in pine species.

Lodgepole pine was a difficult species to study for pressure-volume analysis. One problem was determin e balancing pressure of the shoots, since at low chamber pressures (shoot near full turgor), both resin and cell sap was expressed. Although differentiating between resin and water extruded was possible, the resin 'bubble' made detection of water difficult. Once the resin had emptied out of the resin canals, water was clearly visible on the cut stem at balancing pressure. Another problem was the length of time required for lodgepole pine seedlings to lose turgor. Often plasmolysis did not occur for more than eight hours and, consequently, the linear portion of the curve had to be determined using only two data points (taken 14 and 24 hours after shoot excision). Lodgepole pine results should be interpreted cautiously.

Although Douglas-fir and lodgepole pine were able to maintain turgor by osmotically adjusting cellular sap when drought-stressed, neither species reduced cell wall rigidity to minimize the effect of reduced relative water content on turgor pressure.

The greater reduction in $\psi_{\pi sat}$ in drought-stressed Douglas-fir shoots over time indicates it is a more drought tolerant species than lodgepole pine. Since

Douglas-fir seedlings had lower $\psi_{\pi sat}$ than lodgepole pine seedlings before drought-stress treatments began, Douglas-fir seedlings either maintain lower osmotic potentials than lodgepole pine or they were previously drought-stressed. Independent of this, after drought treatments had begun ψ_{π} in Douglas-fir seedlings decreased by 0.9 MPa after four drought cycles, whereas lodgepole pine seedlings showed a decrease of only 0.6 MPa. Clearly Douglas-fir can osmotically adjust, although not necessarily more than lodgepole pine, but $\psi_{\pi sat}$ in Douglas-fir seedlings is generally lower than lodgepole pine indicating that Douglas-fir is able to tolerate drier soils.

Chapter 5

DROUGHT EFFECTS ON ROOT HYDRAULIC CONDUCTANCE OF DOUGLAS-FIR, LODGEPOLE PINE, AND WHITE SPRUCE SEEDLINGS

5.1 INTRODUCTION

Roots provide a major resistance to water flow in the soil-plant-atmosphere continuum (SPAC) due to the presence of Casparian strips radially surrounding endodermal cells. Other possible major resistances in the SPAC are in the soil, at the soil-root interface, in the xylem, and at the stomata. Under well-watered conditions, when soil has high moisture content and the stomata are open, the greatest resistance to water movement is at the endodermis. Running (1980) found resistance in the roots of mature lodgepole pine trees to be 52-74% of the total resistance in the tree.

Under drought conditions, resistances in the soil, soil to root interface, xylem and stomata may increase, causing reduced hydraulic conductance (L_p, inverse of resistance) in the SPAC. As soil moisture decreases, movement of water in the soil decreases due to increasing number and size of air pockets (Taiz & Zeiger, 1991), roots may shrink, reducing contact between soil water and root surface (Faiz & Weatherley, 1977; 1978; 1982), embolisms may form in xylem tracheids, reducing pathways for water flow (Tyree & Ewers, 1991), and stomata may close in response to hydraulic (Livingston, pers. comm.) or chemical (Davies & Zhang, 1991) signals. Little is known of the importance of root resistances, relative to the increased resistances in these other regions of the SPAC as soil moisture is depleted.

Root hydraulic conductance (L_{proot}) can be calculated using Ohm's law (equation 7). Measurements of water flow through roots in a pressure chamber

have shown that L_{proot} is variable at low pressures and, therefore L_{proot} should be determined from flow rate measurements at pressures greater than 0.3 MPa (Markhart & Smit, 1990). To explain the variable L_{proot} at low pressures, models were developed by Fiscus (1975, 1977), Dalton *et al.* (1975), and Newman (1976). These models suggested that, at low pressures solute build-up at the endodermis causes an ψ_π gradient to form. Therefore, using equation 7, at high pressures $\Delta\psi_\pi$ is negligible relative to ΔP and the slope of flow rate versus hydrostatic pressure (root hydraulic conductance) is linear, and at low pressures, when $\Delta\psi_\pi$ has a significant value, L_{proot} is not linear. Although these models do not completely explain changes in L_{proot} at low pressures (Passioura, 1984), movement of solutes is probably an important factor.

Root hydraulic conductance is commonly determined from flow rate data for de-topped roots of conifer seedlings in pressure chambers (Sands *et al.*, 1982; Colombo & Asselstine, 1989; Coleman *et al.*, 1990; Grossnickle & Russell, 1990). Conifers are believed to have lower L_{proot} than deciduous species due to their lack of vessels, which have lower resistance to water movement than tracheids. Sands *et al.* (1982) found that bean roots had eight times the root axial conductance of loblolly pine roots.

Root hydraulic conductance was determined for first year seedlings of three conifer species, collected from wet and dry sites and grown under two soil water regimes. Of the three conifers, coastal Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) is found in moister habitats than interior lodgepole pine (*Pinus contorta* Dougl.) and white spruce (*Picea glauca* (Moench) Voss). The hypothesis of this study was to test whether interior species are able to grow in drier sites due to their ability to reduce L_{proot} at low soil moisture. The study was continued in the

following growing season to determine the effect of age on L_{proot}, but only well-watered seedlings were measured in the second year.

5.2 MATERIAL AND METHODS

5.2.1 Plant Material and Culture

Douglas-fir, lodgepole pine, and white spruce seeds from wet and dry sites, supplied by the British Columbia Ministry of Forests, were grown in 1989 and 1990 (Table 5). All seeds were soaked on February 28 or March 1, 1989 for 24 hours and stratified in a 5°C refrigerator for 24 days, then sown by seedlot into 11 L pots containing moist, coarse sand. Pots were covered with foil to reduce water loss by evaporation until seeds began to germinate. At the cotyledon stage, 36 seedlings from each seedlot were individually transplanted into 3.2 L pots containing 3-6 cm coarse gravel and 12-15 cm coarse sand. All pots were placed on wooden pallets to allow air circulation under pots. Lodgepole pine seedlings germinated one week earlier than Douglas-fir and white spruce and, consequently, were the first seedlings to be transplanted, on April 14, 1989.

Seedlings were watered every 3-4 days and fertilization (150 ppm 20-20-20 NPK) was begun two to three weeks after transplanting and continued at weekly intervals until the drought treatment was begun. To ensure adequate iron, sulphur, and calcium supply in the sandy soil, 30 ppm $FeSO_4$ and 100 ppm $CaCl_2$ were supplied on alternate weeks until the drought treatment began. In the spring of 1990 watering was begun on March 5 and fertilization with 20-20-20 NPK, $FeSO_4$, and $CaCl_2$ was begun on March 29, April 26, and May 3 respectively, and followed the same timetable as in 1989.

Pots were separated into two groups and randomly placed on pallets such that half the seedlings of each seedlot were watered and the remaining seedlings

Table 5. Location and annual precipitation for seedlots of Douglas-fir, lodgepole pine, and white spruce seeds from wet and dry ecotypes. Seeds were sown in spring 1989 and used in 1989 and 1990 studies of root hydraulic conductance.

Location of Seed Source (latitude, longitude)	Seedlot(s)	Ecotype	Annual[1] Precip.(mm)
1989:			
Douglas-fir:			
Nootka Island (49°45',126°,45')	220X[2]	Wet	3829
Gold River (49°41',126°06')	604	Wet	2721
Goldstream (48°29',123°33')	305X	Dry	1157
Courtenay (49°41',126°06')	45	Dry	1503
Lodgepole pine:			
Suskwa River (55°17',127°15')	2108	Wet	625
Lily Lake (53°56',124°39')	29092	Dry	464
White spruce:			
Prince Rupert (54°,130°)	33 X 63	Wet	2523
East Kootenays (50°,115°)	31 X 126	Dry	547
1990:			
Douglas-fir:			
Kyoquot (49°59',127°18')	218X	Wet	3319
Kyoquot (49°59',127°18')	217	Wet	3319
Sechelt (49°36',123°48')	543X	Dry	1099
Campbell River (50°01',125°20')	331	Dry	1581
Lodgepole pine:			
Clive Creek (54°52',128°23')	28832	Wet	1155
Community Lake (50°55',120°05')	2175	Dry	270
White spruce:			
Prince Rupert (54°,130°)	96 X 35	Wet	2523
East Kootenays (50°,115°)	30 X 29	Dry	547

1 Environment Canada. 1989. Temperature and precipitation, 1951-1980, British Columbia. Atmospheric Environment Service. Downsview, Ontario.

2 'X' indicates Douglas-fir seeds were produced by crossing adjacent seedlots in table.

became drought-stressed. On July 12, 1989 Peters trace elements (4.33 g m^{-3} of soil) were applied to pots and on July 17, 150 ppm 20-20-20 NPK was added to all pots on the final watering before initiating the drought treatment. On July 21, benlate (0.62 g L^{-1} H_2O) was applied to seedlings to prevent fungal infection. Control seedlings continued to be watered every 3-4 days until September 19 when a weekly watering regime maintained soil moisture. All seedlings were supplied with 150 ppm 20-20-20 solution every second week.

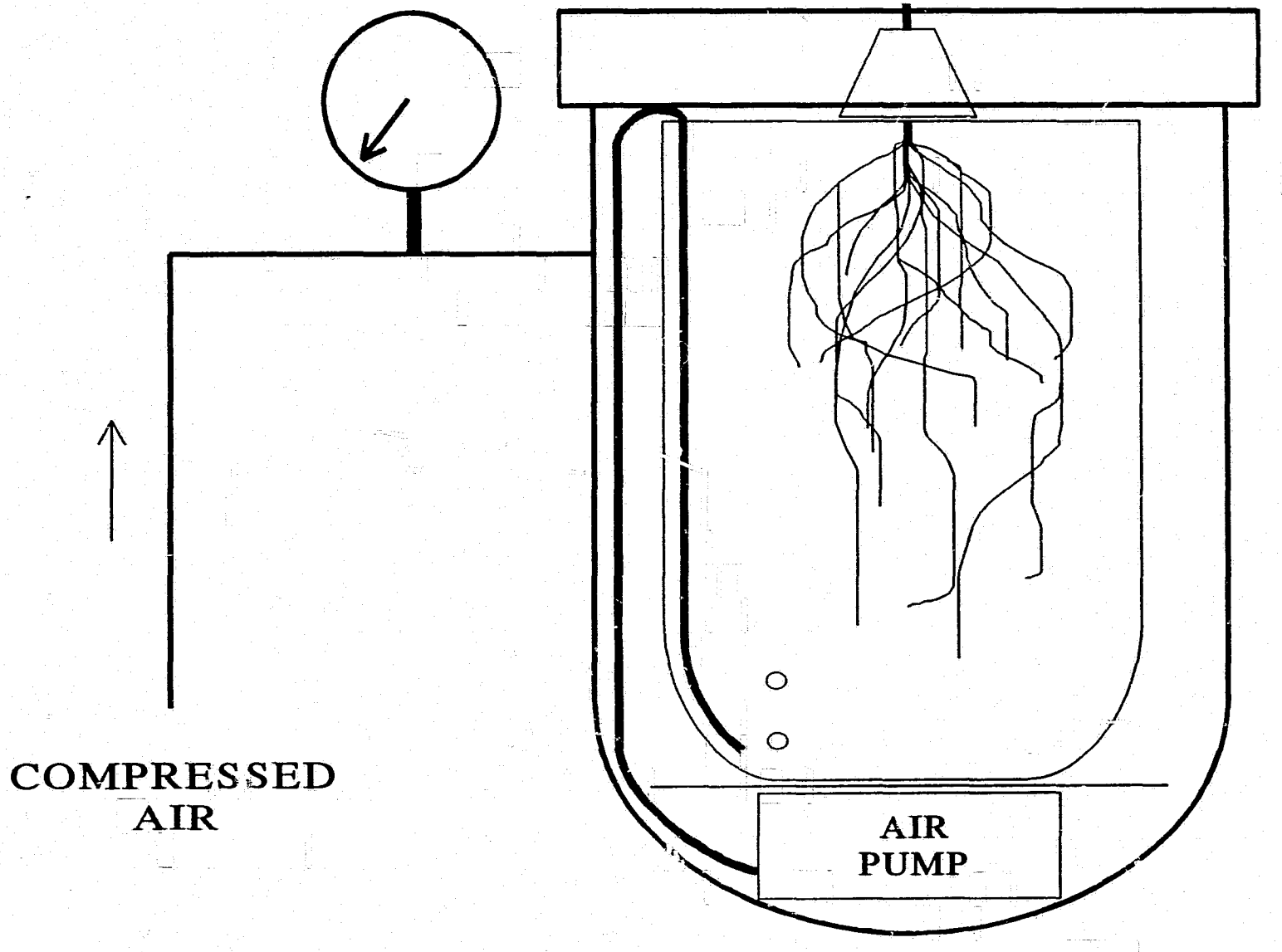
During winter 1989 to spring 1990 seedlings remained dormant in the greenhouse. Due to low survival of drought-stressed seedlings over winter, only control seedlings were measured during the 1990 growing season.

Measurements in 1989 began later in the season than in 1990 due to implementation of the drought treatment. In 1989 seedlings were measured on August 24-27, September 9-11, September 23-25, and October 14-16, and in 1990 seedlings were measured on June 19-20, July 31-August 3, and September 11-12. At each measurement three seedlings were randomly chosen from wet and dry seedlots of each species (Table 5) and measurements of hydraulic flow rates at three pressures were collected using a pressure chamber.

5.2.2 Pressure Chamber Assembly

A 20.0 cm diameter, 25.4 cm tall, 1.3 cm thick steel pressure chamber was built to withstand pressures up to 2.5 MPa (Fig. 8). Pressure was controlled using manual inlet and outlet valves and a Cantech safety valve prevented pressure from exceedings 2.5 MPa (Fig. 8). A 7 mm hole was bored into the centre of the lid, through which the cut end of the root (root stump) protruded. The lid was secured to the chamber with six 0.8 mm diameter bolts and an O-ring provided a good seal. An aquarium air pump at the bottom of the pressure chamber pumped air through

Figure 8. Equipment design to measure flow rates of 1989 and 1990 Douglas-fir, lodgepole pine, and white spruce seedling roots at three pressures.


COMPRESSED
AIR
AIR
PUMP

an air lift pump and circulated the water surrounding the suspended roots in the 5.45 L container.

Preparation of seedlings for pressure chamber measurements and collection of data was the same for each seedling at all harvests. Using a sharp razor, the shoot was removed near the stem base approximately 2.5 cm above soil level and the roots were gently removed from the soil and washed. Root damage was minimal since little of the coarse sand rooting medium adhered to the roots. The root stump was gently inserted through the centre of a rubber stopper which was placed into the underside of the lid so that the root stump protruded from the upper side of the lid and the roots were suspended in the water-filled container inside the pressure chamber (Fig. 8).

Pressure was slowly increased inside the pressure chamber to 0.75, 1.00, and 1.25 MPa for the three sets of flow rate measurements. At each pressure, a pre-weighed plastic tube, ranging from 1 - 2 cm in length and containing a swab of tissue, was placed over the root stump for a timed interval (ranging from 30 seconds to 2 minutes, depending on flow rate) to collect xylem exudate forced through the root system. When the plastic tube was removed, the tube + tissue was reweighed and a dry, pre-weighed plastic tube with tissue was placed on the root stump. The gain in weight for a tube + tissue was taken to represent the weight of xylem exudate forced through the root system during the timed interval. Five measurements were taken at each pressure. Water temperature in the pressure chamber was between 18^{o}C and 23^{o}C.

5.2.3 Preliminary Experiments

During late spring and early summer 1989 four experiments were carried out to determine the best materials and methods for measuring hydraulic conductance:

(1) comparison of oxygen content in tap, distilled, and stagnant water (left overnight), (2) root uptake of oxygen from water at atmospheric pressure, (3) comparison of water versus 120 ppm 20-20-20 (NPK) nutrient solution environment in which to suspend roots, (4) comparison of nitrogen gas and compressed air on root water flow rates, and (5) effect of pressure on flow rate through roots (also determined for one year old seedlings in May 1990).

5.2.3.1 Oxygen content of water:

Using a dissolved oxygen meter (Yellow Springs Instrument Co., Ohio), oxygen concentration was measured in distilled water, tap water, and stagnant water to determine which water supplied the most oxygen to the seedling roots. Several values of oxygen concentration were verified using the Winkler test (Strickland & Parsons, 1972). Mean values of oxygen concentration for distilled, tap, and stagnant water were 103, 269, and 203 μM O_2 respectively for water temperatures ranging from 21^{o} to 24^{o}C. When water was circulated using an air pump, oxygen concentrations for all three types of water ranged from 250 to 269 μM O_2. As a result of these tests, tap water, having the highest mean oxygen concentration for circulated and non-circulated water, was used in all subsequent experiments.

5.2.3.2 Root uptake of oxygen:

Oxygen uptake by de-topped seedling roots, suspended in a 3.58 L glass jar of tap water, was measured at atmospheric pressure over 4-8 hour periods on three seedlings from each species to indicate O_2 requirements of root systems. Temperature was between 16^{o}-24^{o}C throughout the experiment, increasing by 2^{o}C to 7^{o}C during 4 to 8.5 hour time periods. Oxygen concentrations, measured

using an oxygen meter, were corrected for temperature and normalized by root dry weight. Oxygen uptake by white spruce root systems were significantly lower than root systems in the other two species (Table 6). This is probably due to the smaller root dry weight and root diameters in the white spruce seedlings, which indicates a lower root surface area relative to the other species. Although the high O_2 concentrations measured in the water would provide aerobic conditions for more than the one to two hours required to collect measurements for each seedling, an air pump was used to maintain a constant flow of air, containing O_2, to the water. Markhart & Smit (1990) found that an 100 kPa O_2 concentration under 500 kPa pressure had no detrimental effect on hydraulic flow rates of de-topped roots.

Table 6. Mean oxygen uptake per hour for whole root systems of Douglas-fir (DF), lodgepole pine (LP), and white spruce (WS) seedlings. Standard errors (SE) are in parentheses.

Species	mean (SE) O_2 uptake per root system per hour ($\mu M\ hr^{-1}$)	mean (SE) dry weight (g)
DF	14 (4)a[1]	1.710 (0.181)
LP	17 (2)a	1.706 (0.257)
WS	7 (1)b	1.110 (0.209)

[1] means followed by the same letter are not significantly different at the 5% level

5.2.3.3 Comparison of Water versus Nutrient Solution:

It has been suggested that the environment surrounding seedling roots should be the same in the pressure chamber as it was during growth and development (Markhart & Smit, 1990). The osmotic potential of the coarse sand medium in

which the seedlings were grown was determined by cryoscopy to be equivalent to that of 125 ppm 20-20-20 (NPK) nutrient solution. Flow rates for two seedlings of each species were measured twice, once when the container was filled with water and a second time when the container was filled with 120 ppm 20-20-20. No significant difference was found between flow rates. Consequently, water was used as the medium into which seedling roots were suspended for all subsequent experiments.

5.2.3.4 Comparison of Nitrogen Gas versus Compressed Air:

To study the effects of nitrogen gas versus compressed air on water flow rates through roots, flow rates for roots from two seedlings of each species were measured at 1.4 and 3.5 kPa pressures, in a pressure chamber made of PVC, with both gases. Roots equilibrated in the pressure chamber at each pressure for 15 minutes before measurements were taken. No significant differences between flow rates using the two gases were found (means (SD) = 0.0129 (0.0021) and 0.0132 (0.0030) g min^{-1} at 1.4 kPa and 0.0325 (0.0079) and 0.0322 (0.0089) g min^{-1} at 3.5 kPa in compressed air and nitrogen gas respectively). However, Smit and Stachowiak (1988) and Swietlik (1989) found that nitrogen gas had an adverse effect on root flow rate when applied to *Populus* and sour orange seedlings. Swietlik (1989) found flow rates decreased within 30 minutes of applying nitrogen gas at 0.5 MPa in sour orange seedlings, whereas Smit and Stachowiak (1988) observed that 12 hours were required before flow rates in a *Populus* species decreased. Differences between oxygen and nitrogen gas were not observed over 30 minutes in the present study, however compressed air was used in all our subsequent experiments so that the oxygen level in the bathing solution could be maintained with the circulating pump.

5.2.3.5 Pressure Effects on Root Flow Rates:

Root hydraulic conductance is calculated as the change in flow rate with change in pressure, ie. slope of flow rate versus pressure (see Chapter 2). To determine the range at which hydraulic conductance is constant (slope is linear), flow rates were measured for two seedlings from each species at pressures ranging from 0 to 2.5 MPa at each date (Fig. 9). In 1989, all roots reached their maximum flow rates before maximum pressure was applied, however, only a few seedlings showed increasing root hydraulic conductance at low pressures (Fiscus, 1975; Dalton *et al.*, 1975). Most seedlings showed a linear to logarthmic curve shape at low pressures. For all 1989 curves produced, the curves appeared linear between 0.75 and 1.25 MPa. Consequently, all measurements of flow rates were made in this range of pressures in subsequent work.

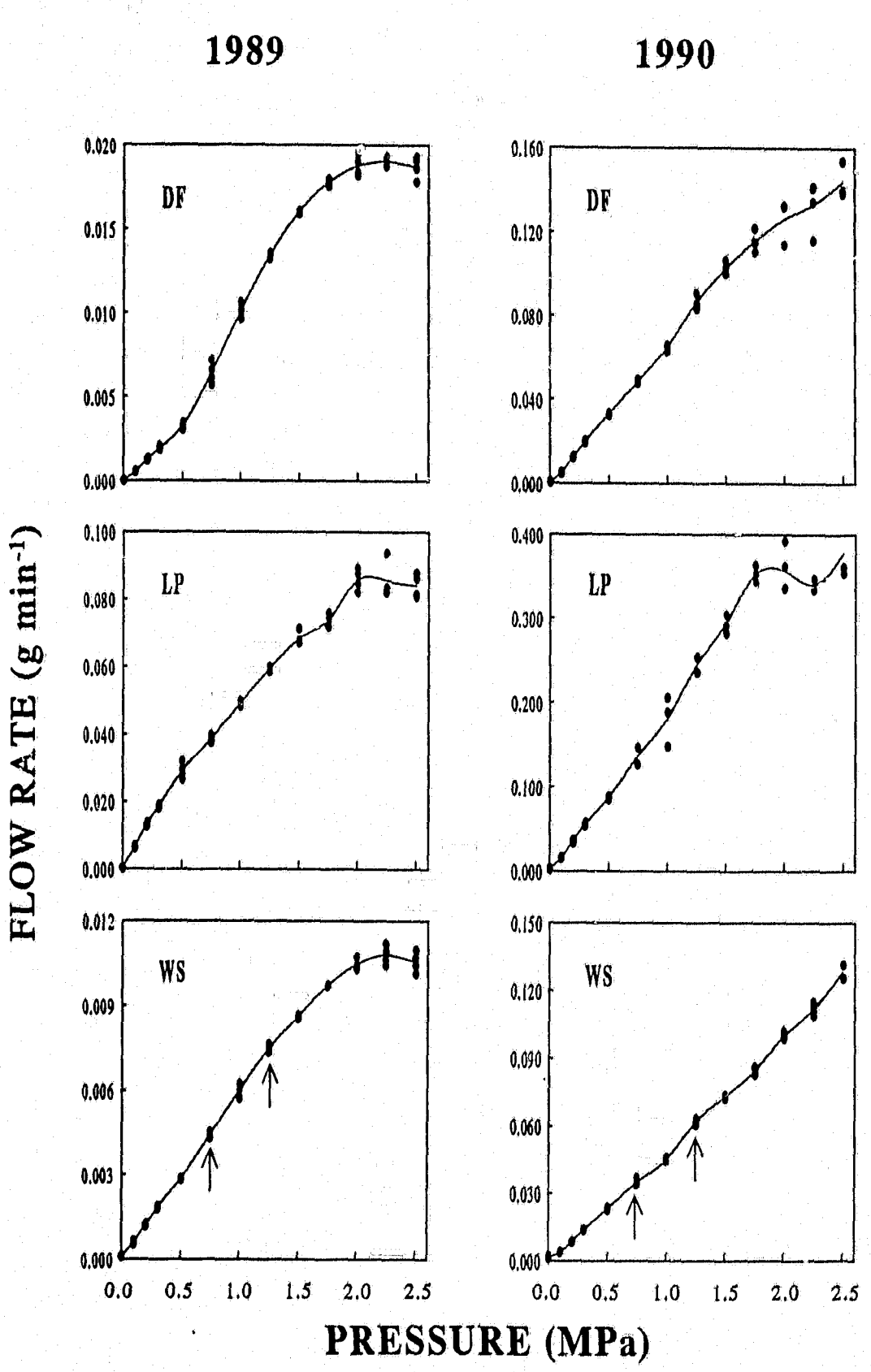
5.3 Statistical Analysis:

Flow rates at each pressure were calculated for each of the plastic tube+tissue measurements using the equation:

$$F = (W_w - W_d)/(t_w - t_d) \quad (13)$$

where F=flow rate in g s^{-1}, W_d=dry weight of tissue (g) and tube at time t_d (s), when tube was placed on the stump, and W_w=wet weight of tissue (g) and tube at time t_w (s), when tube was removed from the stump. Flow rates for each seedling were then normalized by root length (Tennant, 1975) or root dry weight. Hydraulic conductance (g s^{-1} MPa^{-1} m^{-1} or g s^{-1} MPa^{-1} g^{-1}) was calculated as the slope of the best fit curvilinear equation for change in flow rate (g s^{-1} or g s^{-1}) with change in applied pressure (MPa), using the SAS regression procedure (SAS Institute, Cary NC). The hydraulic conductance values were analyzed for species,

Figure 9. Flow rates measured for a Douglas-fir (DF), lodgepole pine (LP), and white spruce (WS) seedling root system at 0 to 2.5 MPa. Flow rates were measured at 30, 60, or 120 second time intervals. Arrows indicate the range of pressures at which flow rates were measured.


1989
1990
DF
LP
WS
FLOW RATE (g min-1)
PRESSURE (MPa)
0.020
0.015
0.010
0.005
0.000
0.160
0.120
0.080
0.040
0.100
0.060
0.020
0.400
0.300
0.200
0.012
0.009
0.006
0.003
0.150
0.090
0.030
0.0
0.5
1.0
1.5
2.0
2.5

ecotype, watering regime, and harvest date main and interactive effects, using the general linear model of SAS for a split plot design, with harvest as the split plot.

5.3 RESULTS

By final harvest on October 14-16, 1989, lodgepole pine and Douglas-fir seedlings had 1.5 to 3 times the dry weights of white spruce seedlings and well-watered seedlings had significantly greater dry weight biomass than drought-stressed seedlings (Fig. 10). Over all species, seedlings from wet ecotypes were more affected by the drought treatment than seedlings from dry ecotypes for all three species (Fig. 11; Appendix, Table 3.1), i.e. seedlings from wet ecotypes had significantly reduced seedling dry weights when drought-stressed, whereas seedlings from dry ecotypes produced the same amount of biomass under both watering regimes.

Root lengths and root dry weights increased significantly with time ($p < 0.001$) during the 1989 growing season. Although white spruce produced the least dry weight (Fig. 10), this species produced the greatest specific root length (m g^{-1}) (Fig. 12). Douglas-fir had the lowest specific root length, indicating that it required the greatest investment of biomass in roots to produce root lengths equivalent to those of the other two species.

Analysis of L_{proot} for all seedlings harvested in 1989 revealed that no ecotype main or interaction effect was significant (Appendix, Table 3.2), consequently the ecotype variable was removed from all analyses of L_{proot}. Although there was a significant watering regime main effect (Appendix, Table 3.3) over the four harvests, lodgepole pine was the only species which showed a response to the drought treatment effect, having a 50% lower L_{proot} under drought-stressed conditions than under well-watered conditions (Table 7). All three species had

Figure 10. Mean root, stem, and needle dry weights for Douglas-fir (DF), lodgepole pine (LP), and white spruce (WS) seedlings at final harvest (October 14-16) in 1989. Means are backtransformed from ln(dry wt+1) results. Root, stem, and needle dry weights were significantly different for species ($p = < 0.03$) and watering treatments ($p = < 0.0007$).

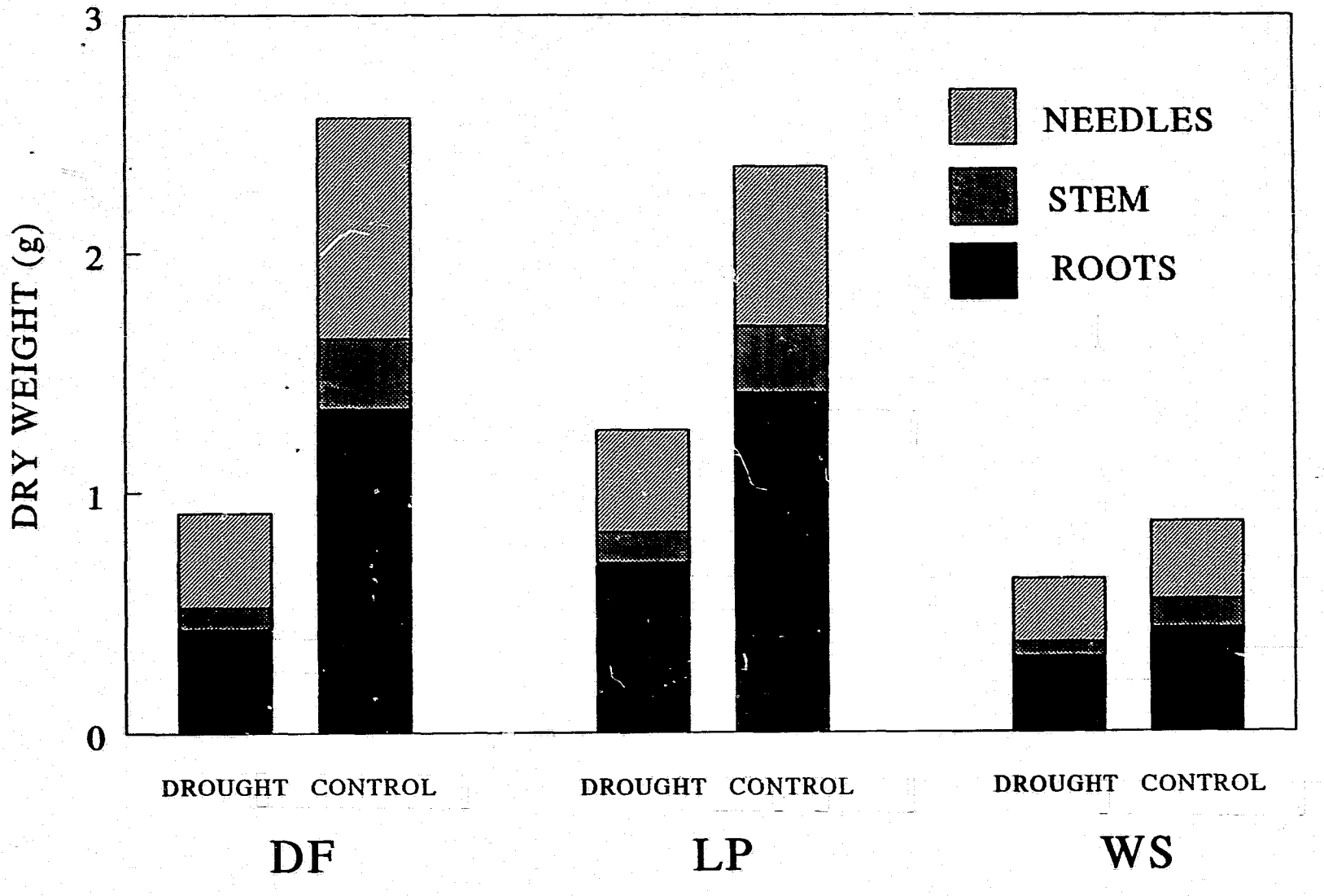

3
2
1
0
DRY WEIGHT (g)
NEEDLES
STEM
ROOTS
DROUGHT CONTROL
DROUGHT CONTROL
DROUGHT CONTROL
DF
LP
WS

Figure 11. Drought treatment effects on dry weights of organs and whole seedlings from wet and dry ecotypes, measured at final harvest. Means have been backtransformed from ln(dry wt+1) results and bars are 95% confidence limits. All dry weights were significantly different for ecotype x watering treatment interaction ($p < 0.03$).

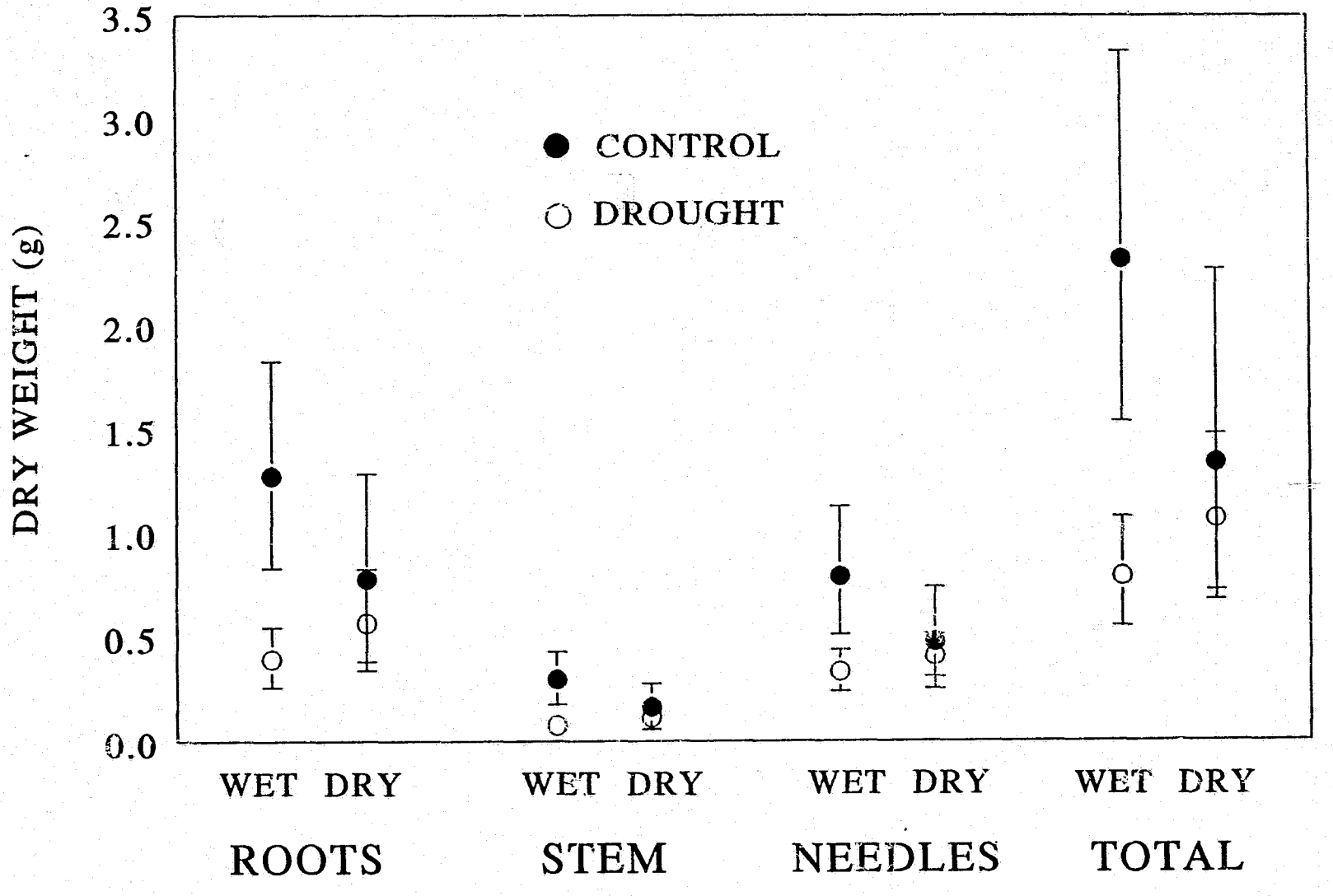

DRY WEIGHT (g)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
CONTROL
DROUGHT
WET DRY
WET DRY
WET DRY
WET DRY
ROOTS
STEM
NEEDLES
TOTAL

Figure 12. Relationship between root length and root weight (specific root length) for seedlings harvested in 1989, by species. Regression slopes between species were significantly different ($p < 0.001$) because the equation for white spruce seedlings had a steeper slope than the equations for Douglas-fir and lodgepole pine seedlings. Best fit regressions had $r^2 = 0.91$, 0.78, and 0.74 for Douglas-fir (DF), lodgepole pine (LP), and white spruce (WS) roots respectively and $p < 0.01$ for each species).

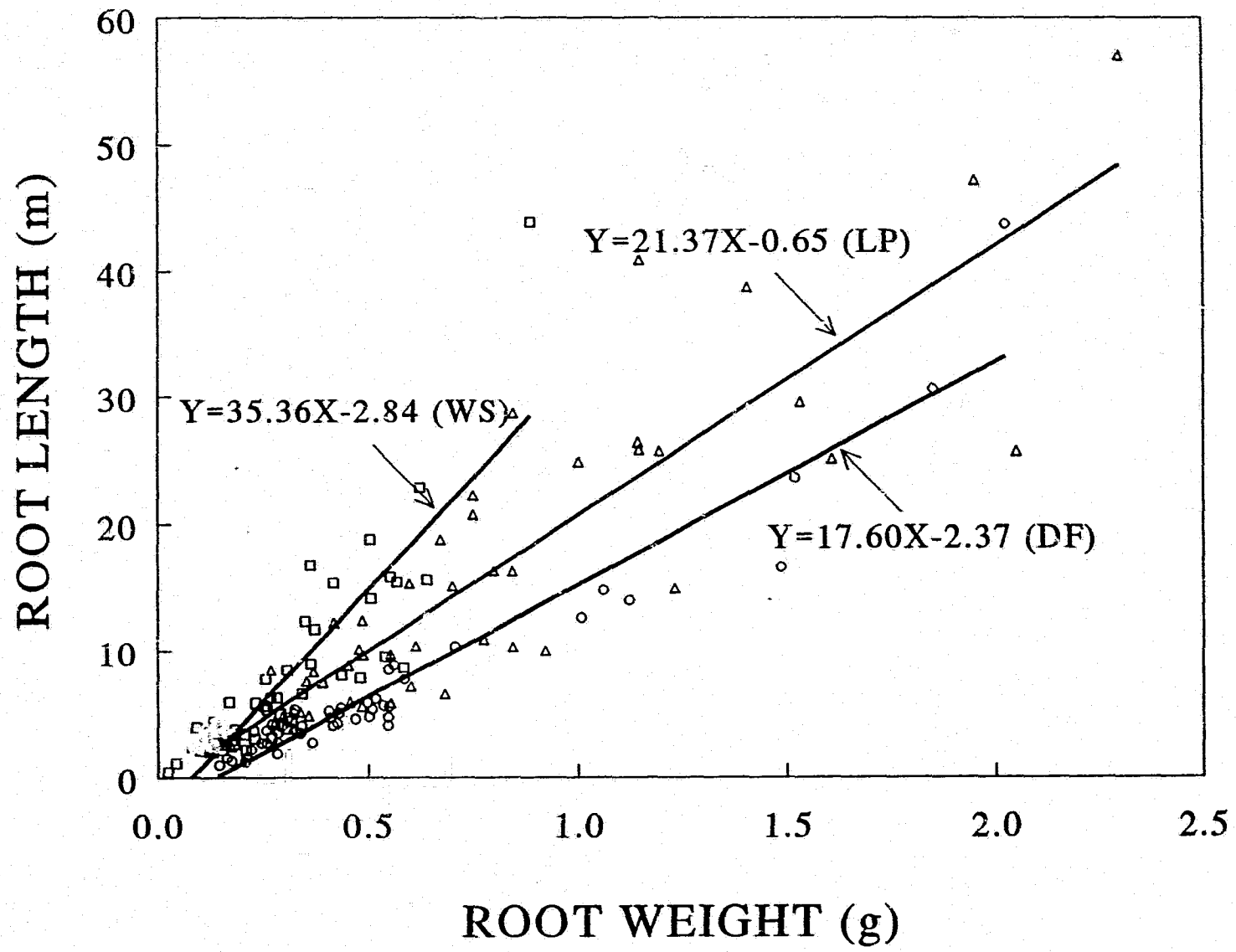

ROOT LENGTH (m)
60
50
40
30
20
10
0
0.0
0.5
1.0
1.5
2.0
2.5
ROOT WEIGHT (g)
Y=21.37X-0.65 (LP)
Y=35.36X-2.84 (WS)
Y=17.60X-2.37 (DF)

Table 7. Mean root hydraulic conductances for Douglas-fir (DF), lodgepole pine (LP), and white spruce (WS) seedlings, grown under two watering regimes. Values in parentheses are one standard error.

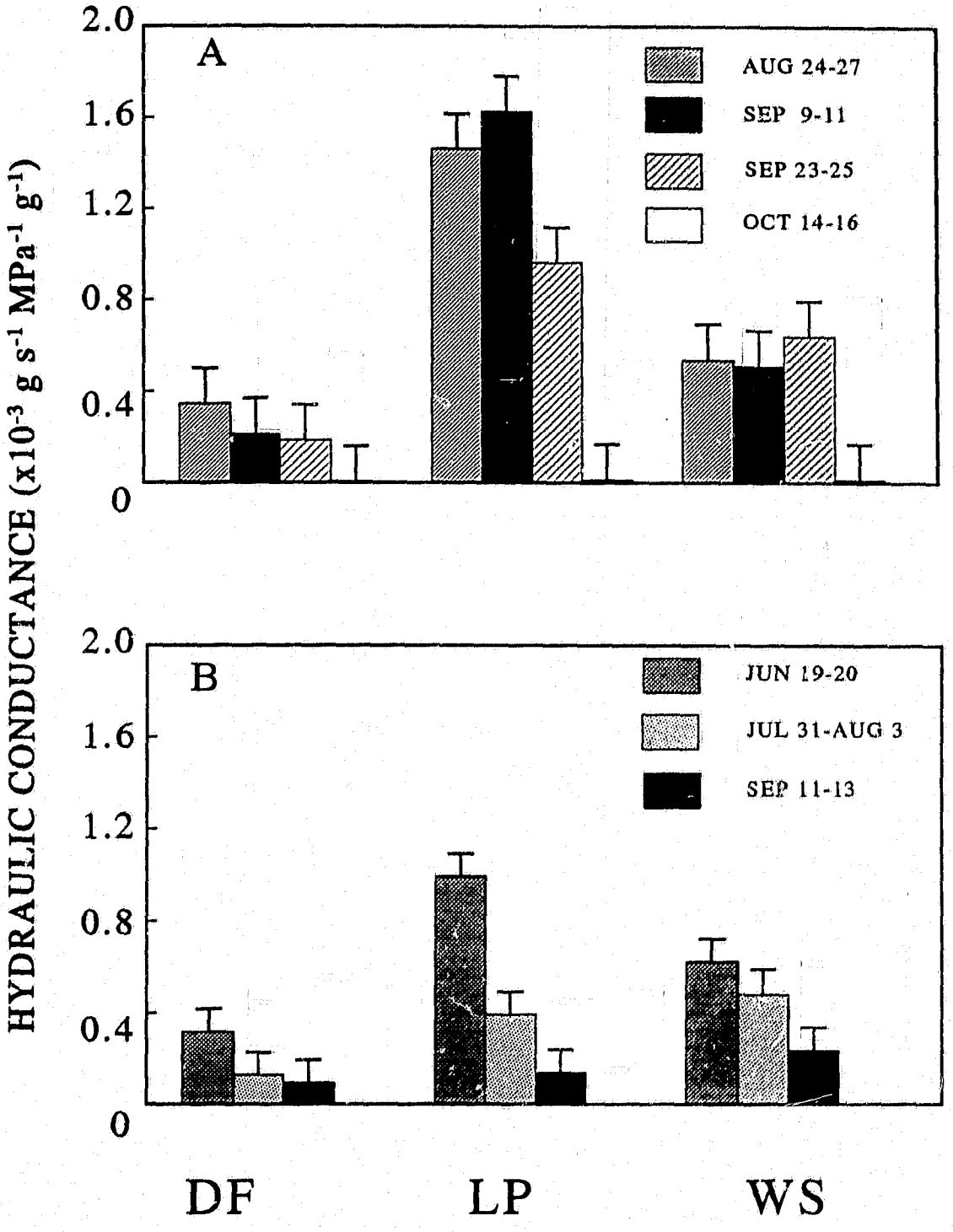
Species	Treatment	mean Root Hydraulic Conductance ($\times 10^{-5}$ g $s^{-1}MPa^{-1}m^{-1}$)
DF	Drought	1.53 (1.03)a[1]
	Control	1.44 (0.97)a
LP	Drought	2.17 (1.00)a
	Control	5.47 (0.97)b
WS	Drought	1.58 (0.97)a
	Control	1.67 (0.97)a

[1] means followed by the same letter are not significantly different at the 5% level

similar L_{proot} under drought conditions but lodgepole pine seedlings were able to conduct twice as much water as the other two species under well-watered conditions (Table 7). Over all three species, no significant differences were found between L_{proot} for the first three harvests, however, all seedlings showed large reductions in L_{proot} between the final two harvests, decreasing from a mean L_{proot} of 4.833×10^{-5} to 5.544×10^{-7} g $m^{-1}s^{-1}MPa^{-1}$.

Comparisons of seedling L_{proot} between 1989 and 1990 was done on well-watered seedlings only, due to the low survival of drought-stressed seedlings over winter. In 1989, well-watered lodgepole pine seedlings had greater L_{proot} than the other species and, except for the reduction in L_{proot} at the third harvest in lodgepole pine seedlings, all species maintained a constant L_{proot} until the fall (Fig. 13A). In 1990, the only significant decrease in L_{proot} was in lodgepole pine seedlings between the first and second harvests, however, larger sample sizes may

Figure 13. Mean hydraulic conductance of roots, normalized by weight, for well-watered Douglas-fir (DF), lodgepole pine (LP) and white spruce (WS) seedlings harvested in (A) 1989 and (B) 1990. Hydraulic conductance was significantly different for species by harvest date interaction in both years ($p = < 0.0001$ and $p = < 0.02$ for 1989 and 1990 respectively). Bars indicate one standard error.


HYDRAULIC CONDUCTANCE (x10-3 g s-1 MPa-1 g-1)
A
AUG 24-27
SEP 9-11
SEP 23-25
OCT 14-16
B
JUN 19-20
JUL 31-AUG 3
SEP 11-13
2.0
1.6
1.2
0.8
0.4
0
DF
LP
WS

have shown lodgepole pine and white spruce seedlings to decrease throughout the 1990 season (Fig. 13B).

In comparing early September harvests for the two years, Douglas-fir seedlings had the lowest L_{proot} throughout both 1989 and 1990 growing seasons (Table 8; Fig. 13A&B) and it was the only species to maintain L_{proot}. Mean L_{proot} in 1990 for lodgepole pine and white spruce were less than 10% and 50% respectively of their 1989 L_{proot} (Table 4).

Table 8. Mean root hydraulic conductance ($g\ s^{-1}\ MPa^{-1}\ g^{-1}$) for the three species in September 1989 and September 1990.

	Means for years	Douglas-fir	lodgepole pine	white spruce
Means for species		0.55[1]	3.18	1.42
1989	2.82[2]	0.76a[3]	5.85c	1.83b
1990	0.56	0.34a	0.50a	0.84a

[1] lodgepole pine had higher root hydraulic conductance than the other species ($p<0.001$)
[2] Differences between years were significant ($p<0.001$)
[3] means followed by the same letter are not significantly different at the 5% level

5.4 DISCUSSION

Although seedlings of all three species produced significantly less dry weight under drought than under well-watered conditions, only lodgepole pine had reduced L_{proot} under drought conditions. Results for lodgepole pine seedlings agree with studies of drought effects on L_{proot} in citrus (Ramos & Kaufmann, 1979; Levy & Syvertsen, 1983), sugarcane (Saliendra & Meinzer, 1992), a desert

succulent (Huang & Nobel, 1992; Nobel & Cui, 1992), sorghum (Cruz *et al.*, 1992), and shortleaf pine seedlings (Brissette & Chambers, 1992). In these studies, L_{proot} decreased when plants were drought-stressed. Lower L_{proot} under drought conditions has been due to early suberization of roots (Cruz *et al.*, 1992; Huang & Nobel, 1992) and cavitation of root xylem (Tyree & Ewers, 1991; Saliendra & Meinzer, 1992).

Our results indicate that the reduced hydraulic conductance in the soil-plant-atmosphere continuum observed under drought conditions (Gollan *et al*, 1986; Jarvis & McNaughton, 1986; Passioura, 1988) is not due to changes in L_{proot} in Douglas-fir and white spruce.

Although seedlots from dry ecotypes of each species showed less reduction in dry weight production when grown under drought-stressed conditions than in well-watered conditions, relative to wet ecotypes, no ecotype main or interaction effects were found for L_{proot}. This suggests that differences in dry weight production between wet and dry ecotypes are not a result of differences in L_{proot}.

It is difficult to compare these results with those found in the literature since L_{proot} can be normalized by root surface area, root weight, and whole root systems, as well as by root length. Only studies where L_{proot} was normalized by root length were compared with our values of L_{proot}. Coleman *et al.*, (1990) found Douglas-fir seedlings to have a mean L_{proot} of 0.3 g $s^{-1}MPa^{-1}m^{-1}$, ten fold higher than the Douglas-fir seedlings in our study. Other studies found a desert succulent species to have L_{proot} 10-100 fold lower than these conifer seedlings (Nobel & Cui, 1992) and citrus seedlings were found to have L_{proot} less than 10^{-8} g $s^{-1}MPa^{-1}m^{-1}$ (Levy & Syvertsen, 1983). Results from the citrus seedlings are surprising since angiosperms are expected to have higher L_{proot} due to the presence of lower resistance vessels in the xylem. Differences between tracheid

(and vessel) size and number were not accounted for in this experiment nor in the above studies.

Lower L_{proot} was found in the second growing season than in the first growing season for lodgepole pine and white spruce seedlings. The decrease in L_{proot} between years may have been due to changes in the proportion of water-absorbing root surface area with age. Anderson *et al.* (1988) found L_{proot} to decrease with age in ash seedlings. Studies on loblolly pine have shown that white roots take up more than four times as much water as brown (suberized) roots (Kramer & Bullock, 1966; Chung & Kramer, 1975) and Sands *et al.* (1982) found L_{proot} of white roots to be 2.5 times greater than in brown roots.

Pressurization of de-topped root systems, as a method to measure L_{proot}, has come under considerable criticism. Areas of concern mainly deal with the artificial conditions under which hydraulic conductance of roots must be measured, such as removal of the shoot and application of high positive pressures to roots. Lack of a shoot may be detrimental to de-topped root metabolism due to discontinuation in supply of carbon and chemical messages from the shoot to roots (Markhart & Smit, 1990). Use of a positive pressure to push water through roots, rather than the negative pressure found in nature, can cause water to move through roots along unnatural pathways, such as along cell walls of cortical cells (Salim & Pitman, 1984; Koide, 1985; Markhart & Smit, 1990). Precautions to take when using the pressure chamber include keeping water aerated to prevent anoxia, suspending roots into dilute solutions to prevent solute concentration gradients, and collecting data at two or more pressures greater than 0.3 MPa to ensure that L_{proot} is constant (Markhart & Smit, 1990). Based on preliminary experiments, a constant supply of oxygen was available to roots by maintaining aeration of the tap water and pressurizing the chamber with compressed air, and that hydraulic

conductance was constant over the range of applied pressures used to measure flow rate. The constant supply of O_2 to the water may have caused partial pressure of O_2 to be high, and possibly detrimental to the seedlings. Since dilute nutrient solution had no effect on water flow rates through roots, tap water was used. Although some of the above criticisms may be valid, this pressure chamber system is precise and permitted direct measurement of L_{proot}, independent of L_p in the shoot. Measurements of water movement through regions of the SPAC are necessary to determine how whole seedlings control water uptake and loss.

Since Douglas-fir and white spruce roots were able to maintain root hydraulic conductance when grown under low soil moisture conditions, any reduction in conductance for whole seedlings would not be due to increased resistance to water flow through roots. However, lodgepole pine, which is able to maintain high L_{proot} under well-watered conditions, shows increased resistance to water flow in the roots under low soil moisture. This reduction may be due to increased suberization of the roots or the formation of air embolisms in the xylem.

Chapter 6

LOW TEMPERATURE AND PRE-CONDITIONING EFFECTS ON WATER FLOW RATES THROUGH ROOTS OF DOUGLAS-FIR, LODGEPOLE PINE, AND WHITE SPRUCE SEEDLINGS

6.1 INTRODUCTION

Conifer seedlings are often planted in early spring when soil temperature is low. Low temperature can induce drought-stress in seedlings, since water uptake is reduced, and seedlings grown at low temperatures have reduced growth and lower stomatal conductance and transpiration rates (Lopushinsky & Kaufmann, 1984; Teskey *et al.*, 1984).

Hydraulic conductance and flow rates in conifer roots decrease with decreasing temperature (Running & Reid, 1980; Smit-Spinks *et al.*, 1984; Teskey *et al.*, 1984; Grossnickle, 1988). Running & Reid (1980) found roots of lodgepole pine to contribute 67% to 93% of the resistance to water flow in the soil-plant-atmosphere continuum as temperatures decreased from 7^{o} to 0^{o}C.

Pre-conditioning seedlings at low temperatures increases their resistance to chilling stress (Levitt, 1980). Conifer seedlings pre-conditioned at lower temperatures have lower stomatal conductance and transpirational water loss than seedlings pre-conditioned at higher temperatures (Teskey *et al.*, 1984; Grossnickle & Blake, 1985), however, conflicting results have been reported for pre-conditioning effects on water movement through roots. Water flow rates through roots of crop species, pre-conditioned at low temperatures, tend to have the same (Bolger *et al.*, 1992) or higher flow rates (Clarkson, 1976) than plants pre-conditioned at high temperatures, depending on the sensitivity of the species to chilling stress (Markhart *et al.*, 1979). The only study of a conifer species found

that scotch pine seedlings, pre-conditioned at low temperatures, had lower flow rates through roots than seedlings grown at high temperatures (Smit-Spinks *et al.*, 1984).

The objective of this work was to see whether pre-conditioning at a low temperature increased water flow through de-topped roots of three commercially important conifer species, Douglas-fir (*Pseudotsuga menzieseii* (Mirb.) Franco), lodgepole pine (*Pinus contorta* Dougl.), and white spruce (*Picea glauca* (Moench) Voss). A subsample of these seedlings was measured at three pressure chamber temperatures to determine whether pre-conditioning resulted in adaptation to low temperature.

6.2 MATERIAL AND METHODS

6.2.1 Plant Material and Experimental Design

Seeds of Douglas-fir, lodgepole pine and white spruce were collected from trees representing wet and dry ecotypes (Table 9). All seeds, except the white spruce from the dry site ecotype, were stored at -2°C for six weeks, soaked for 24 hours, then stratified at 5°C for 40 days. The white spruce seeds from the dry ecotype were soaked and stratified for only 15 days since they were a substitute for a seedlot which had damped off during germination. After chilling, seeds were sown into 30 cm pots of coarse sand (covered with foil until germination) in a glass house, and later in a polyethylene-covered greenhouse. On May 6, a dilute solution of benlate (4.3 g m^{-3} soil) was applied to all seedlings to prevent fungal attack.

At the cotyledon stage, seedlings were transplanted singly into 3.2 L pots containing 3-6 cm deep coarse gravel and 12-15 cm deep coarse sand, and grown in

Table 9. Location and annual precipitation for seedlots of Douglas-fir, lodgepole pine, and white spruce seeds from wet and dry ecotypes. Seedlings were sown in 1990.

Location of Seed Source (latitude, longitude)	Seedlot(s)	Ecotype	Annual[1] Precip.(mm)
Douglas-fir:			
Tahsis Inlet (50°43',126°37')	201 X[2]	Wet	1157
Head Bay (49°48',126°29')	638	Wet	1503
Goldstream (48°29',123°33')	305 X	Dry	1157
Courtenay (49°41',126°06')	45	Dry	1503
Lodgepole pine:			
Suskwa River (55°17',127°15')	2108	Wet	625
Lily Lake (53°56',124°39')	29092	Dry	464
White spruce:			
Prince Rupert (54°,130°)	33 X 63	Wet	2523
East Kootenays (50°,115°)	31 X 126	Dry	547

[1] Environment Canada. 1989. Temperature and precipitation, 1951-1980, British Columbia. Atmospheric Environment Service. Downsview, Ontario.
[2] Douglas-fir seeds were from crosses of adjacent seedlots in table

a greenhouse. All seedlings were watered twice per week throughout the experiment. Thirteen to eighteen days after transplanting, a fertilizer solution (150 ppm 20-20-20 (NPK)) was applied weekly during one of the watering treatments and 30 mg L^{-1} $FeSO_4 \cdot H_2O$ and 100 mg L^{-1} $CaCl_2 \cdot 2H_2O$ were applied on alternate weeks to supply iron, sulphur, and calcium to the seedlings.

Between May 15 and 17 thirty-six pots from each provenance were randomly chosen and transferred to four growth chambers of 25°/20°C (HT) growth chambers and 16 hour photoperiod at University of Victoria and BC Ministry of Forests Research Laboratory. All growth chambers were set at 75% relative humidity and a quantum flux density of close to 200 μmol m^{-2} s^{-1}, under

fluorescent and incandescent lamps. After 5 weeks, the temperature of one growth chamber at each location was reduced to 15°/10°C (LT). Each temperature was therefore replicated twice and the seedlings grew under these temperatures for 32 weeks.

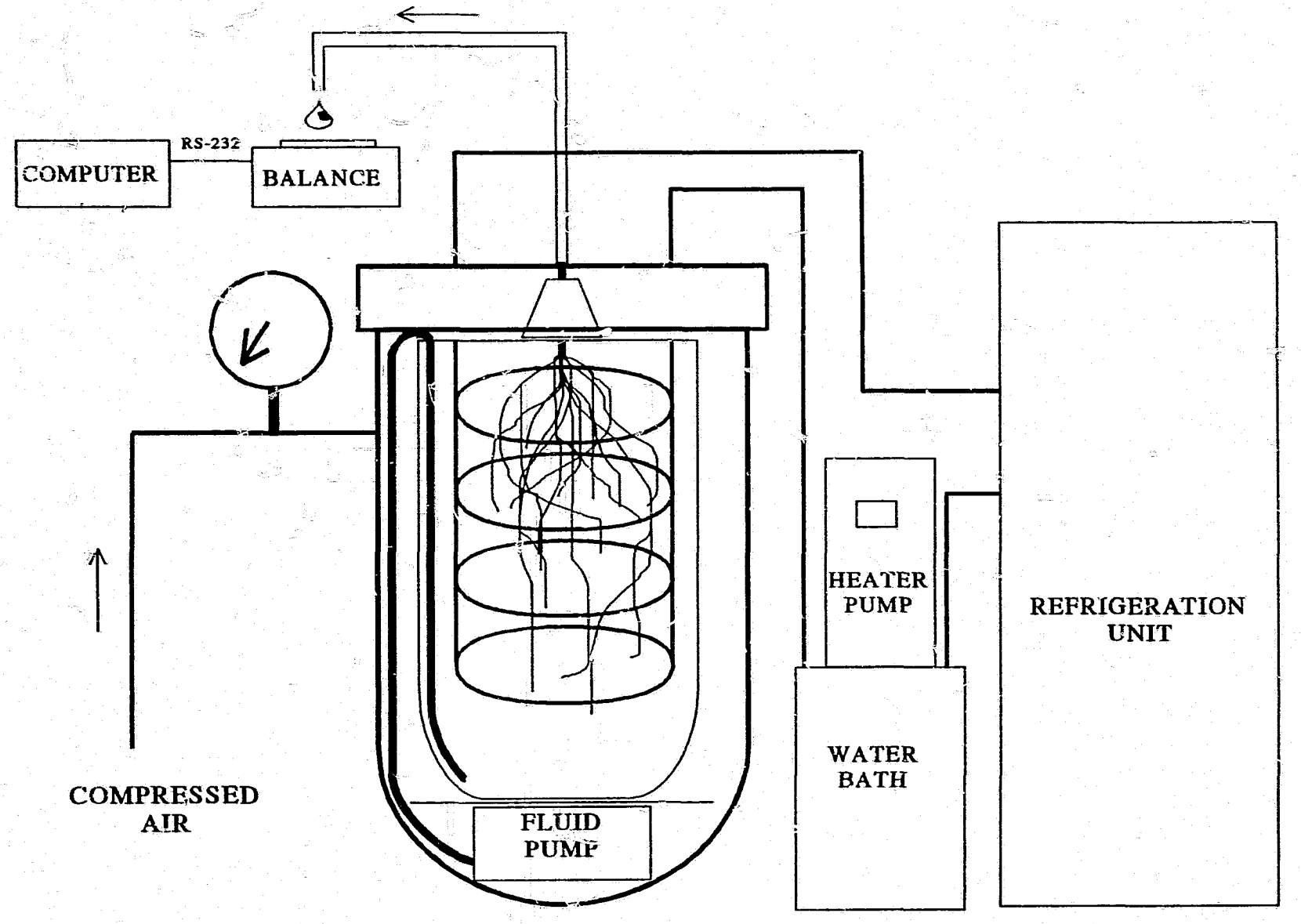
6.2.2 Pressure Chamber Assembly

Water temperature inside a pressure chamber (see section 5.2.2) could be controlled and monitored using a heater pump (Haake, model #001-2826) and a flow-through chiller (PolyScience, model #KR-30A) (Fig. 14). The lid of the steel pressure chamber (see section 5.2.2) was modified in two ways: (1) a nickel-plated copper coil was suspended from the lid into the 5.45 L container so that fluid pumped through the coil controlled water temperature and (2) a small hole was bored into the lid for insertion of a thermocouple to record water temperature in the container.

After washed and detopped roots were inserted into the rubber bung in the lid and the lid was closed, the roots and the coil were suspended in the water-filled root container. The polyethylene glycol:water solution (50:50) circulating through the heater unit, refrigeration unit, and copper coil, controlled the temperature of the water in the container. Water in the root container was circulated using a fluid pump and pressure inside the chamber was regulated with compressed air.

A small-diameter plastic tube was placed onto the root stump to collect exuded sap. The other end of the tube was positioned above a container placed on a balance. As pressure was slowly increased, water was pushed from the water container, through the roots, out of the cut root stump, through the plastic tube, and into the container on the balance. A laptop computer recorded the balance weight every two minutes. Each root system was measured at either 20°C, 12°C,

Figure 14. Pressure chamber assembly used to control pressure chamber temperature and measure water flow rates through roots at three temperatures over a 48 hour period.


COMPUTER
RS-232
BALANCE
HEATER
PUMP
REFRIGERATION
UNIT
WATER
BATH
COMPRESSED
AIR
FLUID
PUMP

and 4°C for 18, 15, and 15 hour intervals respectively or at 20°C for 24 hours. Roots were kept at -5°C until root length was measured (Tennant, 1975). Shoots and roots were placed in an oven at 80°C for 24 hours prior to dry weight measurements.

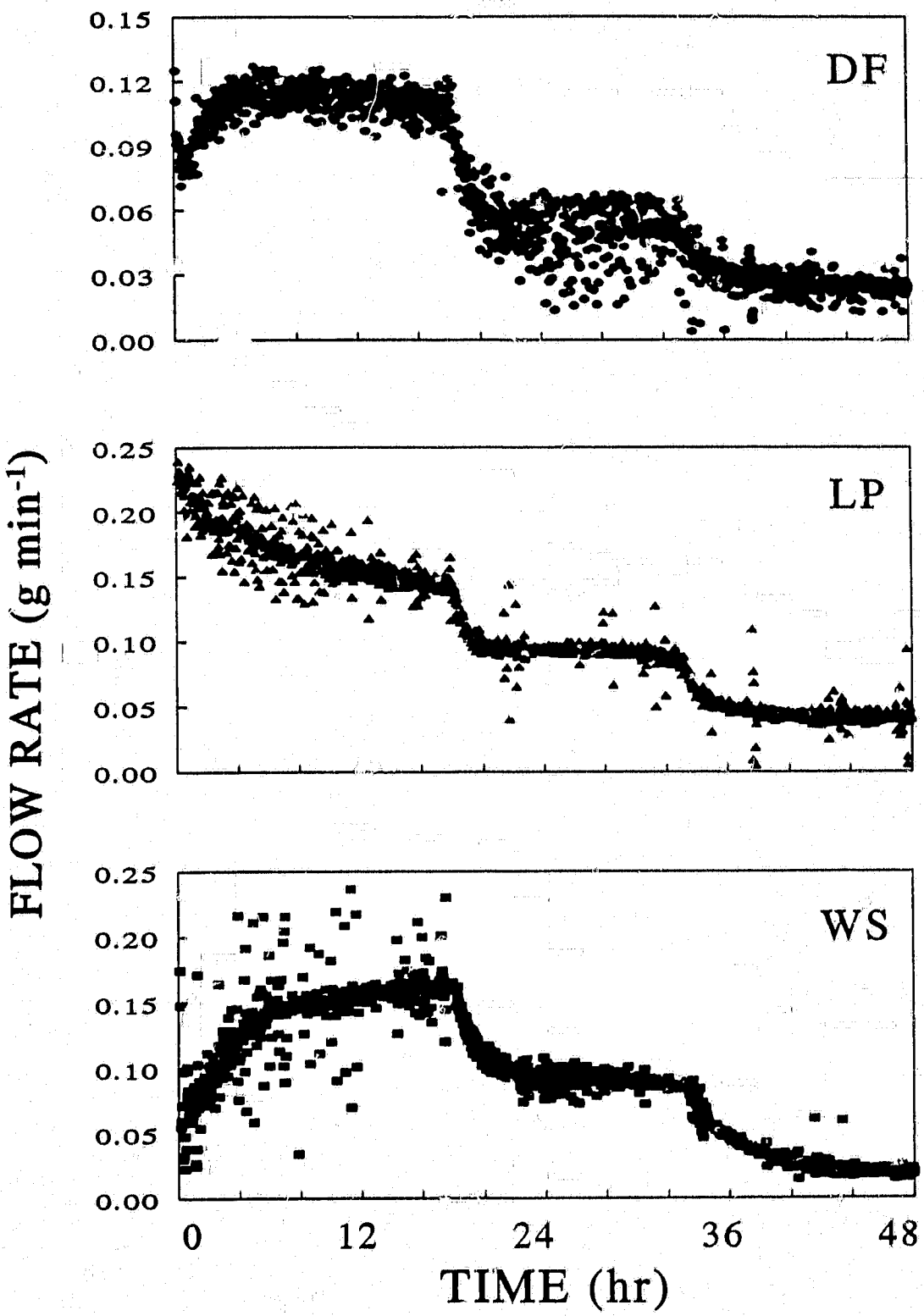
Based on preliminary experiments (see section 5.2.2), roots were immersed in tap water and measurements were taken at 1.0 MPa pressure. Due to long equilibration times (Fig. 15), measurements were taken at only one pressure.

6.2.3 Statistical Analysis

Seedling dry weights were transformed using ln(variable +1), to reduce heteroscedasticity, and analyzed for species, ecotype, and growth chamber temperature main and interaction effects (Appendix, Table 4.1) using a general linear model in SAS (SAS Institute, Cary, NC). Ecotype differences for Douglas-fir could not be determined since only seedlings from dry sites were measured.

Seedling roots from dry ecotypes, measured in the 17th hour at 20°C in the pressure chamber, were analysed for species and growth chamber temperature effects (3 X 2 factorial design) and lodgepole pine and white spruce seedlings were also analysed for ecotype effects. Seedling roots from dry ecotypes, measured in the 17th, 14th, and 14th hour at 20°C, 12°C, and 4°C pressure chamber temperatures respectively, were analysed for pressure chamber temperature effects using a split plot design, with pressure chamber temperature as the split plot. All analyses were run using the general linear model in SAS (SAS Institute, Cary, NC) at University of Victoria. Flow rates were normalized by seedling root length and cross-sectional area of the root stump. Tukey's method was used for multiple comparison tests where required.

Figure 15. Sample flow rates for a Douglas-fir (DF), lodgepole pine (LP), and white spruce (WS) seedling root system at 20°C, 12°C, and 4°C pressure chamber temperatures and 1.0 MPa. The highest flow rates were measured at 20°C and the lowest flow rates were measured at 4°C. Note the long equilibration times at each temperature.


DF
LP
WS
FLOW RATE (g min-1)
TIME (hr)
0.15
0.12
0.09
0.06
0.03
0.00
0.25
0.20
0.15
0.10
0.05
0.00
0
12
24
36
48

In analyses comparing flow rates at three pressure chamber temperatures, flow rates were corrected for increased viscosity of water with decreasing temperature using the equation:

$$J_c = J_m \times \eta_{temp}/\eta_{20} \quad (14)$$

where J_c=corrected flow rate (g s^{-1}), J_m=measured flow rate (g s^{-1}), η_{temp}=viscosity (MPa s) at temperature measured, and η_{20}=viscosity at 20°C pressure chamber temperature.

6.3 RESULTS

Douglas-fir and lodgepole pine seedlings had greater dry weight than white spruce seedlings (Fig. 16A). Investigation of the significant three-way interaction term (Appendix, Table 4.1) indicated that only white spruce seedlings had a significant ecotype by growth chamber temperature effect (Fig. 16B), with seedlings from wet ecotypes producing greater biomass in the LT growth chamber and dry ecotypes producing greater biomass in the HT growth chamber. Analyses of root length and stem cross-sectional area showed significant three-way interactions and species main effects, as was found for seedling dry weight analysis (Appendix, Table 4.2).

Root weight was linearly related to root length for each species for the range of dry root weights measured (Fig. 17). However, extrapolation of the lines for lodgepole pine and Douglas-fir assigns y-intercept values far from the origin, indicating that root length to root weight ratio was not linear when seedlings were small. Slope comparisons of root length to root weight for each species showed that, although white spruce seedlings produced the lowest biomass, they had the highest specific root length (root length per unit root weight) of the three species (Fig. 17).

Figure 16. Root and shoot dry weights for (A) Douglas-fir (DF), lodgepole pine (LP), and white spruce (WS) seedlings and (B) wet and dry ecotypes of white spruce seedlings grown in high and low temperature growth chambers. A: white spruce produced significantly less root and shoot dry weights than the other species ($p<0.05$). B: White spruce seedlings from wet ecotypes produced more dry weight when grown in the low temperature growth chamber and seedlings from dry ecotypes produced more dry weight when grown in the high temperature growth chamber ($p<0.01$). Dry weights were backtransformed from ln(variable+1) and bars represent 95% confidence limits.

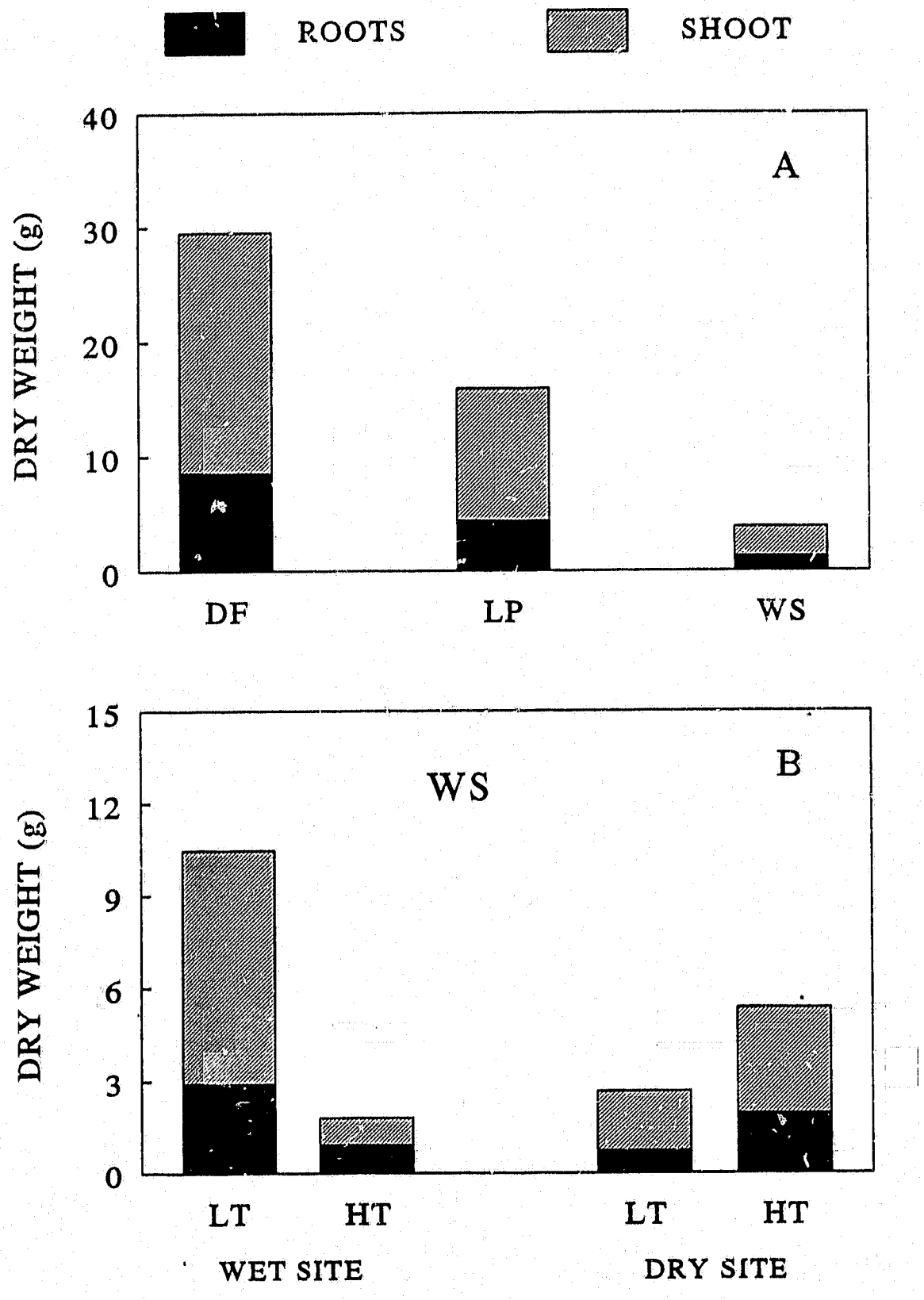
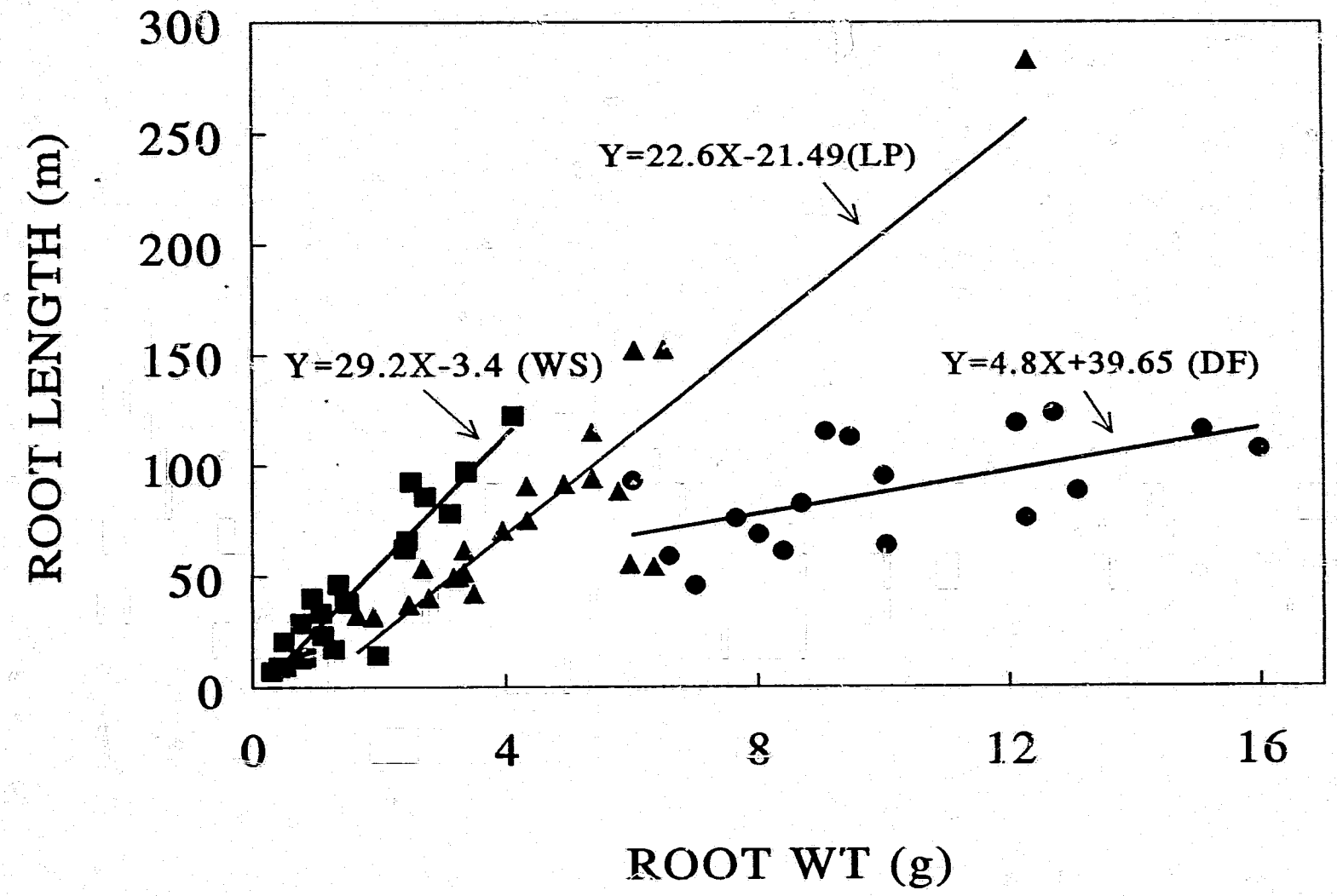

ROOTS
SHOOT
DRY WEIGHT (g)
40
30
20
10
0
A
DF
LP
WS
DRY WEIGHT (g)
15
12
9
6
3
0
WS
B
LT
HT
LT
HT
WET SITE
DRY SITE

Figure 17. Relationship between root length and root weight for all seedlings of each species harvested. Regression slopes between species were significantly different ($p<0.001$) because lodgepole pine and white spruce equations had steeper slopes than the Douglas-fir equation. Best fit curves had $r^2=0.37$, 0.81, 0.86 and $p<0.07$, 0.001, and 0.001 for Douglas-fir (DF), lodgepole pine (LP), and white spruce (WS) seedlings respectively).


300
250
200
150
100
50
0
ROOT LENGTH (m)
Y=22.6X-21.49(LP)
Y=29.2X-3.4 (WS)
Y=4.8X+39.65 (DF)
0
4
8
12
16
ROOT WT (g)

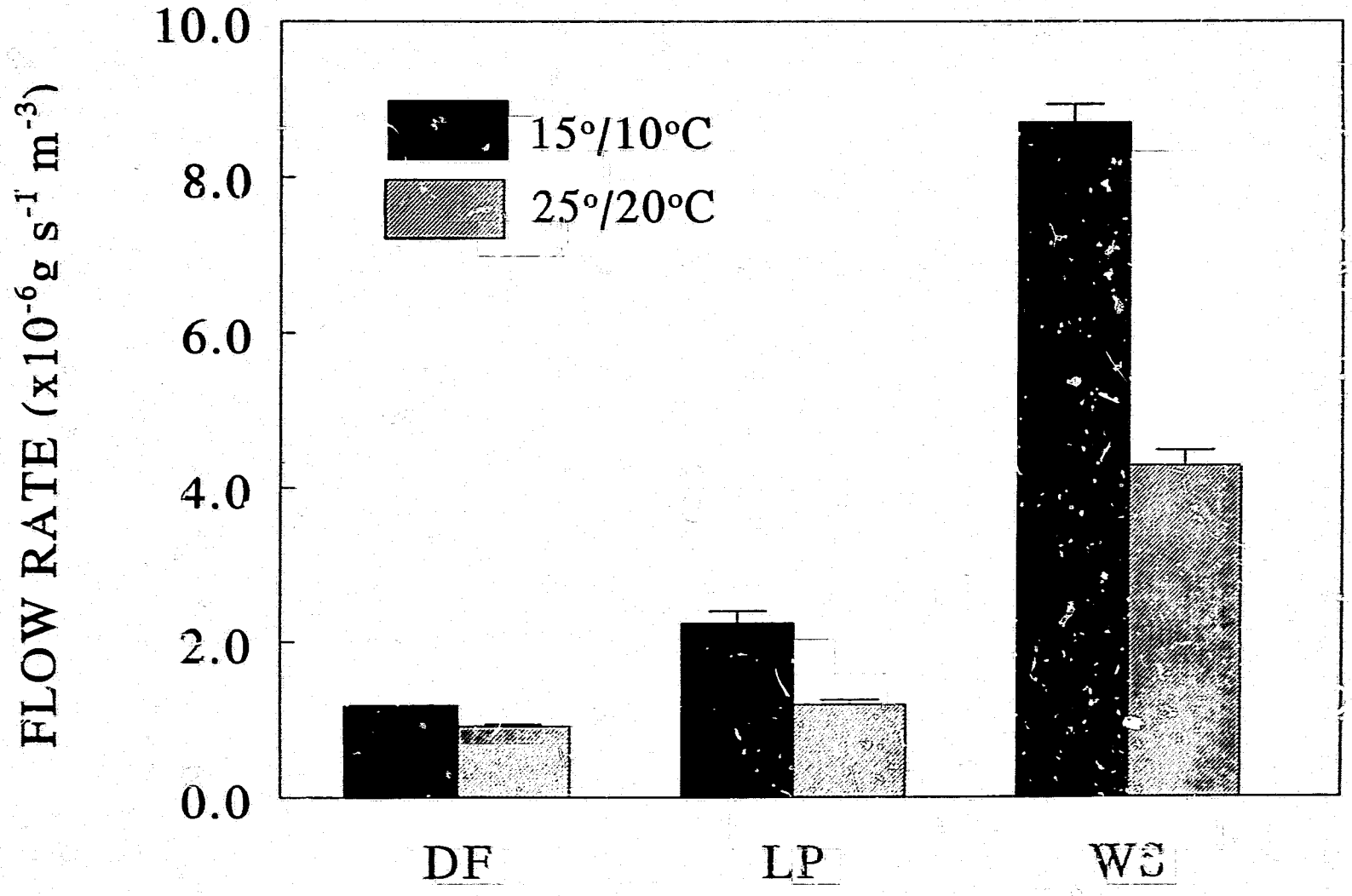
Five Douglas-fir seedlings and ten seedlings of each of the other two species were measured at 20°C in the pressure chamber. Due to the rapid growth of Douglas-fir seedlings in the growth chambers, only two dry ecotype seedlings from the LT growth chamber were measured and no wet ecotype seedlings from either growth chamber were measured. The stem diameters of the Douglas-fir seedlings were too large for the hole in the pressure chamber lid. Consequently, comparisons of species, growth chamber, and pressure chamber temperature were completed on seedlings from dry ecotypes only.

In the 20°C pressure chamber, roots of white spruce seedlings from the dry ecotype had the highest water flow rate, followed by lodgepole pine roots, and Douglas-fir roots had the lowest flow rate (Fig. 18). Investigation into the significant species x growth chamber temperature interaction effect (Appendix, Table 4.3) showed that both lodgepole pine and white spruce seedlings grown in the LT growth chamber had higher flow rates than those grown in the HT growth chamber (Fig. 18).

Lodgepole pine and white spruce were analysed for differences between ecotypes. Although there was no significant difference between ecotypes for water flow rates through roots in lodgepole pine seedlings, white spruce seedlings from the dry ecotype had higher water flow rates when grown in the LT growth chamber and white spruce seedlings from the wet ecotype had higher flow rates when grown in the HT growth chamber (Table 10).

Water flow rates through roots, normalized by root length and stem cross-sectional area decreased with decreasing measurement temperature for seedlings roots of the three species from dry ecotypes (Fig. 19A). When flow rates were corrected for viscosity at the three pressure chamber temperatures, there were significant main effects and two and three-way interaction effects for species,

Figure 18. Mean water flow rates through roots of Douglas-fir (DF), lodgepole pine (LP), and white spruce (WS) seedlings grown in high and low temperature growth chambers. Water flow rates were normalized by root length and stem cross-sectional area. Seedling roots were measured at 20°C in the pressure chamber. Species, growth chamber temperature, and their interaction were significantly different ($p < 0.001$). Tukey's multiple range test indicated that lodgepole pine and white spruce seedlings grown at lower temperatures had higher flow rates. Bars are one standard error.


FLOW RATE (x10^{-6}g s^{-1} m^{-3})
10.0
8.0
6.0
4.0
2.0
0.0
15°/10°C
25°/20°C
DF
LP
WS

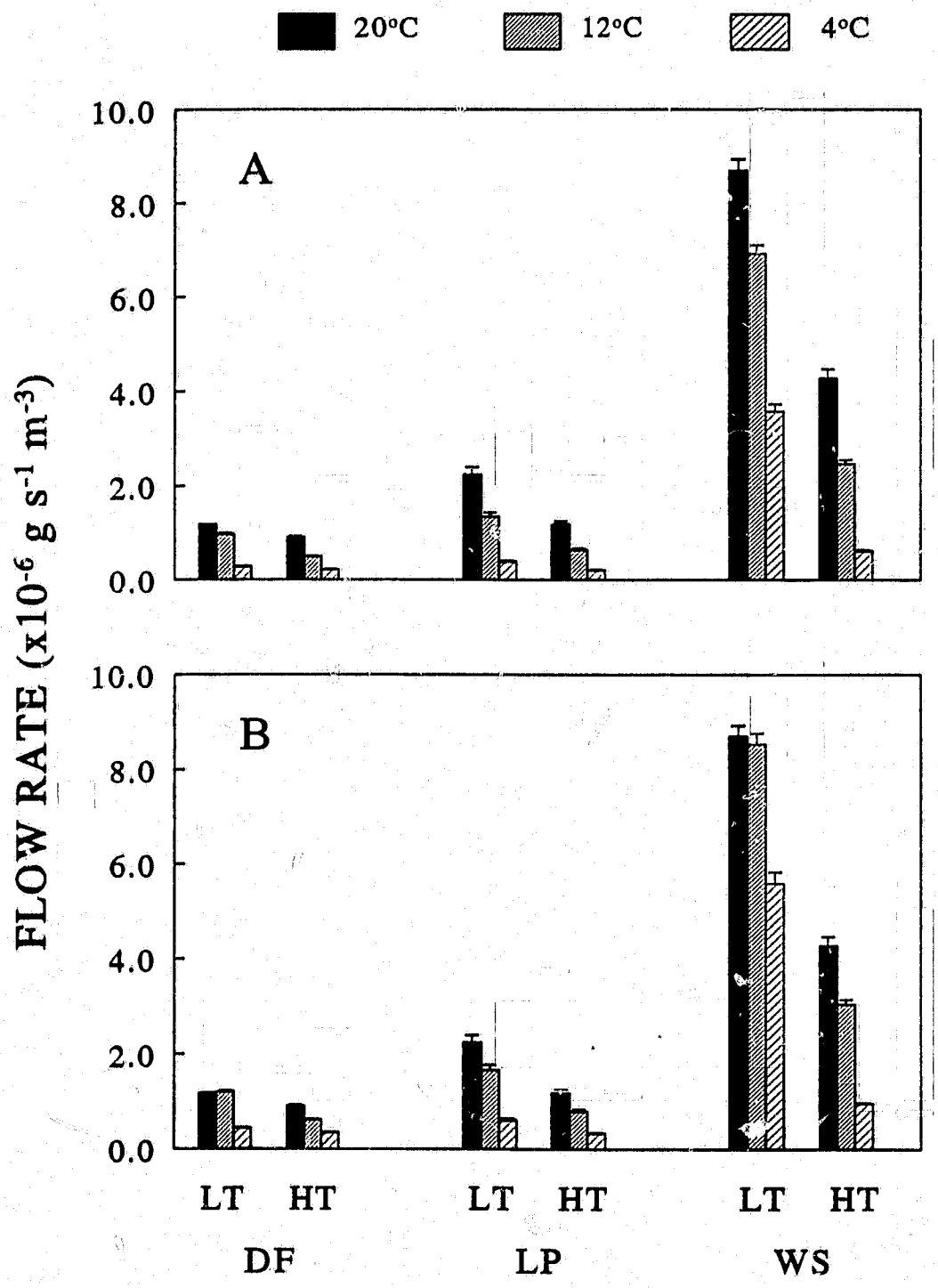
growth chamber temperature, and pressure chamber temperature variables (Appendix, Table 4.4). After correcting for viscosity, water flow rates through roots measured at 20°C and 12°C showed no significant differences in either Douglas-fir or white spruce from LT growth chambers (Fig. 19B). Lodgepole pine seedlings grown in both growth chambers had lower flow rates at 12°C in the pressure chamber than at 20°C, as did Douglas-fir and white spruce seedlings grown in the HT growth chamber. All seedlings had lower flow rates through roots at the 4°C temperature in the pressure chamber than at the 12°C temperature (Fig. 19B).

Table 10. Mean flow rates through seedling roots for wet and dry ecotypes of lodgepole pine (LP) and white spruce (WS) seedlings, grown in high temperature (HT) and low temperature (LT) growth chambers, and measured at 20°C in the pressure chamber. Measurements were taken at 1.0 MPa pressure. Values in parentheses are standard errors.

Species	Ecotype	Growth Chamber	Flow Rate ($\times 10^{-6}$ g s^{-1} m^{-3})
LP	WET	LT	1.86 (0.28)cd[1]
LP	WET	HT	1.46 (0.71)cd
LP	DRY	LT	2.24 (1.50)c
LP	DRY	HT	1.19 (0.60)d
WS	WET	LT	2.46 (0.07)c
WS	WET	HT	9.67 (4.01)a
WS	DRY	LT	8.70 (2.23)a
WS	DRY	HT	4.28 (1.85)b

[1] means followed by the same letter are not significantly different at the 5% level

Figure 19. Mean water flow rates through roots of Douglas-fir (DF), lodgepole pine (LP), and white spruce (WS) seedlings grown in high temperature (HT) and low temperature (LT) growth chambers. A: Flow rates have not been corrected for viscosity differences. B: Flow rates have been corrected for viscosity differences. The three-way interaction effect was significant ($p < 0.001$). Bars are one standard error. LT = $15^{o}/10^{o}$C and HT = $25^{o}/20^{o}$C


20°C
12°C
4°C
A
B
FLOW RATE (x10-6 g s-1 m-3)
10.0
8.0
6.0
4.0
2.0
0.0
LT
HT
DF
LP
WS

6.4 DISCUSSION

In the 20°C pressure chamber, lodgepole pine and white spruce seedlings from dry ecotypes, grown at 15/10°C growth chamber temperatures, had higher water flow rates through roots than seedlings grown at 25/20°C growth chamber temperatures, indicating that, in these species, seedlings pre-conditioned at a low temperature are able to have greater root water uptake under optimal growing conditions than seedlings that are not pre-conditioned. These results agree with pre-conditioning experiments on crops, such as barley and rye (Clarkson, 1976) and soybean (Markhart *et al.*, 1979), although they do not agree with results for scotch pine (Smit-Spinks *et al.*, 1984), where seedlings pre-conditioned at the lower temperature had lower root water flow rates.

No pre-conditioning effect was found for Douglas-fir seedlings. However, Lopushinsky and Kaufmann (1984) found acclimation of water flow through Douglas-fir whole seedlings. The rapid growth and resulting large size of Douglas-fir seedlings may have created conditions which complicated comparisons between seedlings grown in the different temperature growth chambers. The large root biomass for Douglas-fir seedlings may have caused decreased root growth. Since new root length increases white root surface area, which absorbs more water than older suberized roots, seedlings unable to increase new root length are restricted in amount of water uptake. Grossnickle & Russell (1990) found that yellow-cedar seedling hydraulic conductance increased dramatically when new root growth occurred. Another reason Douglas-fir was unable to acclimate at the low temperature is that the large Douglas-fir seedlings in the high temperature growth chamber may have become drought-stressed. Watering was maintained throughout the experiment, however, the high soil water demands by the large

seedlings under the high vapour pressure deficit at the higher temperature may not have been met.

The large differences in seedling size between species suggests that comparisons between species should be interpreted cautiously. Size differences in roots were accounted for by normalizing water flow rates by root length, but the proportion of root length active in water absorption may have been different between species. Since water flow rates through roots is also a function of tracheid number and size (Tyree & Ewers, 1991), flow rates were normalized by stem cross-sectional area. There were obvious differences in stem diameters between white spruce seedlings and seedlings of the two larger species.

The lower water flow rates through roots found at low temperatures in conifers (Running & Reid, 1980; Smit-Spinks *et al.*, 1984), as well as in field crops (Clarkson, 1976) and citrus (Ramos & Kaufmann, 1979), cannot be accounted for by the increased viscosity of water alone. When the temperature falls below a species-specific critical temperature, lipids in cellular membranes within the roots undergo transition from a liquid-crystalline form to a gel(solid)-crystalline form (Lyons, 1973; McWilliam, 1983). Lipid transition can effect flow rates in various ways. When lipids solidify, they contract, reducing permeability across the membrane. If temperature decreases quickly, cracks can form in the membranes and permeability across the membranes may increase. There are numerous secondary responses associated with chilling stress resulting in physiological and biochemical alterations in plants (Lyons, 1973; Wang, 1982; Markhart, 1986).

If lipid transition is responsible for the reduced flow rate in roots, it appears that in this study Douglas-fir and white spruce, grown in the low temperature growth chamber, reach their critical temperatures at temperatures less than 12^{o}C, since viscosity can account for the reduced flow rate between 20^{o}C and 12^{o}C

pressure chamber temperatures. However, the critical temperature for lipid transition in lodgepole pine is higher than 12°C, since after correcting for viscosity, a significant decrease in flow rate occurred when the temperature in the pressure chamber decreased from 20°C to 12°C. These results do not agree with Running and Reid (1980) since they found lodgepole pine seedlings, grown in 19°C greenhouse and 2°C cold room to have a critical temperature of 7°C for lipid transition. It is surprising that they found no effect from pre-conditioning at their low temperature.

Other studies have found critical temperatures of 10°C to 14.7°C for agricultural crops (Markhart *et al.*, 1979; Clarkson, 1976) with 5°C to 7°C reductions in critical temperatures when pre-conditioned at lower temperatures. Ramos and Kaufmann (1979) found that rough lemon seedlings have no critical temperature for lipid transition, however, the increased root resistance with decreasing temperature could not be explained by viscosity and they suggest membrane lipid transition was responsible for the increase in root resistance they found.

It appears that water flow rates in Douglas-fir and white spruce roots, when seedlings are pre-conditioned at lower temperatures, are more insensitive to chilling stress. However, water flow rates in lodgepole pine roots remain sensitive to chilling even when pre-conditioned at low temperatures.

Chapter 7
SUMMARY

This work investigated drought resistance in conifer seedlings and sought to discover the important drought resistance mechanisms by studying root growth, osmotic adjustment and root conductance. Water flow through Douglas-fir, lodgepole pine, and white spruce seedling root systems was measured to ascertain the effect of drought and chilling temperatures on water uptake and movement through roots.

Douglas-fir and lodgepole pine seedlings exhibited both drought avoidance and drought tolerance mechanisms to resist drought. Lodgepole pine avoided drought by producing greater root lengths than Douglas-fir. Greater root length increased surface area for water uptake and permited deeper penetration into the soil for more effective water resource exploitation. Douglas-fir avoided drought by maintaining lower stomatal conductance and higher water use efficiency than lodgepole pine, indicating higher biomass production from the water used. Both species osmotically adjusted when drought stressed, although Douglas-fir was the more drought tolerant species, having lower osmotic potentials than lodgepole pine under drought and well-watered conditions.

Dry matter production for the two species suggests that high root length production by lodgepole pine, despite the associated low WUE, is more effective in resisting the detrimental effects of drought than the avoidance and tolerance mechanisms used by Douglas-fir. Douglas-fir appears to be a conservative species whereas lodgepole pine is a drought avoider by exploiting the soil.

This is the first study of the effect of low soil moisture on root hydraulic conductance in conifers. Hydraulic conductance in lodgepole pine roots, grown

under well-watered conditions was found to be significantly higher than in roots of Douglas-fir and white spruce seedlings. The high rates of root conductance in lodgepole pine may have explained the ability of this species to maintain higher stomatal conductance and ψ than Douglas-fir in the WUE experiment. By contrast, under drought conditions, only lodgepole pine seedlings had reduced root hydraulic conductance. Seedlings of all three species had similar root hydraulic conductance when drought-stressed, suggesting that there may be a minimum root hydraulic conductance for seedling survival.

White spruce seedlings had higher water flow rates through roots than the other two species. This may have been due to the species' high specific root length. All seedlings had reduced water flow rates through roots when measured at decreasing temperatures of 20°C, 12°C, and 4°C. The lower critical temperatures (at which cellular lipids solidify) found for Douglas-fir and white spruce seedlings pre-conditioned at a low temperature, relative to seedlings grown at a high temperature, suggests that these species can adapt to low temperature conditions and become more resistant to chilling stress. Lodgepole pine seedlings had critical temperatures above 12°C, independent of the pre-conditioning temperature. This was surprising since Douglas-fir grows in a more temperate climate than lodgepole pine and white spruce.

Some of the questions studied in this work might be clarified by investigation of root embolism and seasonal changes in root suberization.

LITERATURE CITED

ABRAMS, M.D. 1988. Sources of variation in osmotic potentials with special reference to North American tree species. For. Sci. **34**: 1030-1046.

ANDERSEN, C.P., MARKHART III, A.H., DIXON, R.K., and SUCOFF, E.I. 1988. Root hydraulic conductivity of vesicular-arbuscular mycorrhizal green ash seedlings. New Phytol. **109**: 465-471.

BASSMAN, J.H., and ZWIER, J.C. 1991. Gas exchange characteristics of *Populus trichocarpa*, *Populus deltoides* and *Populus trichocarpa* x. *deltoides* clones. Tree Physiol. **8**: 145-159.

BLACK, C.R. 1979. A quantitative study of the resistances to transpirational water movement in sunflower (*Helianthus annuus* L.). J. Exp. Bot. **30**: 947-955.

BLAKE, T.J., BEVILACQUA, E., and ZWIAZEK, J.J. 1991. Effects of repeated stress on turgor pressure and cell elasticity change in black spruce seedlings. Can. J. For. Res. **21**: 1329-1333.

BLIZZARD, W.E., and BOYER, J.S. 1980. Comparative resistance in the soil and the plant to water transport. Plant Physiol. **66**: 809-814.

BOLGER, T.P., UPCHURCH, D.R., and MCMICHAEL, B.L. 1992. Temperature effects on cotton root hydraulic conductance. Environmental and Experimental Botany. **32**: 49-54.

BRADBURY, I.K., and MALCOLM, D.C. 1977. The effect of phosphorus and potassium on transpiration, leaf diffusive resistance and water-use efficiency in Sitka spruce (*Picea sitchensis*) seedlings. J. Appl. Ecol. **14**: 631-641.

BRISSETTE, J.C., and CHAMBERS, J.L. 1992. Leaf water status and root system water flux of shortleaf pine (*Pinus echinata* Mill.) seedlings in relation to new root growth after transplanting. Tree Physiol. **11**: 289-303.

BUXTON, G.F., CYR, D.R., DUMBROFF, E.B., and WEBB, D.P. 1985. Physiological responses of three northern conifers to rapid and slow induction of moisture stress. Can. J. Bot. **63**: 1171-1176.

CHUNG, H.-H., and KRAMER, P.J. 1975. Absorption of water and 32P through suberized and unsuberized roots of loblolly pine. Can. J. For. Res. **5**: 229-235.

CLARKSON, D.T. 1976. The influence of temperature on the exudation of xylem sap from detached root systems of rye (*Secale cereale*). Planta (Berl.). **132**: 297-304.

CLAYTON-GREENE, K.A. 1983. The tissue water relationships of *Callitris columellaris*, *Eucalyptus melliodora* and *Eucalyptus microcarpa* investigated using the pressure-volume technique. Oecologia. **57**: 368-373.

COLEMAN, M.D., BLEDSOE, C.S., and SMIT, B.A. 1990. Root hydraulic conductivity and xylem sap levels of zeatin riboside and abscisic acid in ectomycorrhizal Douglas fir seedlings. New Phytol. **115**: 275-284.

COLOMBO, S.J. 1987. Changes in osmotic potential, cell elasticity, and turgor relationships of 2nd-year black spruce container seedlings. Can. J. For. Res. **17**: 365-369.

COLOMBO, S.J., and ASSELSTINE, M.F. 1989. Root hydraulic conductivity and root growth capacity of black spruce (*Picea mariana*) seedlings. Tree Physiol. **5**: 73-81.

CRUZ, R.T., JORDAN, W.R., and DREW, M.C. 1992. Structural changes and associated reduction of hydraulic conductance in roots of *Sorghum bicolor* L. following exposure to water deficit. Plant Physiol. **99**: 203-212.

DALTON, F.N., RAATS, P.A.C., and GARDNER, W.R. 1975. Simultaneous uptake of water and solutes by plant roots. Agronomy Journal. **67**: 334-339.

DAVIES, W.J., and ZHANG, J. 1991. Root signals and the regulation of growth and development of plants in drying soil. Ann. Rev. Plant Physiol. Plant Mol. Biol. **42**: 55-76.

DAVIES, W.J., METCALFE, J., LODGE, T.A., and DA COSTA, A.R. 1986. Plant growth substances and the regulation of growth under drought. Aust. J. Plant Physiol. **13**: 105-125.

DIXON, H.H. 1914. Transpiration and the ascent of aap in plants. MacMillan & Co., Ltd., London, England.

ESAU, K. 1977. Anatomy of seed plants. John Wiley & Sons, New York.

EVERARD, J.D., and DREW, M.C. 1989. Mechanisms controlling changes in water movement through the roots of *Helianthus annuus* L. During continuous exposure to oxygen deficiency. J. Exp. Bot. **40**: 95-104.

EWERS, F.W., and CRUZIAT, P. 1991. Measuring water transport and storage. *In* Lassoie, J.P., and Hinckley, T.M. (*Editors*) Techniques and methodologies in tree ecophysiology. CRC Press, New York.

FAIZ, S.M.A., and WEATHERLEY, P.E. 1977. The location of the resistance to water movement in the soil supplying the roots of transpiring plants. New Phytol. **78**: 337-347.

---. 1978. Further investigations into the location and magnitude of the hydraulic resistances in the soil:plant system. New Phytol. **81**: 19-28.

---. 1982. Root contraction in transpiring plants. New Phytol. **92**: 333-343.

FERRELL, W.K., and WOODARD, E.S. 1966. Effects of seed origin on drought resistance of Douglas-fir (*Pseudotsuga menziesii*) (Mirb.) Franco. Ecology. **47**: 499-503.

FISCHER, R.A., and TURNER, N.C. 1978. Plant productivity in the arid zones. Ann. Rev. Plant Physiol. **29**: 277-317.

FISCUS, E.L. 1975. The interaction between osmotic- and pressure-induced water flow in plant roots. Plant Physiol. **55**: 917-922.

---. 1977. Determination of hydraulic and osmotic properties of soybean root systems. Plant Physiol. **59**: 1013-1020.

FREUND, R.J., LITTELL, R.C., and SPECTOR, P.C. 1986. SAS system for linear models. Sas Institute Inc., Cary, NC.

GOLLAN, T., PASSIOURA, J.B., and MUNNS, R. 1986. Soil water status affects the stomatal conductance of fully turgid wheat and sunflower leaves. Aust. J. Plant Physiol. **13**: 459-464.

GRIEU, P., GUEHL, J.M., and AUSSENAC, G. 1988. The effects of soil and atmospheric drought on photosynthesis and stomatal control of gas exchange in three coniferous species. Physiol. Plant. **73**: 97-104.

GROSSNICKLE, S.C. 1988. Planting stress in newly planted jack pine and white spruce. 1. Factors influencing water uptake. Tree Physiol. **4**: 71-83.

---. 1989. Shoot phenology and water relations of *Picea glauca*. Can. J. For. Res. **19**: 1287-1290.

GROSSNICKLE, S.C., and BLAKE, T.J. 1985. Acclimation of cold-stored jack pine and white spruce seedlings: Effect of soil temperature on water relation patterns. Can. J. For. Res. **15**: 544-550.

GROSSNICKLE, S.C., and RUSSELL, J.H. 1990. Water movement in yellow-cedar seedlings and rooted cuttings: comparison of whole plant and root system pressurization methods. Tree Physiol. **6**: 57-68.

---. 1991. Gas exchange processes of yellow-cedar (*Chamaecyparis nootkatensis*) in response to environmental variables. Can. J. Bot. **69**: 2684-2691.

HINCKLEY, T.M., RICHTER, H., and SCHULTE, P.J. 1991. Water relations. *In* Raghavendra, A.S. (*Editor*) Physiology of trees. John Wiley & Sons, Inc.

HSIAO, T.C., ACEVEDO, E., FERERES, E., and HENDERSON, D. 1976. Stress metabolism. Water stress, growth, and osmotic adjustment. Phil. Trans. R. Soc. Lond. B. **273**: 479-500.

HUANG, B., and NOBEL, P.S. 1992. Hydraulic conductivity and anatomy for lateral roots of *Agave deserti* during root growth and drought-induced abscission. J. Exp. Bot. **43**: 1441-1449.

JARVIS, N.J., and MULLINS, C.E. 1987. Modelling the effects of drought on Sitka spruce in Scotland. Forestry. **60**: 13-30.

JARVIS, P.G. 1986. Coupling of carbon and water interactions in forest stands. Tree Physiol. **2**: 347-368.

JOHNSON-FLANAGAN, A.M. 1984. Growth and development of styroplug white spruce (*Picea glauca* (Moench) Voss) seedling roots. Ph.D. Thesis. University of Victoria.

JOLY, R.J., and ZAERR, J.B. 1987. Alteration of cell-wall water content and elasticity in Doulgas-Fir during periods of water deficit. Plant Physiol. **83**: 418-422.

JOLY, R.J., ADAMS, W.T., and STAFFORD, S.G. 1989. Phenological and morphological responses of mesic and dry site sources of coastal Douglas-fir to water deficit. For. Sci. **35**: 987-1005.

JONES, H.G. (*Editor*). 1992. Plants and microclimate, 2nd ed. Cambridge University Press, Cambridge.

KAISER, W.M. 1987. Effects of water deficit on photosynthetic capacity. Physiol. Plant. **71**: 142-149.

KANDIKO, R.A., TIMMIS, R., and 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.

KAUFMANN, M.R. 1968. Water relations of pine seedlings in relation to root and shoot growth. Plant Physiol. **43**: 281-288.

KAUSHAL, P., and AUSSENAC, G. 1989. Transplanting shock in Corsican pine and cedar of Atlas seedlings: internal water deficits, growth and root regeneration. For. Ecol. Manage. **27**: 29-40.

KOIDE, R. 1985. The nature and location of variable hydraulic resistance in *Helianthus annus* L. (Sunflower). J. Exp. Bot. **36**: 1430-1440.

KOZLOWSKI, T.T., KRAMER, P.J., and PALLARDY, S.G. 1991. The physiological ecology of woody plants. Academic Press, San Diego, California.

KRAMER, P.J. 1942. Species differences with respect to water absorption at low soil temperatures. Amer. J. Bot. **29**: 828-832.

KRAMER, P.J., and BULLOCK, H.C. 1966. Seasonal variations in the proportions of suberized and unsuberized roots of trees in relation to the absorption of water. Amer. J. Bot. **53**: 200-204.

KUBISKE, M.E., and ABRAMS, M.D. 1991. Seasonal, diurnal and rehydration-induced variation of pressure-volume relationships in *Pseudotsuga menziesii*. Physiol. Plant. **83**: 107-116.

LANDSBERG, J.J. 1986. Physiological ecology of forest production. Academic Press, London, England.

LEDIG, F.T., and BOTKIN, D.B. 1974. Photosynthetic CO2-uptake and the distribution of photosynthate as related to growth of larch and sycamore progenies. Silvae Genetica. **23**: 188-192.

LEVITT, J. 1980. Responses of plants to environmental stresses. Chilling, freezing, and high temperature stresses. vol.1. Academic Press, New York.

LEVY, Y., and SYVERTSEN, J.P. 1983. Effect of drought stress and vesicular-arubscular mycorrhiza on citrus transpiration and hydraulic conductivity of roots. New Phytol. **93**: 61-66.

LIVINGSTON, N.J., and BLACK, T.A. 1987. Water stress and survival of three species of conifer seedlings planted on a high elevation south-facing clear-cut. Can. J. For. Res. **17**: 1115-1123.

---. 1988. The growth and water use of three species of conifer seedlings planted on a high-elevation south-facing clearcut. Can. J. For. Res. **18**: 1234-1242.

LIVINGSTON, N.J., and DE JONG, E. 1991. The use of unsaturated salt solutions to generate conifer needle water-release curves. Can. J. For. Res. **21**: 53-57.

LIVINGSTON, N.J., VON ADERKAS, P., FUCHS, E.E., and REANEY, M.J.T. 1992. Water relation parameters of embryogenic cultures and seedlings of larch. Plant Physiol. **100**: 1304-1309.

LOPUSHINSKY, W., and KLOCK, G.O. 1974. Transpiration of conifer seedlings in relation to soil water potential. For. Sci. **20**: 181-186.

LOPUSHINSKY, W., and KAUFMANN, M.R. 1984. Effects of cold soil on water relations and spring growth of Douglas-fir seedlings. For. Sci. **30**: 628-634.

LYONS, J.M. 1973. Chilling injury in plants. Ann. Rev. Plant Physiol. **24**: 445-466.

MARKHART, A.H. 1986. Chilling injury: A review of possible causes. HortSci. **21**: 1329-1333.

MARKHART III, A.H., and SMIT, B. 1990. Measurement of root hydraulic conductance. HortSci. **25**: 282-287.

MARKHART III, A.H., FISCUS, E.L., NAYLOR, A.W., and KRAMER, P.J. 1979. Effect of temperature on water and ion transport in soybean and broccoli systems. Plant Physiol. **64**: 83-87.

MATTHEWS, R.G. 1983. Seedling production for crown lands in British Columbia: guidelines for commercial container nurseries. British Columbia Ministry of Forests, Silviculture Branch, Victoria, B.C., 45 p.

MCWILLIAM, J.R. 1983. Physiological basis for chilling stress and the consequences for crop production. *In* Raper, C.D.J., and Kramer, P.J. (*Editors*) Crop reactions to water and temperature stresses in humid, temperate climates. Westview Press, Boulder, Colorado.

MEES, G.C., and WEATHERLEY, P.E. 1957. The mechanism of water absorption by roots. I. Preliminary studies on the effects of hydrostatic pressure gradients. Proc. Royal Soc. Lond. Series B. **147**: 367-380.

MORESHET, S., COHEN, Y., GREEN, G.C., and FUCHS, M. 1990a. The partitioning of hydraulic conductances within mature orange trees. J. Exp. Bot. **41**: 833-839.

MORESHET, S., HUCK, M.G., HESKETH, J.D., and PETERS, D.B. 1990b. Relationships between sap flow and hydraulic conductivity in soybean plants. Agronomie. **10**: 381-389.

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

MUNNS, R. 1988. Why measure osmotic adjustment? Aust. J. Plant Physiol. **15**: 717-726.

MUNNS, R., and PASSIOURA, J.B. 1984. Hydraulic resistance of plants. III. Effects of NaCl in barley and lupin. Aust. J. Plant Physiol. **11**: 351-359.

NAMBIAR, E.K.S., BOWEN, G.D., and SANDS, R. 1979. Root regeneration and plant water status of *Pinus radiata* D. Don. seedlings transplanted to different soil temperatures. J. Exp. Bot. **30**: 1119-1131.

NEWMAN, E.I. 1976. Interaction between osmotic- and pressure-induced water flow in plant roots. Plant Physiol. **57**: 738-739.

NI, B.-R., and PALLARDY, S.G. 1991. Response of gas exchange to water stress in seedlings of woody angiosperms. Tree Physiol. **8**: 1-9.

NOBEL, P.S. 1991. Physicochemical and environmental plant physiology. Academic Press, San Diego.

NOBEL, P.S., and CUI, M. 1992. Hydraulic conductances of the soil, the root-soil air gap, and the root: changes for desert succulents in drying soil. J. Exp. Bot. **43**: 319-326.

PALLARDY, S.G., PEREIRA, J.S., and PARKER, W.C. 1991. The state of water in tree systems. *In* Lassoie, J.P., and Hinckley, T.M. (*Editors*) Techniques and methodologies in tree ecophysiology. CRC Press, New York.

PARKER, W.C., and 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**: 535-549.

---. 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.

PASSIOURA, J.B. 1980. The transport of water from soil to shoot in wheat seedlings. J. Exp. Bot. **31**: 333-345.

---. 1982. Water in the soil-plant-atmosphere continuum. *In* Lange, O.L., Nobel, P.S., Osmond, C.B., and Zeigler, H. (*Editors*) Physiological plant ecology. II. water relations and carbon assimilation, Encyclopedia of Plant Physiology ed., vol. 12B. Springer, New York.

---. 1984. Hydraulic resistance of plants. I. Constant or variable? Aust. J. Plant Physiol. **11**: 333-339.

---. 1988. Water transport in and to roots. Ann. Rev. Plant Physiol. Plant Mol. Biol. **39**: 245-265.

PASSIOURA, J.B., and MUNNS, R. 1984. Hydraulic resistance of plants. II. Effects of rooting medium, and time of day, in barley and lupin. Aust. J. Plant Physiol. **11**: 341-350.

PHARIS, R.P., and FERRELL, W.K. 1966. Differences in drought resistance between coastal and inland sources of Douglas fir. Can. J. Bot. **44**: 1651-1659.

RAMOS, C., and KAUFMANN, M.R. 1979. Hydraulic resistance of rough lemon roots. Physiol. Plant. **45**: 311-314.

RICHTER, H. 1978. A diagram for the description of water relations in plant cells and organs. J. Exp. Bot. **29**: 1197-1203.

RITCHIE, G.A., and RODEN, J.R. 1985. Comparison between two methods of generating pressure-volume curves. Plant, Cell and Environment. **8**: 49-53.

RITCHIE, G.A., and SHULA, R.G. 1984. Seasonal changes of tissue-water relations in shoots and root systems of Douglas-fir seedlings. For. Sci. **30**: 538-548.

ROBERTS, D.R., and DUMBROFF, E.B. 1986. Relationships among drought resistance, transpiration rates, and abscisic acid levels in three northern conifers. Tree Physiol. **1**: 161-167.

RUNNING, S.W. 1980. Field estimates of root and xylem resistances in *Pinus contorta* using root excision. J. Exp. Bot. **31**: 555-569.

RUNNING, S.W., and REID, C.P. 1980. Soil temperature influences on root resistance of *Pinus contorta* seedlings. Plant Physiol. **65**: 635-640.

SALIENDRA, N.Z., and MEINZER, F.C. 1992. Genotypic, developmental and drought-induced differences in root hydraulic conductance of contrasting sugarcane cultivars. J. Exp. Bot. **43**: 1209-1217.

SALIM, M., and FITMAN, M.G. 1984. Pressure-induced water and solute flow through plant roots. J. Exp. Bot. **35**: 869-881.

SANDFORD, A.P., and JARVIS, P.G. 1986. Stomatal responses to humidity in selected conifers. Tree Physiol. **2**: 89-105.

SANDS, R., FISCUS, E.L., and REID, C.P.P. 1982. Hydraulic properties of pine and bean roots with varying degrees of suberization, vascular differentiation and mycorrhizal infection. Aust. J. Plant Physiol. **9**: 559-569.

SCHOLANDER, P.F., HAMMEL, H.T., HEMMINGSEN, E.A., and BRADSTREET, E.D. 1964. Hydrostatic pressure and osmotic potential in leaves of mangroves and some other plants. Procedures of the National Academy of Science. **52**: 119-125.

SCHOLANDER, P.F., HAMMEL, H.T., BRADSTREET, E.D., and HEMMINGSEN, E.A. 1965. Sap pressure in vascular plants. Science. **148**: 339-346.

SCHULTE, P.G., and HINCKLEY, T.M. 1985. A comparison of pressure-volume curve data analysis techniques. J. Exp. Bot. **30**: 1590-1602.

SCHULZE, E.-D. 1986. Carbon dioxide and water vapor exchange in response to drought in the atmosphere and in the soil. Ann. Rev. Plant Physiol. **37**: 247-274.

SCHURR, U., GOLLAN, T., and SCHULZE, E.-D. 1992. Stomatal response to drying soil in relation to changes in the xylem sap composition of *Helianthus annuus*. II. Stomatal sensitivity to abscisic acid imported from the xylem sap. Plant, Cell and Environment. **15**: 561-567.

SEILER, J.R., and JOHNSON, J.D. 1988. Physiological and morphological responses of three half-sib families of loblolly pine to water-stress conditioning. For. Sci. **34**: 487-495.

SHERIFF, D.W., NAMBIAR, E.K.S., and FIFE, D.N. 1986. Relationships between nutrient status, carbon assimilation and water use efficiency in *Pinus radiata* (D. Don) needles. Tree Physiol. **2**: 73-88.

SINCLAIR, R., and VENABLES, W.N. 1983. An alternative method of analysing pressure-volume curves produced with the pressure chamber. Plant, Cell and Environment. **6**: 211-217.

SLATYER, R.O. 1967. Plant-water relationships. Academic Press, London, England.

SMIT, B., and STACHOWIAK, M. 1988. Effects of hypoxia and elevated carbon dioxide concentration on ater flux through *Populus* roots. Tree Physiol. **4**: 153-165.

SMIT-SPINKS, B., SWANSON, B.T., and MARKHART III, A.H. 1984. Changes in water relations, water flux, and root exudate abscisic acid content with cold acclimation of *Pinus sylvestris* L. Aust. J. Plant Physiol. **11**: 431-441.

SOKAL, R.R., and ROHLF, F.J. 1981. Biometry, 2nd ed. W.H. Freeman and Co., San Francisco, California.

SPERRY, J.S., DONNELLY, J.R., and TYREE, M.T. 1988. A method for measuring hydraulic conductivity and embolism in xylem. Plant, Cell and Environment. **11**: 35-40.

SPITTLEHOUSE, D.L. 1985. Determination of the year-to-year variation in growing season water use in a Douglas-fir stand. *In* Hutchinson, B.A., and Hicks, B.B. (*Editors*) The forest-atmosphere interaction. D. Reidel Publishing Co., Dordrecht, Netherlands.

SQUIRE, R.O., NEALES, T.F., LOVEYS, B.R., and ATTIWILL, P.M. 1988. The influence of water deficits on needle conductance, assimilation rate and abscisic acid concentraiton of seedlings of *Pinus radiata* D. Don. Plant, Cell and Environment. **11**: 13-19.

STEUDLE, E., and JESCHKE, W.D. 1983. Water transport in barley roots. Measurements of root pressure and hydraulic conductivity of root in parallel with turgor and hydraulic conductivity of root cells. Planta (Berl.). **158**: 237-248.

STRICKLAND, J.D.H. and PARSONS, T.R. 1972. A practical handbook of seawater analysis. Fisheries Research Board of Canada. Bulletin 67.

SWIETLIK, D. 1989. Pressure-induced water fluxes through root systems of sour orange seedlings. J. Amer. Soc. Hort. Sci. **114**: 139-143.

TAIZ, L. and ZEIGER, E. 1991. Plant physiology. Benjamin/Cummings Co., Redwood City.

TALBOT, A.J.B., TYREE, M.T., and DAINTY, J. 1975. Some notes concerning the measurement of water potentials of leaf tissue with specific reference to *Tsuga canadensis* and *Picea abies*. Can. J. Bot. **53**: 784-788.

TENNANT, D. 1975. A test of a modified line intersect method of estimating root length. J. Ecol. **63**: 995-1001.

TESKEY, R.O., HINCKLEY, T.M., and GRIER, C.C. 1984. Temperature-induced change in the water relations of *Abies amabilis* (Dougl.) Forbes. Plant Physiol. **74**: 77-80.

TURNER, N.C. 1986. Adaptation to water deficits: a changing perspective. Aust. J. Plant Physiol. **13**: 175-190.

---. 1988. Measurement of plant water status by the pressure chamber technique. Irrig. Sci. **9**: 289-308.

TYREE, M.T., and EWERS, F.W. 1991. The hydraulic architecture of trees and other woody plants. New Phytol. **119**: 345-360.

TYREE, M.T., and 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**: 267-282.

VAN DEN DRIESSCHE, R. 1987. Importance of current photosynthate to new root growth in planted conifer seedlings. Can. J. For. Res. **17**: 776-782.

---. 1989. Changes in osmotic potential of Douglas-fir *Pseudotsuga menziesii* seedlings in relations to temperature and photoperiod. Can. J. For. Res. **19**: 413-421.

---. 1991. Influence of container nursery regimes on drought resistance of seedlings following planting. I. Survival and growth. Can. J. For. Res. **21**: 555-565.

WANG, C.Y. 1982. Physiological and biochemical responses of plants to chilling stress. HortScience. **17**: 173-186.

WARING, R.H., and FRANKLIN, J.F. 1979. Evergreen forests of the Pacific Northwest. Science. **204**: 1380-1386.

ZAVITKOVSKI, J., and FERRELL, W.K. 1968. Effect of drought upon rates of photosynthesis, respiration, and transpiration of seedlings of two ecotypes of Douglas-fir. Bot. Gaz. **129**: 346-350.

ZIMMERMANN, U. 1978. Physics of turgor- and osmoregulation. Ann. Rev. Plant Physiol. **29**: 121-148.

ZIMMERMANN, U. 1983. Xylem structure and the ascent of sap. Springer-Verlag, Berlin.

ZWIAZEK, J.J., and BLAKE, T.J. 1989. Effects of preconditioning on subsequent water relations, stomatal sensitivity, and photosynthesis in osmotically stressed black spruce. Can. J. Bot. **67**: 2240-2244.

APPENDIX

Table 1.1 Analysis of variance for seedling dry weight, lengths of new roots produced, and water use for Douglas-fir and lodgepole pine seedlings, grown in tubes having high and low soil moisture treatments.

Table 1.2 Analysis of variance for stomatal conductance of Douglas-fir and lodgepole pine seedlings, from two ecotypes, grown under wet and dry watering regimes during last three harvest intervals.

Table 1.3 Analysis of variance for soil moisture of 0-20, 20-40, 40-60, 60-80, and 80-97 cm depth in tubes at harvest.

Table 2.1 Analysis of variance for osmotic potential at full saturation, osmotic potential at the turgor loss point, symplastic fraction, and bulk elastic modulus near full saturation for Douglas-fir and lodgepole pine seedlings from two ecotypes, grown under wet and dry watering regimes at three harvests.

Table 2.2 Analysis of variance for osmotic potential at full saturation, osmotic potential at the turgor loss point, symplastic fraction, and bulk elastic modulus near full saturation for Douglas-fir and lodgepole pine seedlings grown under wet and dry watering regimes at the three harvests.

Table 3.1 Analysis of variance for dry weights at final harvest of Douglas-fir, lodgepole pine, and white spruce seedlings from wet and dry ecotypes, grown under two watering regimes.

Table 3.2 Analysis of variance for root hydraulic conductance, normalized by root length, of Douglas-fir, lodgepole pine, and white spruce seedlings from wet and dry ecotypes and grown under two watering regimes.

Table 3.3 Analysis of variance for hydraulic conductance, normalized by root length, of Douglas-fir, lodgepole pine, and white spruce roots grown under two watering regimes, and harvested on four dates.

Table 3.4 Analysis of variance for root hydraulic conductance, normalized by root weight for Douglas-fir, lodgepole pine, and white spruce seedlings, grown under well-watered conditions for one to two growing seasons.

Table 3.5 Analysis of variance for hydraulic conductance of roots, normalized by root weight, for Douglas-fir, lodgepole pine, and white spruce seedlings from wet and dry ecotypes, harvested in early September 1989 and 1990.

Table 4.1 Analysis of variance for dry weights of Douglas-fir, lodgepole pine, and white spruce seedlings from wet and dry ecotypes, grown at high and low growth chamber temperatures.

Table 1.1. Analysis of Variance for seedling dry weight (SDW), lengths of new roots produced (NRL), and water used (in kg) by Douglas-fir and lodgepole pine seedlings, grown in tubes having high and low soil moisture treatments.

	ln(SDW)			ln(NRL)		water used		
Source*	DF	MS	p	MS	p	DF	MS	p
SP	1	2.698	0.0001	11.413	0.0001	1	0.154	0.0001
EC	1	0.201	0.0188	0.031	0.578	1	0.578	0.669
SW	1	1.397	0.0001	8.001	0.0001	1	0.257	0.0001
SPxEC	1	0.007	0.632	0.000	0.970	1	0.970	0.909
SPxSW	1	0.008	0.607	0.528	0.032	1	0.032	0.997
ECxSW	1	0.009	0.581	0.001	0.917	1	0.917	0.295
SPxECxSW	1	0.000	0.904	0.044	0.506	1	0.506	0.836
ERROR I	16	0.029	-----	0.096	----	16	0.015	----
HV	4	7.965	0.0001	271.705	0.0001	3	0.111	0.0001
SPxHV	4	0.463	0.0001	1.685	0.0001	3	0.154	0.0001
ECxHV	4	0.036	0.299	0.164	0.116	3	0.116	0.673
SWxHV	4	0.246	0.0001	0.713	0.0001	3	0.257	0.0001
SPxECxHV	4	0.038	0.268	0.055	0.628	3	0.628	0.910
SPxSWxHV	4	0.024	0.511	0.063	0.568	3	0.568	0.478
ECxSWxHV	4	0.039	0.255	0.121	0.235	3	0.235	0.067
SPxECxSWxHV	3	0.034	0.325	0.119	0.249	2	0.249	0.571
ERROR II	62	0.028	----	0.085	----	46	0.015	----

*SP=species, EC=ecotype, SW=soil water treatment, HV=harvest date

Table 1.2. Analysis of Variance for stomatal conductance of Douglas-fir and lodgepole pine seedlings, from two ecotypes, grown under wet and dry watering regimes during last three harvest intervals.

Source* (xPressure)	DF	MS	p
SP	1	65600.4	0.0001
EC	1	973.6	0.168
SW	1	49196.0	0.0001
SPxEC	1	495.1	0.319
SPxSW	1	6.1	0.910
ECxSW	1	1.4	0.956
SPxECxSW	1	2.2	0.947
ERROR I	16	467.2	----
HV	2	60072.6	0.0001
SPxHV	2	18863.0	0.0001
ECxHV	2	2624.2	0.0004
SWXHV	2	3144.5	0.0001
SPxECxHV	2	1273.1	0.0221
SPxSWxHV	2	971.6	0.0539
ECxSWxHV	2	1964.9	0.0029
SPxECxSWxHV	2	2451.4	0.0007
ERROR II	240	328.7	----

*SP=species, EC=ecotype, SW=soil water treatment, HV=harvest date

Table 1.3. Analysis of Variance for soil moisture at 0-20 (20), 20-40 (40), 40-60 (60), 60-80 (80), and 80-97 (97) cm depth in tubes at harvest.

Source*	DF	MS	p	MS	p	MS	p
		20		40		60	
SP	1	0.084	0.386	1.416	0.0028	5.887	0.0001
EC	1	0.055	0.484	0.000	0.997	0.001	0.907
SW	1	0.745	0.0175	0.805	0.0173	1.626	0.0001
SPxEC	1	0.040	0.546	0.002	0.888	0.033	0.394
SPxSW	1	0.011	0.753	0.025	0.644	0.078	0.197
ECxSW	1	0.132	0.281	0.125	0.312	0.028	0.431
SPxECxSW	1	0.021	0.661	0.006	0.824	0.025	0.452
ERROR I	16	0.106	----	0.114	----	0.043	----
HV	3	14.030	0.0001	25.000	0.0001	15.468	0.0001
SPxHV	3	0.104	0.680	0.075	0.0036	2.224	0.0001
ECxHV	3	0.016	0.430	0.010	0.585	0.062	0.498
SWxHV	3	0.069	0.0006	0.060	0.0001	2.417	0.0001
SPxECxHV	3	0.062	0.286	0.009	0.360	0.003	0.989
SPxSWxHV	3	0.113	0.029	0.102	0.232	0.134	0.174
ECxSWxHV	3	0.040	0.582	0.045	0.082	0.260	0.027
SPxECxSWxHV	2	0.000	0.028	0.002	0.590	0.079	0.368
ERROR II	46	0.028	----	0.051	----	0.077	----
		80		97			
SP	1	5.571	0.0001	8.189	0.0001		
EC	1	0.023	0.518	0.014	0.698		
SW	1	4.787	0.0001	15.122	0.0001		
SPxEC	1	0.049	0.352	0.052	0.453		
SPxSW	1	0.039	0.407	0.135	0.236		
ECxSW	1	0.094	0.203	0.355	0.063		
SPxECxSW	1	0.019	0.560	0.017	0.668		
ERROR I	16	0.106	----	0.089	----		
HV	3	13.454	0.0001	19.378	0.0001		
SPxHV	3	3.943	0.0001	5.825	0.0001		
ECxHV	3	0.022	0.832	0.142	0.244		
SWxHV	3	3.366	0.0001	7.358	0.0001		
SPxECxHV	3	0.055	0.542	0.031	0.813		
SPxSWxHV	3	0.039	0.670	0.123	0.304		
ECxSWxHV	3	0.194	0.066	0.301	0.038		
SPxECxSWxHV	2	0.008	0.906	0.006	0.945		
ERROR II	46	0.076	----	0.099	----		

*SP=species, EC=ecotype, SW=soil water treatment, HV=harvest date

Table 2.1. Analysis of Variance for $\psi_{\pi sat}$, $\psi_{\pi tlp}$, SYMFR, and E_{max} for Douglas-fir and lodgepole pine seedlings from two ecotypes, grown under wet and dry watering regimes at the three harvests.

		$\psi_{\pi sat}$		$\psi_{\pi tlp}$		SYMFR		E_{max}	
Source*	DF	MS	p	MS	p	MS	p	MS	p
SP	1	1.738	0.000	0.805	0.016	0.309	0.001	351.8	0.000
EC	1	0.046	0.383	0.000	0.952	0.071	0.050	12.2	0.334
WR	1	0.560	0.007	0.793	0.016	0.026	0.220	11.9	0.340
SPxEC	1	0.064	0.308	0.234	0.164	0.010	0.442	5.5	0.513
SPxWR	1	0.018	0.588	0.002	0.888	0.011	0.406	14.2	0.297
ECxWR	1	0.028	0.492	0.020	0.672	0.002	0.743	1.7	0.718
SPxECxWR	1	0.000	0.984	0.010	0.771	0.013	0.379	29.2	0.143
ERROR I	15	0.057	----	0.109	----	0.016	----	12.2	----
HV	2	0.799	0.000	1.475	0.000	0.019	0.164	65.0	0.002
SPxHV	2	0.104	0.045	0.075	0.256	0.006	0.557	20.2	0.078
ECxHV	2	0.016	0.577	0.010	0.820	0.003	0.726	3.6	0.577
WRxHV	2	0.069	0.114	0.060	0.330	0.025	0.100	4.0	0.571
SPxECxHV	1	0.062	0.155	0.009	0.682	0.002	0.692	18.2	0.120
SPxWRxHV	1	0.113	0.060	0.102	0.173	0.112	0.003	6.4	0.347
ECxWRxHV	2	0.040	0.267	0.045	0.433	0.001	0.897	11.1	0.224
SPxECxWRxHV	2	0.000	0.902	0.002	0.856	0.002	0.679	66.9	0.006
ERROR II	18	0.028	----	0.051	----	0.010	----	6.8	----

*SP=species, EC=ecotype, WR=watering regime, HV=harvest date

Table 2.2 Analysis of Variance for $\psi_{\pi sat}$, $\psi_{\pi tlp}$, SYMFR, and E_{max} for Douglas-fir and lodgepole pine seedlings grown under wet and dry watering regimes at the three harvests

		$\psi_{\pi sat}$		$\psi_{\pi tlp}$		SYMFR		E_{max}	
Source*	DF	MS	p	MS	p	MS	p	MS	p
SP	1	2.539	0.0001	1.054	0.0164	0.428	0.0014	456.8	0.0005
WR	1	0.497	0.0171	0.664	0.0431	0.0421	0.170	2.1	0.713
SPxWR	1	0.067	0.303	0.045	0.551	0.005	0.604	2.5	0.687
ERROR I	8	0.055	----	0.115	----	0.019	----	14.2	----
HV	2	0.971	0.0001	1.727	0.0001	0.034	0.074	65.2	0.0061
SPxHV	2	0.154	0.0258	0.140	0.096	0.003	0.753	23.1	0.138
WRxHV	2	0.091	0.104	0.119	0.134	0.016	0.269	0.8	0.928
SPxWRxHV	2	0.050	0.281	0.047	0.441	0.056	0.0161	8.5	0.470
ERROR	35	0.038	----	0.056	----	0.012	----	11.0	----

*SP = species, WR = watering regime, HV = harvest date

Table 3.1 Analysis of variance for dry weights at final harvest of Douglas-fir, lodgepole pine, and white spruce seedlings from wet and dry ecotypes, grown under two watering regimes.

		ln(root)		ln(stem)		ln(needle)		ln(total)	
Source*	DF	MS	p	MS	p	MS	p	MS	p
SP	2	0.400	0.0001	0.028	0.0293	0.154	0.0009	0.612	0.0002
EC	1	0.036	0.244	0.012	0.198	0.037	0.134	0.083	0.195
WR	1	0.817	0.0001	0.109	0.0006	0.257	0.0005	1.179	0.0001
SPxEC	2	0.044	0.195	0.012	0.201	0.029	0.177	0.088	0.174
SPxWR	2	0.117	0.0215	0.009	0.287	0.052	0.052	0.162	0.0483
ECxWR	1	0.260	0.0042	0.041	0.023	0.128	0.0088	0.490	0.0038
SPxECxWR	2	0.004	0.865	0.007	0.356	0.007	0.656	0.016	0.705
ERROR	21	0.025	----	0.007	----	0.015	----	0.046	----

*SP = species, EC = ecotype, WR = watering regime

Table 3.2 Analysis of variance for root hydraulic conductance, normalized by root length, of Douglas-fir, lodgepole pine, and white spruce seedlings from wet and dry ecotypes and grown under two watering regimes. Hydraulic conductance was calculated as the slope of the water flow rate through de-topped roots at 0.75, 1.0, and 1.25 MPa pressure.

Source* (xPressure)	DF	MS	p
SP	2	0.0537	0.0001
EC	1	0.0002	0.8300
WR	1	0.0202	0.0310
SPxEC	2	0.0021	0.6200
SPxWR	2	0.0231	0.0048
ECxWR	1	0.0030	0.4000
SPxECxWR	2	0.0047	0.3400
HV	3	0.0595	0.0001
SPxHV	6	0.0086	0.0628
ECxHV	3	0.0012	0.8440
WRxHV	3	0.0051	0.3130
SPxECxHV	6	0.0055	0.2690
SPxWRxHV	6	0.0088	0.0579
ECxWRxHV	3	0.0014	0.8020
SPxECxWRxHV	6	0.0017	0.8870
ERROR	1675	0.0148	----

*SP = species, EC = ecotype, WR = watering regime, HV = harvest date

Table 3.3 Analysis of variance for hydraulic conductance through roots, normalized by root length, of Douglas-fir, lodgepole pine, and white spruce roots grown under two watering regimes, and harvested on four dates. Hydraulic conductance was calculated as the slope of the water flow rate through de-topped roots at 0.75, 1.0, and 1.25 MPa pressure.

Source* (xPressure)	DF	MS	p
SP	2	0.0535	0.0001
WR	1	0.0239	0.0400
SPxWR	2	0.0248	0.0126
HV	3	0.0587	0.0001
SPxHV	6	0.0084	0.177
WRxHV	3	0.0053	0.418
SPxWRxHV	6	0.0085	0.176
ERROR	1651	0.0057	----

*SP = species, WR = watering regime, HV = harvest date

Table 3.4 Analysis of variance for root hydraulic conductance, normalized by root weight for Douglas-fir, lodgepole pine, and white spruce seedlings, grown under well-watered conditions for one (1989) to two (1990) growing seasons.

	1989			1990		
Source* (xPressure)	DF	MS	p	DF	MS	p
SP	2	28.55	0.0001	2	3.53	0.0001
HV	3	15.69	0.0001	2	7.11	0.0001
SPxHV	6	4.37	0.0001	4	1.19	0.0153
ERROR	842	0.96	----	636	0.38	----

*SP = species, HV = harvest date

Table 3.5 Analysis of variance for hydraulic conductance of roots, normalized by root weight, for Douglas-fir, lodgepole pine, and white spruce seedlings from wet and dry ecotypes, harvested in early September 1989 and 1990.

Source* (xPressure)	DF	MS	p
SP	2	10.872	0.0001
EC	1	0.227	0.510
SP*EC	2	0.506	0.380
YR	1	22.891	0.0001
SPxYR	2	10.887	0.0001
ECxYR	1	0.030	0.696
SPxECxYR	2	0.877	0.188
ERROR	406	0.522	----

*SP=species, EC=ecotype, YR=year

Table 4.1 Analysis of variance for dry weights of Douglas-fir, lodgepole pine, and white spruce seedlings from wet and dry ecotypes, grown at high and low growth chamber temperatures.

		ln(root)		ln(shoot)		ln(seedling)	
Source*	DF	MS	p	MS	p	MS	p
SP	2	3.617	0.0001	6.173	0.0001	6.413	0.0001
EC	1	0.261	0.120	0.035	0.665	0.112	0.422
GC	1	0.086	0.360	0.444	0.136	0.157	0.344
SPxEC	1	0.006	0.810	0.006	0.862	0.002	0.915
SPxGC	2	0.131	0.286	0.650	0.051	0.520	0.071
ECxGC	1	0.175	0.197	1.114	0.0245	0.992	0.0265
SPxECxGC	1	0.897	0.008	1.109	0.0248	1.356	0.0115
ERROR	15	0.096	----	0.178	----	0.164	----

*SP=species, EC=ecotype, GC=growth chamber temperature

4.2 Analysis of variance for root length and stem cross-sectional area for the three species from two ecotypes, grown in high and low temperature growth chambers and measured at 20°C in the pressure chamber.

	ln(root length)			stem cross-sectional area		
Source*	DF	MS	p	DF	F	p
SP	2	2.314	0.0018	2	30.53	0.0001
EC	1	0.111	0.500	1	0.01	0.919
GC	1	0.133	0.461	1	5.45	0.0363
SPxEC	1	0.021	0.769	1	0.59	0.457
SPxGC	2	0.818	0.056	2	1.66	0.229
ECxGC	1	0.824	0.079	1	3.71	0.076
SPxECxGC	1	2.104	0.0088	1	6.04	0.0288
ERROR	15	0.232	----	13	9.46	----

*SP=species, EC=ecotype, GC=growth chamber temperature

Table 4.3 Analysis of variance for water flow through roots, normalized by root length and stem cross-sectional area, for Douglas-fir, lodgepole pine, and white spruce seedlings grown in high and low growth chamber temperatures.

Source*	DF	F	p
SP	2	769.88	0.0001
GC	1	232.87	0.0001
SPxGC	2	105.14	0.0001
ERROR	503	407.66	----

*SP=species, GC=growth chamber temperature

Table 4.4 Analysis of variance for water flow through roots, normalized by root length and stem cross-sectional area, for Douglas-fir, lodgepole pine, and white spruce seedlings from dry ecotypes, grown in high and low temperature growth chambers and measured at three temperatures in the pressure chamber. Flow rates were corrected for viscosity.

Source*	DF	F	p
SP	2	19.7	0.0003
GC	1	10.6	0.0087
SPxGC	2	5.3	0.0271
ERROR I	10	413.4	----
PC	2	1057.5	0.0001
SPxPC	4	234.2	0.0001
GCxPC	2	36.5	0.0001
SPxGCxPC	4	13.8	0.0001
ERROR II	1500	1236.8	----

*SP=species, GC=growth chamber temperature, PC=pressure chamber temperature