AN ABSTRACT OF THE THESIS OF

Gladwin Joseph for the degree of Doctor of Philosophy in Forest Science presented on November 1, 1996. Title: Water Relations, Gas Exchange, and Growth Responses of Douglas-fir Seedlings to Stresses Associated with Transplanting.

Abstract approved: _____

Joe B. Zaerr

The goal of this thesis was to determine the physiological mechanisms that link adverse preplanting treatments of Douglas-fir, such as exposure and root pruning, to the phenomenon of transplanting shock. The objective of experiments 1 and 2 was to measure the effect of exposure and pruning on the physiology and growth of seedlings. The objective of experiment 3 was to understand the physiological mechanisms that affect shoot elongation. Two-year-old Douglas-fir seedlings were exposed or root pruned and transplanted on different dates from November through April. Water relations, gas exchange, phenology, and growth were measured during the first-year of establishment.

Exposure reduced new root growth. Stomatal conductance decreased regardless of changes in water potential (ψ) during the first 2 months. Time of transplanting significantly affected the stress response of seedlings. Exposed April transplants had reduced stem conducting area, probably due to cavitation. After budbreak, exposed April transplants showed reduced midday ψ and gas exchange, whereas exposed

winter transplants had recovered. Seedlings recovered a favorable ψ and net photosynthesis before shoot elongation ceased. However, exposure caused a reduction in shoot growth and an increase in the root:shoot ratio.

Removing 30-50 % of the original root volume reduced shoot and root growth. Predawn ψ remained unaffected by the root pruning treatments during the first 2 months after planting. After budbreak, midday ψ and gas exchange of root pruned seedlings were low.

Root exposure and root pruning decreased shoot ψ and turgor during elongation of the leader. The reduced turgor did not affect the rate of cell wall hardening as measured by changes in the modulus of elasticity. Changes in cell wall elasticity closely corresponded to the ontogeny of the leader. Current shoots of stressed seedlings did not show osmotic adjustment during the period of low ψ. Photosynthesis of stressed seedlings decreased during elongation as a result of non-stomatal mechanisms.

Reduced terminal elongation induced by preplanting stresses may be caused by a reduction in cell wall extensibility rather than cell wall elasticity as a result of low turgor. Decreased photosynthesis and a higher allocation of resources to roots during establishment may also limit shoot elongation.

Water Relations, Gas Exchange, and Growth Responses of Douglas-fir Seedlings to Stresses Associated with Transplanting.

by

Gladwin Joseph

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APPROVED:

Major Professor, representing Forest Science

Chair of Department of Forest Science

Dean of Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Gladwin Joseph, Author

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" In HIM All things hold together, whether things on earth or things in heaven"

- St.Paul

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Water Relations, Gas Exchange, and Growth Responses of Douglas-fir Seedlings to Stresses Associated with Transplanting

CHAPTER I. INTRODUCTION

Significance and Rationale

In the Pacific Northwest of the United States, millions of Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco) seedlings are transplanted each year to reforest harvested sites. In most situations, transplanting is the method of choice over direct seeding or natural regeneration (Cleary et al. 1978). Therefore, there is a need to produce seedlings that are vigorous and be able to better predict survival and growth of transplanted seedlings. In order to predict transplanting success confidently, knowledge of how seedlings respond to the various stresses associated with transplanting will be useful. However, information necessary to predict seedling performance from seedling quality assessment prior to planting is still lacking (Lavender 1989).

Tree seedlings are vulnerable to several kinds of stress during transplanting and the first growing season thereafter. Such stresses that impact seedlings prior to planting can be termed pre-planting stresses, while those after planting may be called post-planting stresses. Pre-planting stresses, such as exposing seedlings to drying, root loss and damage, mechanical injury, and storage damage, occur during lifting, grading, storing, transporting, and planting. They may also predispose seedlings to post-planting stresses (Grossnickle and Folk 1993). On the other hand, post-planting

stresses, such as extremes of soil and air temperature, moisture and nutritional deficiencies or surplus, or resource limitation due to competing vegetation, and animal damage can also affect seedlings adversely (Lavender 1990, Hobbs et al. 1992).

The degree to which seedlings tolerate these stresses is influenced by their physio-morphological quality. The physio-morphological quality of seedlings is a dynamic suite of physiological and morphological characteristics that determine their vigor (Sutton 1979, Bunting 1980, Ritchie 1984, Duryea 1984, Cleary et al. 1978). The physio-morphological quality of the seedlings can be shaped to a certain extent by genetic selection and by manipulating how they are grown (Duryea 1984, Lavender 1984). The combined effect of the seedlings' vigor and the stresses they encounter is reflected in survival and growth (Ritchie 1984).

Of the stresses that seedlings encounter prior to transplanting, exposure to dry air and root loss may be the two most significant factors affecting their performance when transplanted (Rietveld 1989; Stoneham and Thoday 1985). Seedling exposure prior to planting has detrimental effects on the growth of Douglas-fir (Hermann 1964, 1967), Sitka spruce (Coutts 1981), and loblolly pine seedlings (Feret et al. 1985). Of particular interest is the work done by Hermann (1964, 1967), in which he showed that exposing Douglas-fir seedlings for 30 minutes at 32°C reduced growth. However, it must be noted that he exposed the seedlings at temperatures much higher than the ambient temperatures during the transplanting period in the Pacific Northwest. Secondly, in both experiments, the whole seedling was exposed and the roots were assumed to be the tissue that was primarily damaged by the exposures.

This assumption seems to neglect the degree to which the shoots contribute to the overall damage resulting from exposure.

The effect of root loss on growth depends on the severity of root damage, the time of transplanting, species, and method of pruning. Operational root pruning may occur either when the seedlings are in the soil or at the time of planting. Undercutting and wrenching seedlings in the nursery is common in the Northwest and apparently done to increase the fibrosity of the root system (Duryea 1984). However, there are conflicting reports on the effects of undercutting and root wrenching on growth after transplanting. For instance Duryea and Lavender (1982) report that the first year's growth of Douglas-fir seedlings was consistently greater for unwrenched seedlings than for wrenched seedlings. On the other hand, Tanaka et al. (1976) found that wrenched seedlings showed higher growth after 5 years in the field. However, Hobbs et al. (1987) found that both Douglas-fir and ponderosa pine seedlings showed no difference in growth between several undercut treatments or controls after 4 years in the field. Root loss due to lifting and table pruning before planting can be substantial (Burdett and Simpson 1984). Sitka spruce seedlings had lower survival when they were root pruned at the time of planting (Mullin 1973). However, the root pruning treatment was not clearly described and would be hard to repeat. Surprisingly, there is no published information on the effects of root pruning at planting on subsequent growth of Douglas-fir seedlings.

The effect of pre-planting stresses on seedling growth is influenced by the season when seedlings are lifted and transplanted. Seedlings lifted between

December and February are apparently better able to tolerate stresses than seedlings either transplanted in fall or in spring (Hermann 1967, Ritchie 1984). Seedlings are operationally lifted in December and transplanted during a narrow window between January and April. Due to frozen soils, the planting season often has to be extended to May or June. Fall transplanting may be feasible although little is known about how it may affect seedling physiology and growth. Seedlings transplanted in January are often planted in cold soils and cool ambient temperatures, whereas spring transplants are planted in warmer soils and air temperatures. Although delay in spring planting reduces survival and growth, there is little information on the physiological response of Douglas-fir seedlings transplanted at different dates, particularly when seedlings are either transplanted in spring or fall.

A common symptom observed in transplanted Douglas-fir seedlings is a stunting of the first-year spring growth, with densely packed, short needles. These first-year needles appear like a `bottle brush'. Frequently, needles are pale green to yellowish in color. These typical signs of stress define what is often called transplant shock or transplanting stress (Rietveld 1989). Depending on the degree of stress, seedlings may take a year or more to recover. Some seedlings do not survive this first year after transplanting. The stunting of the first year growth concerns forest managers and has been a focus of research.

Reduced growth can cause the seedlings to lose their competitive edge over adjacent weeds, prolong the period that they remain susceptible to deer browsing, and in the long run, delay establishment and increase rotation length (Cleary et al. 1978).

First-year height growth of Douglas-fir was positively correlated with tree size after 4 years, suggesting that increasing initial seedling growth after transplanting may improve plantation development (Wagner and Radosevich 1991). This reduced growth of transplanted Douglas-fir delays the harvest of Christmas tree plantations, where fast growth is of exceptional economical importance.

The elongation of the terminal leader in spring is a complex process that dependents on several interacting factors such as light, water, and nutrients. However, in newly planted Douglas-fir seedlings, water stress may be one of the most important factors affecting elongation growth (Grossnickle and Folk 1993). Water stress in these seedlings may be aggravated by damage to the roots during transplanting combined with seasonal changes in the atmospheric vapor pressure deficit. Reduction in shoot elongation as a result of prior exposure or root pruning Douglas-fir seedlings may be associated with a reduction in turgor and/or an increase in the rate of cell wall hardening. If cell walls were to harden at a faster rate in the stressed seedlings than the controls, then elongation growth would be inhibited even if turgor were to increase sufficiently during the elongation process. Several reports have indicated that inhibition of elongation growth in response to water stress can occur without long-term reductions in the turgor pressures of expanding cells (Hsiao and Jing 1987; Nonami and Boyer 1990a; Serpe and Mathews 1992), suggesting that inhibition of cell expansion could be associated with the hardening process.

In mature cells, the modulus of tissue elasticity measures the reversible extensibility of the cell wall (Nonami and Boyer 1990b). A higher cell wall elasticity

(low modulus of elasticity) indicates better turgor maintenance under water deficits, because an elastic tissue will sustain a smaller decrease in turgor (p), as a given volume of water is lost, than will a more rigid tissue (Joly and Zaerr 1987). However, in expanding immature tissue, the changes in the modulus of tissue elasticity could be indicative of cell wall hardening as well as the capacity to maintain turgor. The modulus of tissue elasticity in such tissue may therefore influence turgor mediated processes such as elongation (Hsiao et al 1976). For whole Douglas-fir seedlings, modulus of tissue elasticity (measured by PV curve analysis) increases during the spring flush period suggesting that a decrease in cell wall elasticity is associated with ontogenetic cell wall hardening (Ritchie and Shula 1984). Similarly Tyree et al. (1978) found that the tissue modulus of elasticity increased with leaf age in sugar maple Acer saccharum L. and poplar species Populus spp.

There are few detailed studies of the ontogenetic changes in tissue-water relations of expanding new growth. Ritchie and Shula (1984) measured the tissue-water parameters of Douglas-fir seedlings monthly over the entire year. However, they did not separate the new growth from the older growth, therefore confounding the independent effects of these tissues on the tissue-water relations. Chapter IV describes detailed PV-analysis of expanding new terminals and 1-year-old shoot in order to characterize the ontogenetic and seasonal development of water relations of Douglas-fir seedlings. Of particular interest was how tissue water characteristics are affected by exposure to dry air or root pruning prior to transplanting. This information would be valuable in better understanding some mechanisms involved in

the elongation of terminals during the establishment of transplanted Douglas-fir seedlings.

In summary, the research described in this dissertation attempted to determine the effects of seedling exposure and root pruning at the time of planting on subsequent growth and physiology of 2-year-old Douglas-fir seedlings when transplanted in different seasons. Of special interest were the physiological mechanisms that produce the commonly observed stunted terminal leader of the first-year's growth, which is often associated with transplanting shock.

Objectives and Hypotheses

This research has three broad objectives, each addressed in a separate chapter. The objective of chapter II is to determine how seedling exposure to drying and time of transplanting affect the physiology and growth of Douglas-fir seedlings during the first growing season. The first hypothesis states that exposing seedlings to air affects growth primarily by damaging the root system rather than the shoots. The second hypothesis states that seedling exposure damages and prolongs the physiological recovery of transplanted seedlings by causing a decrease in water potential, and reductions in new root initiation, stem and root hydraulic conductance, leaf stomatal conductance, and net photosynthetic rates. The third hypothesis states that, irrespective of transplanting date, seedlings recover to a favorable water balance and gas exchange rate only after shoot elongation ceases in late spring. The fourth hypothesis states that delayed (spring) transplanting would aggravate the negative

effects of seedling exposure more than winter (January) transplanting, by reducing new root growth, decreasing shoot water potential and stomatal conductance during the first 2 months of establishment.

The objective of chapter III is to determine how the degree of root pruning and time of transplanting affect the physiology and growth of Douglas-fir seedlings during the first growing season. The hypotheses states that root pruning of Douglas-fir at the time of transplanting will significantly decrease new root growth, biomass and elongation growth during the first season. The second hypothesis states that the negative effects of root pruning will be ameliorated for seedlings transplanted in November > January > March. The third hypothesis states that November and March transplants will show higher root growth due to warmer soils than January transplants, but shoot water potentials will be higher for January > November > March transplants during the first 2 months of establishment due to increasing VPD. The fourth hypothesis states that root pruning will decrease shoot water potential, stomatal conductance and net photosynthesis.

The objective of chapter IV is to determine what physiological mechanisms link pre-planting seedling exposure and root pruning to the elongation of the terminal leader. This experiment was specifically designed to test the hypothesis that Douglas-fir seedlings stressed prior to transplanting lose tissue elasticity of elongating spring shoots faster than unstressed controls, and that this increase in the rate of cell wall hardening is associated with a lower measurable turgor pressure in the elongating shoots of Douglas-fir. The second hypothesis states that osmotic

adjustment in stressed newly transplanted Douglas-fir seedlings would account for some degree of turgor maintenance in alleviating internal water deficit. Finally, measurement of gas exchange parameters of mature and expanding needles in stressed and unstressed controls would aid in characterizing carbon acquisition, and stomatal regulation of water loss, during early establishment of transplanted seedlings.

Chapter V summarizes the principal findings from all the experiments and discusses the impact and the nature of damage that pre-planting stress has on seedling physiology during the first year of establishment. The implications that these results may have on forest regeneration are also discussed .

CHAPTER II. EFFECT OF SEEDLING EXPOSURE AND TIME OF TRANSPLANTING ON WATER RELATIONS, GAS EXCHANGE AND GROWTH OF DOUGLAS-FIR SEEDLINGS

Introduction

Exposure to dry air may be the most common source of stress to seedlings during lifting and transplanting (Aldhous 1972). The negative effects of exposure on growth have been known for some time. Hermann (1964) found that a 30 minute exposure of Douglas-fir seedlings at 32°C reduced growth. Feret et al. (1985) found that first-year survival of loblolly pine seedlings declined 7% for each 10 min of root exposure, and new root growth decreased 50% after 35 min. Working with Sitka spruce, Deans et al. (1990) reported that exposure for 1-1.5 hrs not only reduced first-year growth rates but also decreased survival after 3 years. Little, however is understood of how exposure may affect physiological processes that induce the observed reduction in growth.

The severity of damage due to exposure is dependent on the duration, nature, and intensity of exposure, and on the relative degree of stress tolerance of seedlings. Desiccating conditions intensify as temperature, vapor pressure deficit, and wind speed increase. Therefore, under low vapor pressure deficits, seedlings could withstand longer durations of exposure (Cleary et al. 1978). However, increasing wind speeds reduce the boundary layer resistance around seedlings at all levels of vapor pressure deficit, subsequently increasing water loss (Cleary et al. 1978). Coutts (1981) found that the damaging effects of exposure on Sitka spruce seedlings was

dependent on whether internal moisture was lost primarily through the shoots or roots. Root exposure took 2x (6.4 hrs) longer to reduce the water potential to -2.0 MPa than shoot exposure. However, root-exposed seedlings had 50% lower survival and 87% less leader growth than shoot-exposed seedlings. It would be reasonable to expect roots to be similarly sensitive in Douglas-fir, although the relative contribution of roots and shoots to exposure damage is not known. Tabbush (1987) found that Douglas-fir was more sensitive to whole seedling exposure than Sitka spruce, although Douglas-fir maintained a higher root moisture content and ψ was reduced less rapidly than for Sitka spruce. It also seems important to know whether the relative sensitivity of roots and shoots to exposure changes with the season.

The negative effects of seedling exposure can be ameliorated to a certain extent by lifting and transplanting seedlings in winter when they are more tolerant to such stresses (Hermann 1967; Ritchie 1986; Deans et al. 1990). Although specific mechanisms that cause changes in seasonal tolerance are still unknown, stress tolerance peaks in mid-winter concurrently with cold hardiness and root growth potential (RGP) (Ritchie 1984). In the moist temperate northwestern United States, seedlings that are transplanted in winter are often planted in cold, wet soils under high ambient relative humidity (RH). However, if soils are frozen, planting may have to be delayed until spring when soils become warmer and the ambient RH is low, resulting in higher vapor pressure deficits (VPD) (Cleary et al. 1978). This seasonal variation in the operational environment of transplanted seedlings can also influence the degree to which seedlings are able to tolerate stresses. However, there

is very little published information on how different transplanting dates may affect the physiological response of seedlings to stresses such as pre-planting exposure, during the first year of establishment. This information will lead to a better understanding of the causes for the ubiquitous problem of transplanting shock.

Transplanting shock is the term used to describe the phenomenon of stunted growth in recently transplanted seedlings that is often accompanied by needles that are short, chlorotic and densely packed on the stem. In such seedlings, the shoot elongates to only a small portion of its full potential. The needles are characteristically shorter than those of the previous year, and are packed densely on the stem, often appearing as a `bottle brush'. This condition may last for one year in Douglas-fir or in some species, such as Sitka spruce, it may last for several years (Sutton and Tinus 1983). The reduction in shoot growth has been attributed to a deficit of tissue water (Burdett et al. 1984, Kaushal and Aussenac 1989), but no physiological studies support that explanation (Grossnickle and Blake 1987). Cell elongation, unlike cell division, can be very sensitive to internal water deficit (Hsiao 1973; Levitt 1980). Although the relative contribution of cell elongation and cell division to first year growth in Douglas-fir seedlings is unknown, Douglas-fir seedlings suffer physiological damage when the water potential falls below -2.0 MPa (Cleary and Zaerr 1980).

Water deficit within a transplanted seedling may develop because water uptake is impaired, and the root system cannot adequately replenish the water lost due to evapotranspiration from the needle surface (Kozlowski 1982; Margolis and Brand

1990). Water uptake may be critically limiting to newly transplanted seedlings because of the loss of a large number of roots during transplanting, poor initial root-soil contact (Sands 1984), or low water uptake by a root system that is primarily suberized. Chung and Kramer (1975) found that suberized roots of loblolly pine seedlings absorbed 89% less water than unsuberized roots. Although a larger root system would significantly improve water uptake (Carlson 1986), a significant increase in water uptake is dependent on the number of new roots initiated (Carlson 1986; Johnsen et al. 1988). Therefore stresses prior to planting that affect the root system, such as exposure (Coutts 1981), would further decrease water uptake. Although Coutts (1980) reported a slight reduction in water conductivity of Sitka spruce roots as a result of root disturbance, there have been no other reports of the effect of transplanting stresses on water uptake or hydraulic conductance of roots in conifer seedlings.

To minimize the extent and duration of the water deficit in transplanted seedlings, they would have to increase water uptake by initiating new roots or rapidly elongating existing roots (Rietveld 1989). The importance of new root production for increased water absorption is well documented (Chung and Kramer 1975; Weatherly 1975; Carlson 1986; Johnsen et al. 1988). In another study, Omi et al. (1991) have shown that new root initiation and new root weight for ponderosa pine were significantly related to plant water potential and stomatal conductance. New root initiation in post-dormant Douglas-fir is dependent on current photosynthate (van den Driessche 1987; Philipson 1988). Water stress can also indirectly reduce net

photosynthetic rates by inducing stomatal closure (Teskey et al. 1986; Teskey et al. 1987), thereby limiting the photosynthate available for new root initiation.

When water deficits in plants become sufficiently low, xylem conduits cavitate and become embolized (filled with air) (Milburn and Johnson 1966; Tyree and Dixon 1983). Embolization significantly increases the resistance to water flow through the xylem (Tyree and Sperry 1988). Conifer trachieds have been shown to cavitate and become air-filled at water potentials ranging from -0.5 MPa to -5.0 MPa (Tyree and Ewers 1991; Cochard 1992). Recently it has been shown that cavitation induced loss in hydraulic conductivity may be a significant cause of transplanting stress in western hemlock seedlings (Kavanagh 1993). The vulnerability of xylem to cavitation is higher for western hemlock than for Douglas-fir seedlings. While western hemlock starts cavitating at -1.5 MPa, Douglas-fir starts cavitating at -2.0 MPa (Cochard 1992). Complete loss in conductivity occurs at -4.0 MPa in hemlock and at -5.0 MPa in Douglas-fir. However, we do not know to what extent the stresses associated with transplanting induce cavitation and consequently increase stem resistance to water flow. Further, it would be important to know whether a loss in stem conductance affects stem elongation of Douglas-fir.

The focus of the research reported in this chapter was to determine how seedling exposure to drying and time of transplanting affect the physiology and growth of Douglas-fir seedlings during the first growing season. The first hypothesis states that exposing seedlings to air affects growth primarily by damaging the root system rather than the shoots. The second hypothesis states that seedling exposure

damages and prolongs the physiological recovery of transplanted seedlings by causing a decrease in water potential, and reductions in new root initiation, stem and root hydraulic conductance, leaf stomatal conductance, and net photosynthetic rates. The third hypothesis states that, irrespective of transplanting date seedlings recover to a favorable water balance and gas exchange rate only after shoot elongation ceases in late spring. The fourth hypothesis states that delayed (spring) transplanting would aggravate the negative effects of seedling exposure more than winter (January) transplanting by reducing new root growth, decreasing shoot water potential and stomatal conductance during the first 2 months of establishment.

Material and Methods

In order to study the effects of exposing seedlings to dry air, the roots and shoots of 2-year-old Douglas-fir seedlings were exposed separately under controlled conditions and planted in a common garden plot (Fig II.1). Seedlings were exposed on 3 different dates during the normal planting season to evaluate the effect of the time of transplanting. The response of these treated seedlings was evaluated physiologically and morphologically during the first season of growth. The experiments were conducted at the Forest Research Laboratory facilities, Corvallis, Oregon from January through September, 1991.

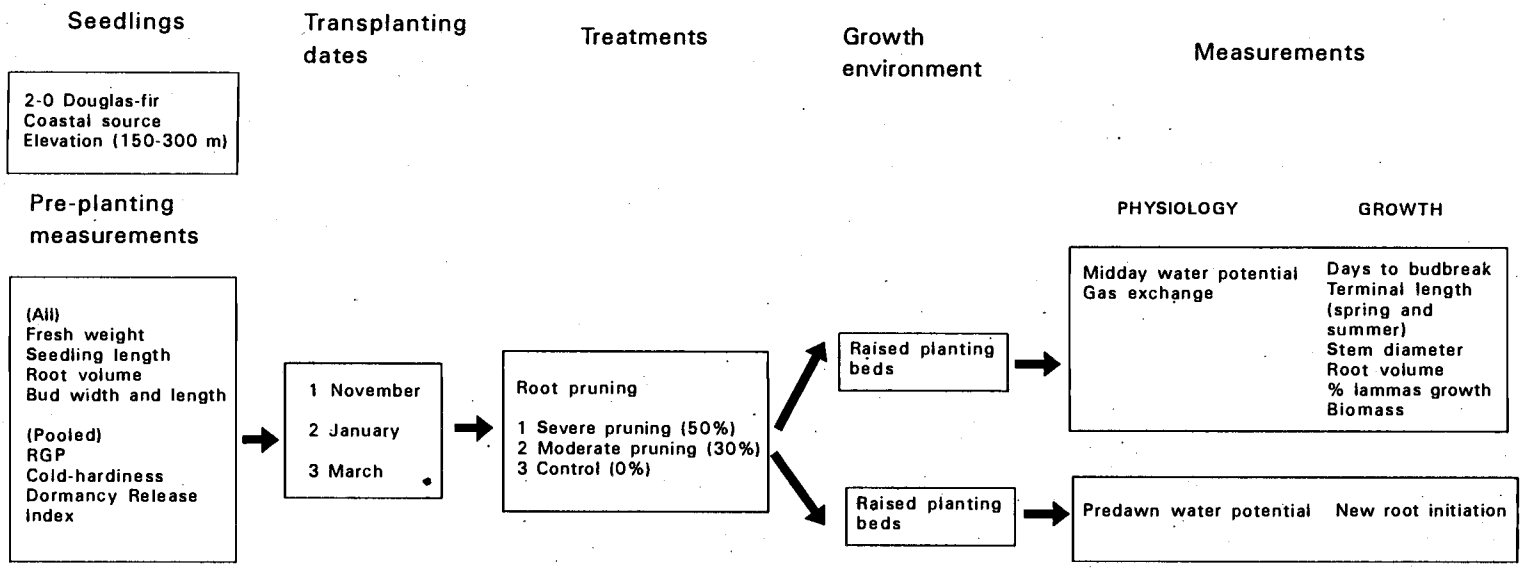

Figure II.1. Layout of the experiments outlining the different treatments, growth conditions, and measurements taken. [1] These measurements were taken only on January and April transplants and only for the untreated controls and RS3 exposure treatments. [2] This measurement was made only on the January transplants.

Seedlings

Two-year-old Douglas-fir seedlings [Pseudotsuga menziesii, (Mirb.) Franco] from a coastal Oregon source (elevation 150-300 m, seed zones 072 and 062) were grown as 2+0 stock type at the International Paper Co. nursery in Kellogg, Oregon.

Transplanting dates

Seedlings were carefully hand-lifted on three dates. The first lift date was January 17th (planted January 25th), the second was February 28th (planted March 6th), and the third was March 22nd (planted April 8th). Seedlings were kept in cold storage (5°C) during the short period between lifting and planting. The transplanting dates for this experiment fall within the normal lifting period for Douglas-fir in the Pacific Northwest. On each lift date, seedlings were randomly assigned to the different treatments. Transplanting was completed within 2 weeks of lifting.

Pre-planting seedling evaluation

Prior to treatment and subsequent transplanting, individual seedlings were weighed and measured. Measurements included total height, fresh weight, stem diameter, root volume (Rose et al. 1991), terminal bud width and length. Bud width was measured at the widest section of the bud with a digital calipers to the nearest 0.01 mm. In addition, a sub-sample of 15-20 seedlings were measured at each transplanting date, for cold-hardiness, root growth potential, and days to budbreak

(DBB) to determine the over all physiological status of the seedlings at each transplanting date (Ritchie 1984). The DBB measurements were converted to Dormancy Release Index DRI (=10/DBB) (Ritchie 1984).

Exposure treatments

Seedlings were exposed in a temperature controlled chamber which was maintained at 3-4°C, RH 85%, and photosynthetically active radiation (PAR) of 7.3μmoles m^{-2} s^{-1}. Two small desk fans were used to generate a wind speed of 0.9-1.3 m s^{-1} (2-3 mi h^{-1}) during the entire exposure period. The stem of each seedling was gently sandwiched between two wooden slats and hung upside down, so that the entire seedling was exposed to the air from all directions. The temperature and humidity during exposure were selected to simulate the operational conditions during winter lifting and planting in the Pacific Northwest. The exposure treatments were, 1) untreated control (no exposure), 2) root exposure (R3), and 3) root+shoot exposure (RS3). For the root exposure treatment, the shoot of the seedling was protected from the air with a plastic bag such that only the roots were exposed. For the root+shoot exposure treatment, both roots and shoots were exposed. Seedlings were uniformly exposed to air from all directions for a period of 3 hrs.

Immediately after the seedlings were exposed, a random sample of 10-15 seedlings from each treatment were chosen for the measurement of plant water potential. Water potential measurements were made on a lateral twig using a pressure chamber apparatus (PMS Instrument Co, Corvallis OR). All the seedlings

were then immediately placed in water for approximately 1-1.5 hrs before they were planted. This process was repeated on every transplanting date.

Planting

Seedlings were grown in a common garden plot that consisted of well-drained, raised planting beds 18 x 1.5 m in size, divided into 4 equal blocks. The soil in the bed was a forest soil of loamy-sand type. In order to measure the predawn water potential (ψ) and stomatal conductance (g_s) prior to budbreak, a sub-sample of 8 seedlings for each treatment on every transplanting date were grown individually in 3.5 liter pots filled with the same soil from the beds (total of 72 seedlings). Two pots of each treatment were placed in each of the 4 blocks. A trench was dug at one corner of the bed in each block to accommodate the pots. The pots were half buried in the soil in the beds so that their soil temperatures were close to that of the planting beds. The experiment was a 3x3 factorial design (3 transplanting dates and 3 exposure treatments) randomized over 4 blocks, with a total of 216 seedlings. On each transplanting date, for every treatment, a total of 24 seedlings were planted. Each row contained 6 seedlings from a particular exposure treatment and transplanting date. The rows were randomized in each block. The inter-row spacing was 30 cm and the intra-row spacing was 20 cm.

During rain-free periods starting in July, the planting beds were watered 1-2 times a week until the end of the experiment in September. The beds were kept well watered to avoid the confounding effect of summer drought on the treatment effects.

Beds were manually kept free of weeds. Soil analysis of beds indicated adequate nutrients, so beds were not fertilized.

Measurement of growth and physiology

All the growth parameters were measured at the end of the first season except percent budbreak, which was scored during the period of flushing. The physiological measurements were made primarily during two periods during the growth of the transplanted seedlings. The first measurements were made during the first 55 days after seedlings were transplanted on each of the transplanting dates. Potted seedlings were brought into a growth room held at constant temperature, and measurements of predawn water potential and midday stomatal conductance were made every 8-10 days (averaging 3-4 times). The second set of measurements were made after all the seedlings for each transplant date had been planted in the raised beds. Gas exchange and midday water potentials were measured only for the untreated control and the RS3 treatment of the January and April transplants. These transplanting dates and treatments were selected because they include the extremes of the range of possible effects.

Growth

For seedlings in the raised beds, growth was measured in terms of rate of percent budbreak and terminal leader growth. Percent budbreak was scored

periodically for each seedling from the time that the first seedling broke bud until all seedlings showed visible budbreak. Budbreak was scored for both the terminal bud and lateral buds when the new spring flush broke through the bud scales. Lateral budbreak was scored when any one of the lateral buds showed spring flush breaking through the scales. Final growth measurements included fresh weight, terminal leader length, needle density, stem diameter, bud width, bud length, root volume, root dry weight and shoot dry weight. Needle density was measured by counting all the needles in a 1 cm segment located at the middle of the leader. This measurement is a modification of the stem-unit length used by Cannell et al. (1976). Most of the growth measurements presented in this paper are in terms of final or relative growth rates. Relative growth rate is a measure of growth that is normalized for starting biomass (Hunt 1982; South 1995), or other appropriate starting growth parameters, therefore eliminating the influence of initial size or growth of seedlings.

$$\text{RGR (biomass,diameter,etc.)} = \frac{\log_e(w_n)-\log_e(w_0)}{(t_n-t_0)}$$

where w_n is the final weight (or other parameters), w_0 is the initial weight (or other parameters), t_n is the date when w_n was measured, and t_0 is the time when w_0 was measured. The dry weights were measured on samples that had been dried in an oven at 70°C for 72 hrs.

Gas exchange and Water Potential

Seedling physiological responses to the various treatments were measured at two different periods after transplanting. The first period was during the first 55 days after transplanting, at which time measurements were made on the potted seedlings. The second period was after budbreak, during active growth, at which time measurements were made on a sub-sample of seedlings in the planting bed.

For the first period, at intervals of 8-10 days after transplanting, seedlings were measured for predawn ψ and g_s. For the January transplants these measurements were made on February 7, February 14, February 21, and March 5. For the March transplants these measurements were made on March 19, March 26, April 2, and April 16. For the April transplants these measurements were made on April 16, May 8, and May 23. In order to eliminate confounding effects of seasonal changes in ambient weather, and to keep the conditions uniform for seedlings from different lift dates, the seedlings were temporarily transferred to a temperature controlled growth chamber between 4:30 and 6:30 pm the evening prior to the measurements. Seedlings were allowed to equilibrate to chamber temperature and humidity for 16-18 hrs in the dark. Seedlings were watered to saturation and allowed to drain overnight before measurements were made the next morning 3-4 hrs after turning on the lights. The growth chambers were maintained at 15-17°C, RH 40-55%, and PAR 150-180 μmoles m^{-2} s^{-1}.

Stomatal conductance was measured between 1030 hrs and 1200 hrs using a steady state porometer (LICOR-1600, Lincoln NE). Lights were turned on 3-4 hrs

prior to measurements. At each time, the conductance was measured on the same tagged section of 1-year-old needles on a lateral branch. Stomatal conductance was calculated from the measured values of relative humidity, leaf and air temperature, and the flow rate of dry air necessary to maintain constant relative humidity inside the cuvette. Needle areas were measured after the last measurement using a leaf area meter (Licor 3100, Lincoln, NE). Predawn ψ was measured using a pressure chamber (PMS Co. Corvallis, OR) just before lights came on in the chamber. After the seedlings were measured, they were transferred back to the planting beds until the next measurement date. After the final measurement, at the end of 40-55 days after transplanting, seedlings were harvested and the number of new roots counted up to a maximum of 50. The number of new roots were separated into those above 1 cm in length and those below 1 cm.

During the second period, seedlings in the planting bed were measured on two dates, May 21 and June 7. For the January lift, May 21 and June 7 were 116 and 139 days after transplanting, respectively, and for the April lift, May 21 and June 7 was 43 and 60 days after transplanting, respectively. Measurements were made on a random sub-sample of 8 seedlings/treatment (2 seedlings/block) from 2 transplanting dates (January and April) and 2 exposure treatments (untreated control, root+shoot exposure). Only these treatments and lift dates were chosen because they included the potential extreme effects of the treatment combinations and also made measurements logistically possible. Measurements included, 1) Net photosynthesis (net CO_2 uptake rate), 2) g_s, 3) midday ψ, and 4) terminal leader length. Gas

exchange was measured with a portable infrared gas analyzer (IRGA) (LICOR 6250, Lincoln NE). The IRGA was calibrated before the measurements against a known concentration of CO_2. Measurements of gas exchange were made between 1030 hrs and 1300 hrs on both the dates. Measurements were done on 1-year-old needles on a lateral branch. Duplicate measurements were made on each seedling on the same section of needles on a twig. Midday ψ was measured on lateral twigs with a pressure chamber just after the gas exchange measurements were made. The terminal lengths were also measured on these seedlings. Using the terminal lengths, terminal elongation rates were calculated from April 20 to May 21 (31 days) and from May 21 to June 7 (17 days).

$$\text{Terminal elongation rate} = (l_n - l_{n-1})/(t_n - t_{n-1})$$

where l_n is the terminal length at time t_n and l_{n-1} is the terminal length and time at t_{n-1}. For the first growth period, April 20 was chosen as the t_{n-1} as this was when <5% of the seedlings had broken bud. After the measurements on June 7, the twigs with the needles were harvested and their needle areas measured using an area meter. During the measurements on May 21, the ambient conditions were as follows: PAR 1109 μmoles m^{-2} s^{-1}, temperature 23.9°C, and VPD 17.1 mb, and on June 7 they were: 1068 μmoles m^{-2} s^{-1}, 25.5°C, and 19 mb.

Stem Conducting Area

Stem conducting area was measured as the cross sectional area of conducting sapwood (dyed) as a percent of the total cross sectional area of the stem. When

seedlings grown in raised beds under ambient conditions were harvested, a sub-sample of 8 seedlings (2/block) from the April and January transplants and untreated control and root+shoot treatments were set aside for these measurements. Seedlings were stored in the cooler in plastic bags at 4°C for 10 days before they were used in determining the area of stem conductance.

A 0.2 % O-Saffranin dye solution was made and vacuum filtered through a 0.22 μm membrane filter. 100 ml of this filtered dye solution was added to a glass test-tube. The seedlings were cut at the root collar under water and inserted into the test-tubes with the dye solution. The seedlings in the test-tubes were allowed to stand in a metal rack and placed in a greenhouse at 20-25°C, 45-50% RH and ambient light. The experiment was commenced on November 27, 1991, and terminated on December 2, 1991. During the course of the experiment the volume of the dye solution in the test-tube was kept constant by adding the solution when needed. After 5 days of allowing the shoot to absorb dye, a 3 cm long segment was removed from the stem at 6 cm from the root collar on all seedlings to measure the percent sapwood dyed. This 3 cm segment was just above the level of the dye solution in the test-tube. The dye had not moved up the whole length of the shoot as expected. It is possible that resin that is secreted on wounding the stem could have clogged the entry pores, thereby reducing dye uptake prematurely. However, at a distance of 6-10 cm from the root collar the dye was uniformly distributed across all treatments. The 3 cm long stem segment was placed in a plastic bag and stored at -20°C.

In November, 1993, cross-sections measuring 1-2 mm width were cut between 1 and 2 cm from the base of the stem segments. These sections were air dried at room temperature for > 24 hrs and the areas measured on two representative cross-sections. The total cross-sectional area of the section, the widest diameter and the area of the dyed section was quantified using a video camera, attached to an image processor (NIH Image Ver 1.52, Rasband and Bright 1995). The area of stem cross-section dyed was expressed as a percent of the total area. Although the pith was only a fraction of the total cross sectional area of the stem, it was still not included in the calculations. The percent dyed sapwood is an estimate of conducting tissue in the stem. The percent undyed area is an estimate of the loss of stem conductance due to cavitation during the growing season. Terminal length was also measured for the above seedlings.

Root Conductance

Root conductance was measured with a Root Hydraulic Conductance Meter (Weyerheuser Co.) (Fig II.3). The instrument consists of a large pressure chamber that is connected to a temperature controlled water circulation system. Pressure in the chamber was adjusted by the flow rate of the circulating water using a simple control valve. Pressures of 0.07 MPa or 0.14 MPa were maintained in the chamber during measurements. The chamber had a lid with three openings for the seedling stems so that three seedlings could be measured simultaneously. For measurements, seedlings were severed at the root collar in water and bark was removed 2 cm from

the top of cut ends. This prevented the exudation of phloem sap which could otherwise interfere with the readings. Root systems were allowed to soak in tap water (at 15-17°C) for a minimum of 30 min before measurements were made, as this reduced the time for the flux of water through the stem to reach a steady state. Once the root systems were cut at the root collar, they were immersed in water in the pressure chamber at a specified temperature. Their cut ends protruded through rubber stoppers in the lid of the vessel. Small amounts of high vacuum grease was applied around the stem to maintain a water tight seal.

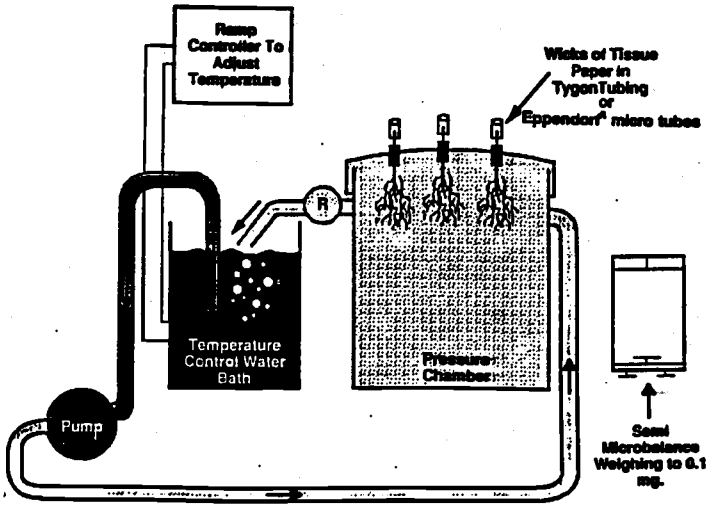

Figure II.2. Schematic diagram of the components of the root hydraulic conductance meter (Weyerheuser Co.).

Once the system was pressurized to the level required, water exuded from the cut end of the seedling. The exuding water was collected in absorbent paper in a 1.5 ml Eppendorf^{R} micro tube (with its tip cut off) at regular intervals. It took 20-30 min for the rates of water flux to reach a steady state. After initially weighing the micro tubes and paper at time 0, the water from the cut end was weighed at regular intervals of time (in this case, 20- to 40-second intervals) using a microbalance (weighing to the nearest 0.1 mg). Between 8 and 10 readings were taken for each seedling. Mean root conductance was expressed per ml of root volume (root hydraulic conductivity; µl of sap exuding ml root volume^{-1} min^{-1}). Before measurements were made the root volumes of all sample seedlings were determined using the volume displacement method (Rose et al. 1991).

Root conductance was measured at the end of 30 days on the exposed and control seedlings transplanted on January 17th and grown under ambient conditions in pots in planting beds. Seedlings were carefully uprooted, washed free of soil and placed in a cooler at 4°C overnight before measurements were made. The number of new root tips less than or > 1cm long were also counted for each of the seedlings that were measured. Root conductance was measured on a random sample of 3 seedlings from each treatment at a pressure = 0.14 MPa, with the water temperature at 15^{0}C with the method described above. Root hydraulic conductance was also measured immediately after the roots and shoots of seedlings were exposed to 23°C for 60 min. Conductance was measured at a pressure = 0.07 MPa and at a water temperature of 15°C.

Statistical Analysis

All statistical analyses were made using SAS (SAS Institute Inc.1989). Percent budbreak scores were ranked and examined by standard ANOVA. Differences between means were separated by the Fisher's Protected Least Significant Difference (FPLSD) at $p<0.05$. A Covariate analysis (ANCOVA) was made to determine which of the initial morphological seedling characteristics were significant covariates of final growth parameters with treatment and transplanting date as the main effects. Total biomass, shoot biomass, root biomass, spring terminal growth, and stem diameter at the end of the growing season were the dependent variables of interest. Type III mean sum of squares, corresponding F ratios and associated P-values are reported for the significant variables ($P<0.05$) in the final covariate model for each of the dependent variables.

For all the physiological measurements, except root conductance, the data were subjected to a standard 2-way ANOVA. Transplanting dates and exposure treatments were the two main factors. No analysis was done on the root conductance measured for the January transplants because the sample size was small. Only means with standard errors are presented. Significant differences among treatment means were estimated using FPLSD. Linear regression analysis between variables using PROC GLM procedures was done using the model $y = \propto + \beta x$.

Results

Pre-planting Seedling Evaluation.

Douglas-fir seedlings transplanted on different dates showed differences in cold hardiness, the degree of dormancy and root growth potential. These measurements were made in order to characterize the physiological condition of the seedlings on different transplanting dates. These data were not subjected to statistical analysis since there were no true replications. January transplanted seedlings had a mean lethal temperature of -21.5°C for 50% mortality (LT_{50}), while those transplanted in March and April had LT_{50}'s of -9 and -6°C respectively. April transplanted seedlings took 14 days (Dormancy Release Index = 10/days to terminal budbreak, DRI=0.71) to break bud under a forcing environment, while January and March transplanted seedlings took 49 (DRI=0.20) and 17 days (DRI=0.58) respectively. On an average, 24 new roots were recorded for the January planted seedlings in the RGP test, while the March and April planted seedlings had 14 and 29 new roots, respectively.

The ψ immediately after exposing the seedlings to air varied with the type of exposure and time of transplanting. The larger the exposed area of the seedlings, the greater the drop in the ψ; however, in April, transplants with only roots exposed (R3) showed the same drop in ψ as exposing both roots and shoots (RS3) (Fig II.3). When both root and shoot were exposed to air, the ψ was lower than -1.1 MPa, while the untreated control had ψ > - 0.3 MPa. Lower ψ for R3 seedlings in April

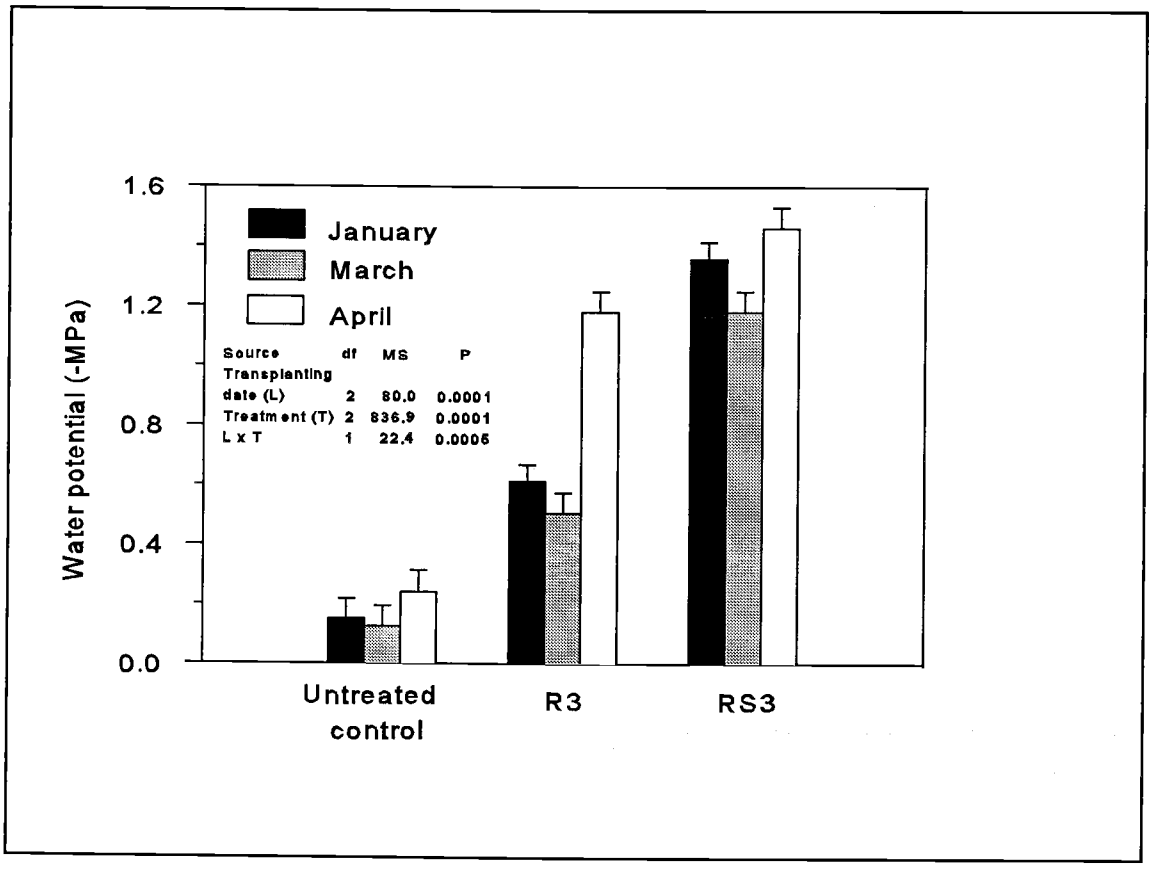

Figure II.3. Immediate changes in ψ after different exposure treatments in a temperature controlled chamber. Untreated control=no exposure; R3=root exposure for 3 hrs; RS3 = root and shoot exposure for 3 hrs. Conditions of exposure t=4°C, RH=85%, Photosynthetically active radiation=7.3 µmoles m^{-2} s^{-1}, and a wind speed= 1-1.5 m sec^{-1} (2-3 mph). Vertical bars represent one standard error of the mean.

indicated that they apparently lost more water through their roots than for R3 seedlings in either of the other transplanting dates.

Initial morphology of seedlings varied with time of transplanting. Seedlings transplanted in April (40 g fresh weight) were significantly ($P<0.05$) heavier than those transplanted in either January (32 g) or March (34 g). Stem diameter and root

volumes also showed similar differences between transplanting dates. Higher root volumes for the April seedlings indicated that between March and April there was a significant growth in new roots.

Budbreak

Table II.1. P-values from ANOVA for the different transplanting dates and treatment effects for percent budbreak. Budbreak scores were Rank transformed prior to ANOVA.

Source of variation	Terminal budbreak	Lateral budbreak
Day of observation (D)	0.0001	0.0001
Transplanting date (L)	0.63	0.02
Exposure treatment (T)	0.09	0.003
L x T	0.78	0.12
L x D	0.93	0.0001
T x D	0.0001	0.0001
L x T x D	0.08	0.11

The effect of exposure treatments on % budbreak (terminal and laterals) was significantly influenced by the day of observation (Table II.1). For the terminal buds, during the first 10 days of observation, a very small percent of the seedlings had broken bud so the difference between the treatments was not obvious (Fig II.4). After April 20, the untreated controls broke bud more rapidly than either R3 or RS3 treatments. For lateral buds, untreated controls showed more rapid bud break after

day 5 than the other treatments. The % terminal bud break was unaffected by transplanting dates. However, the effect of transplanting dates on % lateral budbreak was significantly influenced by the day of observation (Table II.1). The % lateral budbreak of April transplanted seedlings lagged behind both March and January transplants until April 20 (Fig II.4).

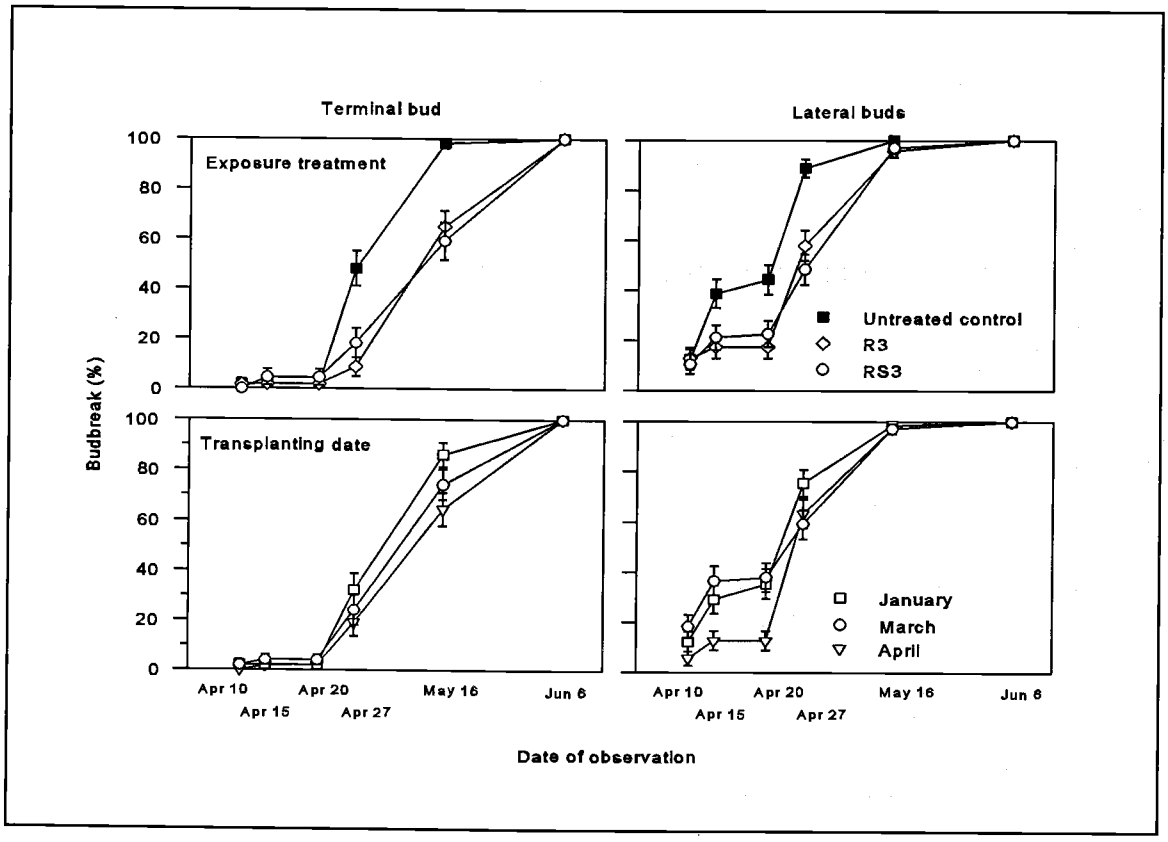

Figure II.4. The effect of different transplanting dates and exposure treatments on the rate of budbreak of terminal and lateral buds of 2-year-old Douglas-fir seedlings. See Table II.1 for the level of significance of treatment effects.

Water Relations and Root Growth During the First 55 Days After Transplanting

Water relations of seedlings during the first 55 days after transplanting were characterized by g_s and predawn ψ (fig II.5). There was no clear difference in the magnitude of g_s values between transplanting dates, although ψ tended to be lower for the April transplants. Seedlings that were exposed showed a lower ψ and g_s than the untreated controls on all transplanting dates. Exposing both roots and shoots did not increase the water deficit or decrease g_s more than did exposing roots alone. Regardless of treatment, larger g_s appeared always to correspond to higher predawn ψ (Fig II.5) and vice-versa. However, for January and March transplants, g_s was highly sensitive to small changes in predawn ψ. For instance between 30 and 40 days after transplanting January seedlings, g_s of untreated controls was 4x higher than the exposed treatments whereas ψ increased by only 0.25x. April transplants, on the other hand, did not show such a relationship between the predawn ψ and g_s. On average, April transplants showed the lowest ψ, especially for the exposed seedlings, indicating an increased water deficit.

New root growth, measured as number of new root initiates at the end of the 40-55 days, was significantly related to predawn ψ (Fig II.6), but was influenced by when seedlings were transplanted. The predawn ψ of April transplants was more sensitive to the number of new root initiates than for either of the other two dates, as observed by a steeper slope for the relationship between new root growth and predawn ψ. Differences between slopes could not be statistically analyzed because the number of new root initiates in April over 50 were not counted. A majority of

the seedlings in April had over 50 new roots initiated at the end of 55 days, whereas on the other two dates very few seedlings had over 50 new roots at the end of 40 days. Differences between treatments for mean number of new root initiates could also not be statistically analyzed because of the inadequate quantification of roots for the April transplants. However, mean number of new root initiates in April was 38 while it was only 14 on the other months. Similarly, mean number of new root initiates was 30 for the untreated control seedlings while only 17 for both R3 and RS3 seedlings.

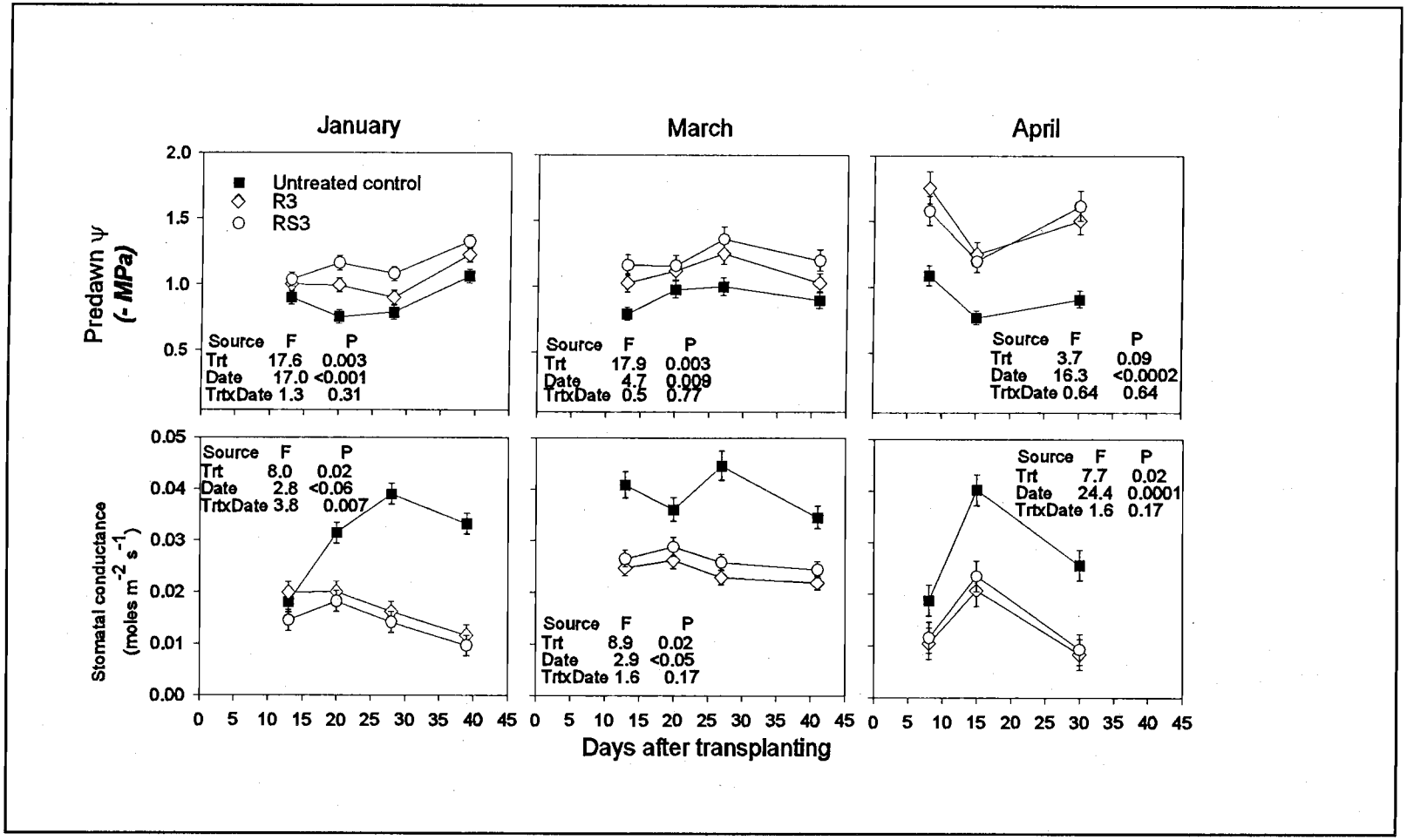

Figure II.5. Effect of different transplanting dates and exposure treatments on patterns of predawn ψ and midday g_s during the first 55 days after transplanting 2-year-old Douglas-fir seedlings. Vertical bars represent one standard error.

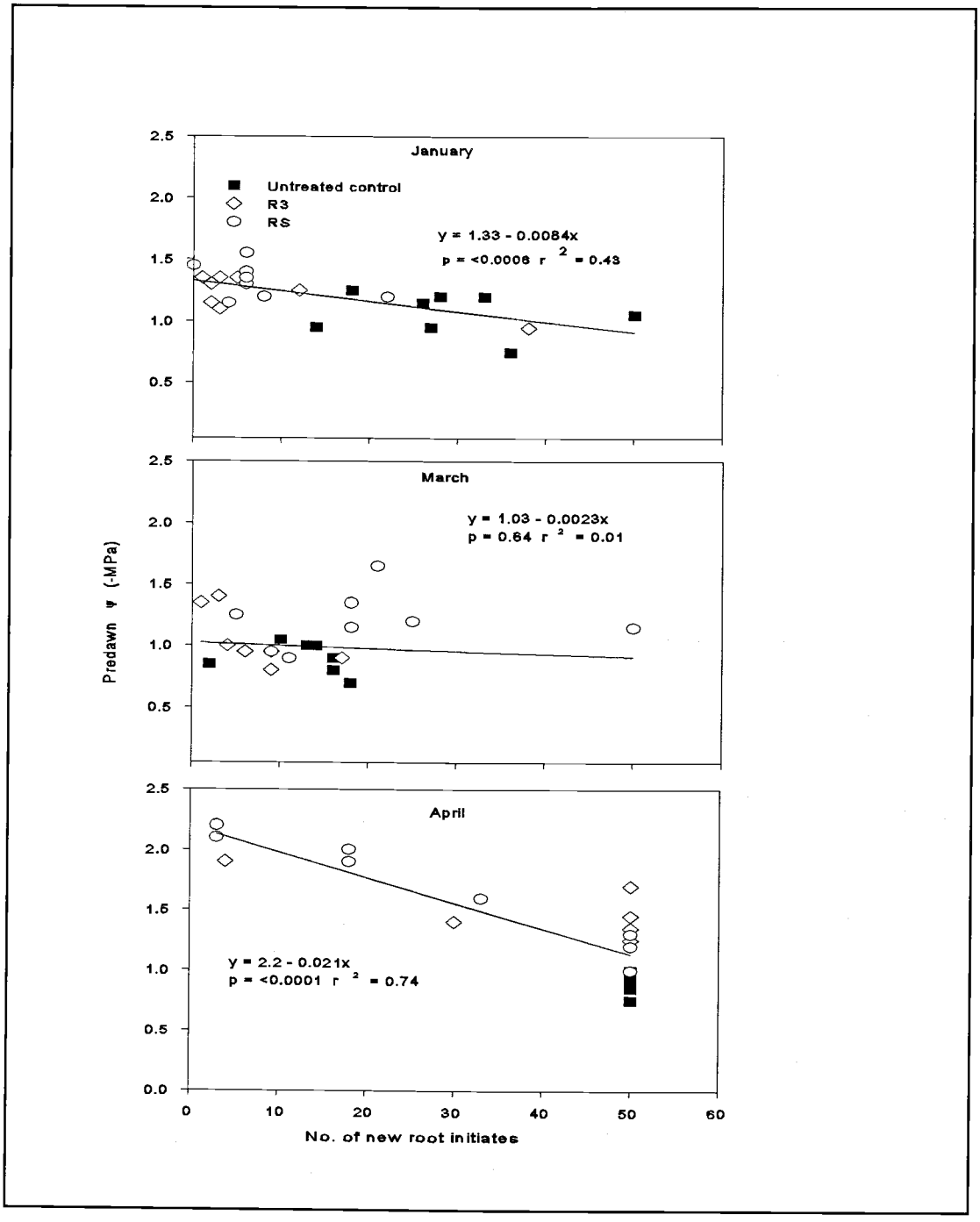

Figure II.6. The relationship of number of new root tips to predawn ψ of 2-year-old Douglas-fir seedlings transplanted on different dates and subjected to different exposure treatments. The line fitting the data was generated by regression analysis using the linear equation of the form $y = \propto + ß(\log x)$.

Water Relations and Net Photosynthesis During Active Leader Elongation

There were significant differences among treatments for midday ψ, g_s, and net photosynthesis (A) on May 21, but there were no such differences on June 7 (> 17 days), indicating that the exposed seedlings had recovered physiologically (Fig II.7, Table II.2). There was a significant difference in midday ψ and net photosynthesis between exposed and untreated control seedlings for the April transplants on May 21, but there was no difference on June 7. Untreated controls of April transplants also had a lower midday ψ than either of the treatments of January transplants. On May 21, the April RS3 seedlings had midday ψ -1.0 MPa lower than January RS3 seedlings, and net photosynthesis that was only 20 % of January RS3 seedlings. Stomatal conductance was unaffected by the treatments (Table II.2). However, on May 21, mean g_s of April seedlings was 50% of January seedlings, but again on June 7 there were no differences between the transplanting dates.

Leader elongation rates between the two periods also parallel this physiological change. Growth rates for the first period were lower than the second period for all treatments (Fig II.7). They ranged from 0.06 cm d^{-1} for the April root+shoot (RS3) exposure to 0.19 cm d^{-1} for the January untreated controls. Growth rates for the second period ranged from 0.21 cm d^{-1} for the April RS3 stress to 0.32 cm d^{-1} for the January RS3 exposure. Both the April treatments and the January RS3 seedlings showed a larger difference in growth rates (0.18 cm d^{-1}) between the first and second period than the January untreated controls (0.08 cm d^{-1}).

Table II.2. P-values from ANOVA for midday water potential (ψ), stomatal conductance (g_s), net photosynthesis (A), and terminal elongation rate as affected by transplanting dates, treatments and the two measurement periods.

Source of variation	Midday ψ	g_s	A	Elongation rate
Transplanting date (L)	0.0001	0.004	0.002	0.02
Treatment (T)	0.0001	0.25	0.009	0.02
L x T	0.01	0.42	0.04	0.08
Day (D)	0.0001	0.77	0.0003	0.0001
D x L	0.0001	0.007	0.0003	0.16
D x T	0.08	0.79	0.01	0.02
D x L x T	0.75	0.13	0.002	0.81

On May 21, decreasing midday ψ significantly explained the variation in stomatal conductance, net photosynthesis and terminal growth rates (Table II.3, fig II.8). However, on June 7, low midday ψ was associated with lower terminal growth rates but showed no relationship with the other variables. Net photosynthesis was limited more by ψ below -1.2 MPa. Of the different treatments, the April RS3 seedlings had midday ψ that were lower than January RS3 seedlings by -1.0 MPa, and net photosynthesis was only 20 % of January RS3 seedlings. Stomatal conductance explained 62 and 53 % of the variation in net photosynthesis on both days, respectively.

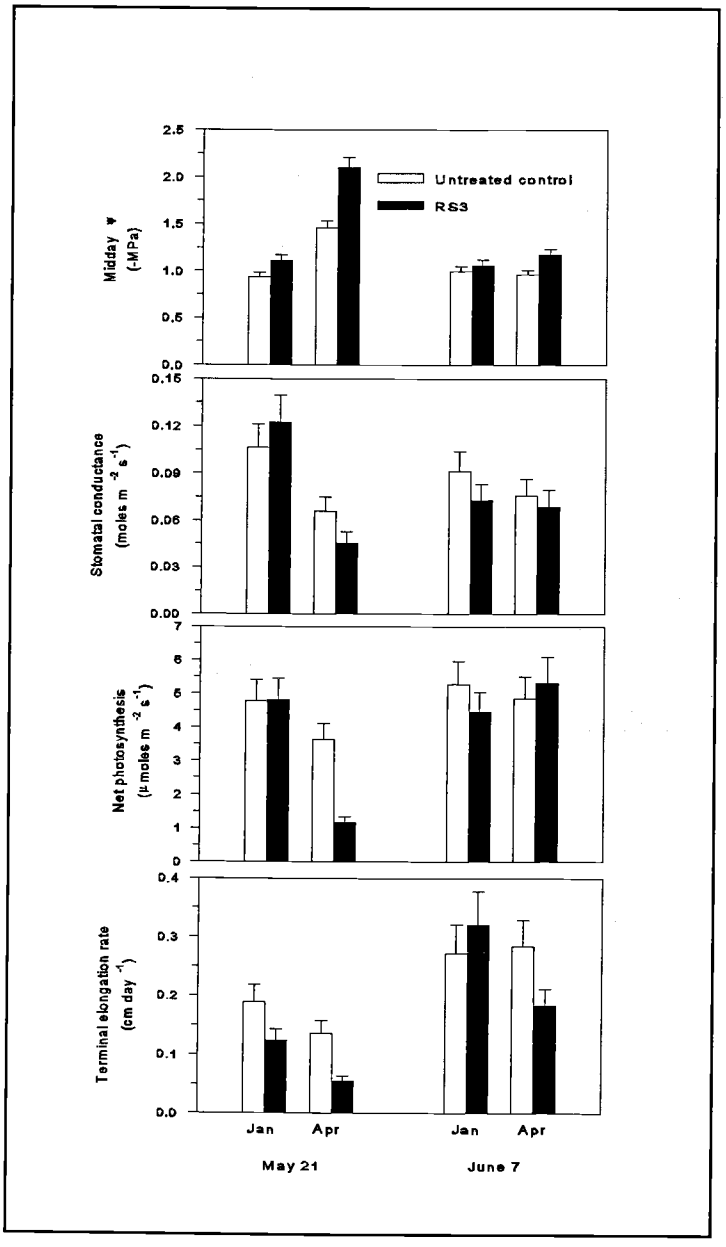

Figure II.7. Effect of different transplanting dates and exposure treatments on midday ψ, g_s, net photosynthesis and terminal elongation rates for 2-year-old Douglas-fir seedlings on May 21 (PAR 1109 μmoles m^{-2} s^{-1}, 23.9°C, 17.1 mbars VPD) and June 7 (PAR 1068 μmoles m^{-2} s^{-1}, 24.5°C, 19 mbars VPD), after they had broken bud (n=8). The vertical bars represent one standard error. See Table II.2 for significance of treatment effects.

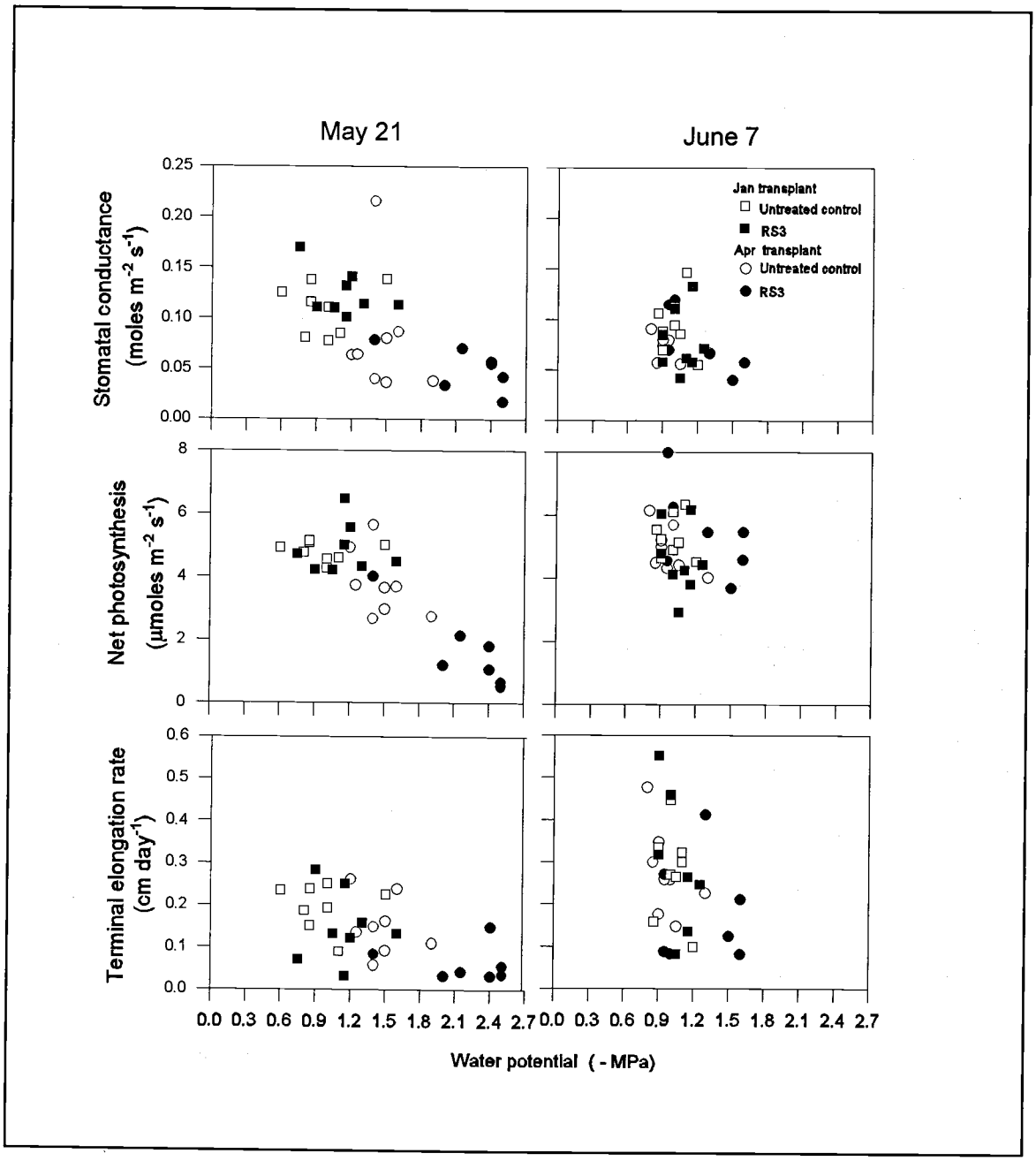

Figure II.8. Effect of transplanting dates and exposure treatments on the relationship of midday ψ to terminal growth rate, g_s and net photosynthesis for 2-year-old Douglas-fir seedlings on May 21 and June 7, after they had broken bud (see table II.3 for regression parameters).

Table II.3. R^2 values and significance levels (p-values) from linear regression analysis between net photosynthesis, plant ψ, stomatal conductance, and terminal growth rate on May 21 and June 7 for transplanted 2-year-old Douglas-fir seedlings (n=32).

Physiological variables[1]	Net photosynthesis		Stomatal conductance		Growth rate	
	May 21[2]	June 7	May 21	June 7	May 21	June7
Water potential	0.60	0.02	0.42	0.11	0.31	0.15
	(0.0001)	(NS)[3]	(0.0001)	(NS)	(0.0001)	(0.03)
Stomatal conductance	0.62	0.53	-	-	0.21	0.02
	(0.0001)	(0.0001)			(0.01)	(NS)
Net photosynthesis	-	-	-	-	0.38	0.04
					(0.0003)	(NS)

1 Net photosynthesis = (μmoles m^{-2} s^{-1}); Water potential = (-MPa); Stomatal conductance = (moles m^{-2} s^{-1}), Growth rate = Rate of terminal elongation (cm d^{-1})
2 Seedlings were planted on January 25 and April 8.
3 NS = Not significant at $p \leq 0.05$.

Stem Conducting area

Untreated controls from both transplanting dates had lost 20% of their stem conducting area, presumably to cavitation. Untreated controls showed no difference

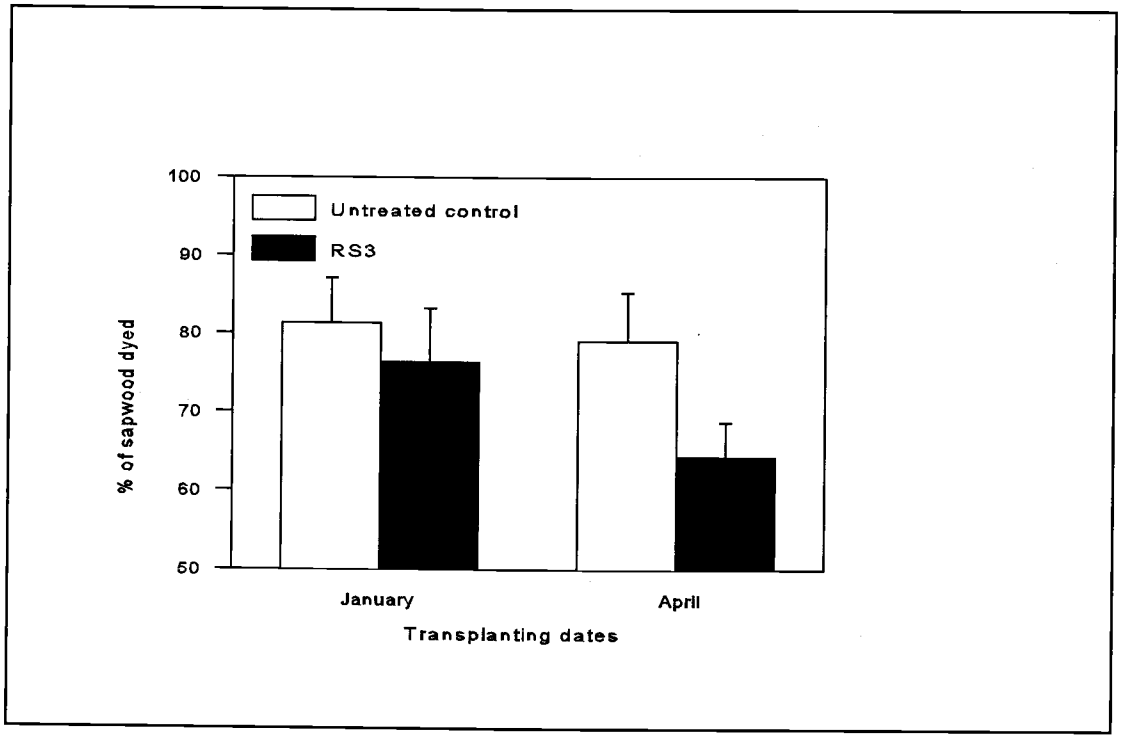

Figure II.9. Mean % dyed stem-sapwood of 2-year-old Douglas-fir seedlings planted on different transplanting dates with different exposure treatments. Bars represent one standard error of the mean.

between January and April transplants (Fig II.9). However, when both roots and shoots of seedlings were exposed for 3 hrs in a cooler at 4°C, the April transplants had lost 35% of its conducting area while the January transplants had lost only 25% ($p<0.15$). The lack of a higher significance in mean differences is

most probably due to the small sample size. There was a small (r^2=0.26) but significant ($p<0.01$) positive correlation between % of functional area and the spring terminal length when all treatments were pooled (Fig II.10)

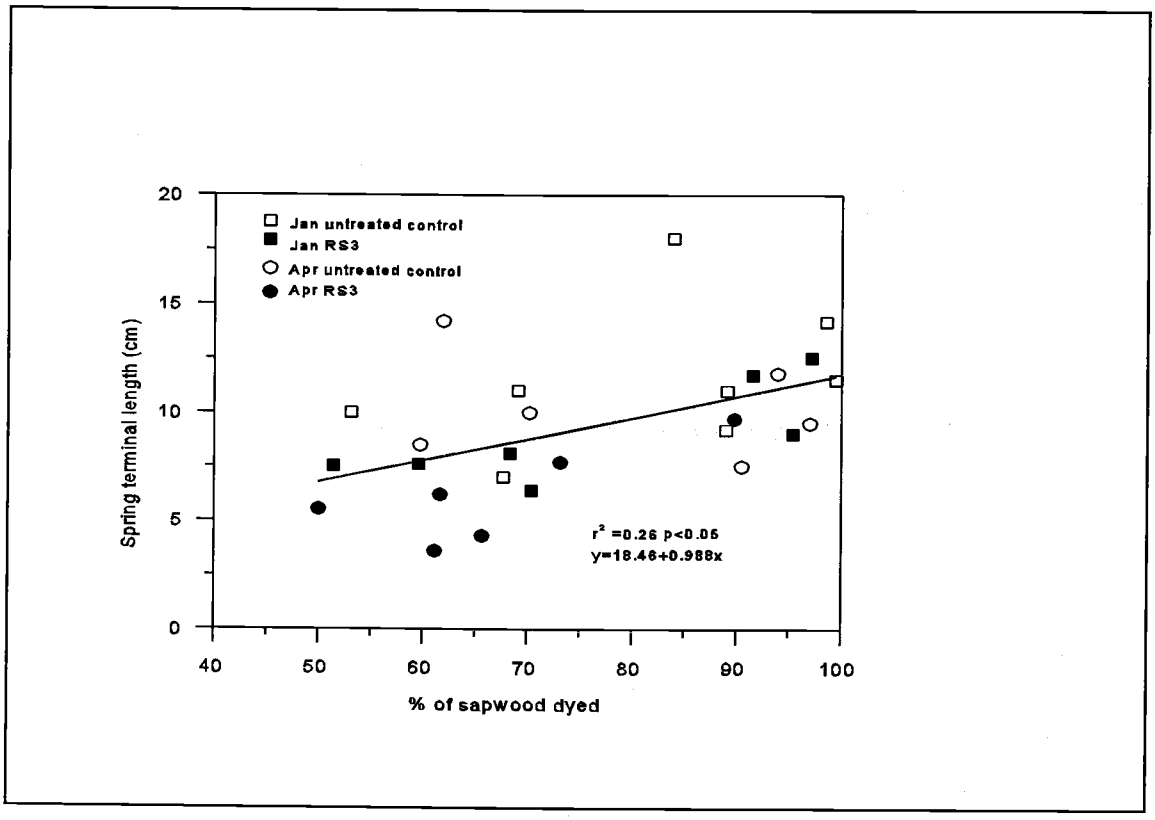

Figure II.10. The relationship of % dyed stem-sapwood and spring terminal length of 2-year-old Douglas-fir seedlings. Data were pooled across transplanting dates and exposure treatments.

Root conductance

After 30 days in the soil, seedlings that had been exposed to dry air had lower root conductance than untreated controls (Fig II.11). The number of new roots was

also considerably reduced relative to the untreated controls. Root conductance tended to increase with increasing root volume (Fig II.12). Root and shoot exposure of seedlings for 60 min, at room temperature (23°C) decreased root conductance by about 60% of the untreated control (Fig II.13). However, the variation between individual seedlings was large.

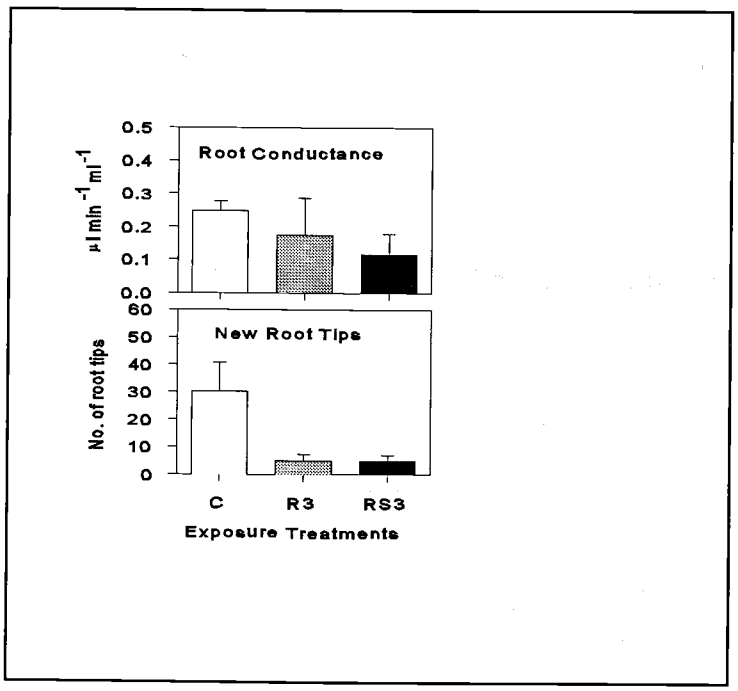

Figure II.11. The effect of exposure on the initiation of new roots and the conductance of the root system of 2-year-old Douglas-fir seedlings, after 30 days under ambient conditions. C= untreated controls, R3= only roots exposed. RS3= root and shoot exposed (whole seedling). Seedlings were exposed in a temperature controlled chamber for 3 hrs at 4°C. Root conductance was measured at a pressure = 0.14 MPa, water temperature=15°C. Vertical bars represent one standard error of the mean.

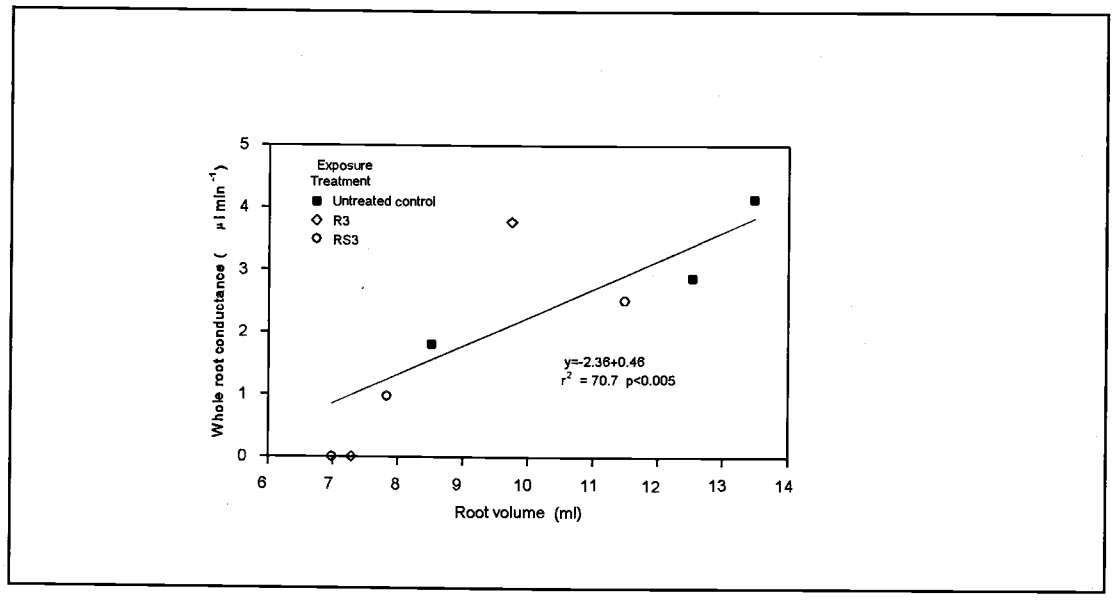

Figure II.12. The relationship of root conductance to root volume in 2-year-old Douglas-fir seedlings for all treatments. Line fitted using a regression model of the form $y = \propto + \beta x$. Conductance was measured at a pressure=0.14 MPa, water temperature=15°C.

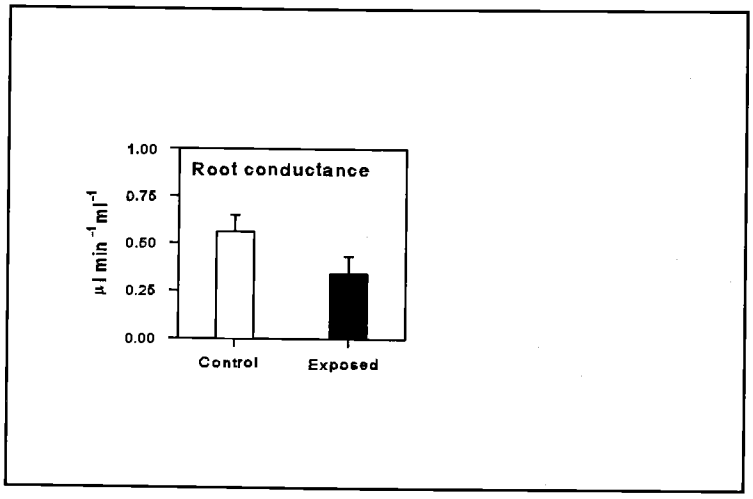

Figure II.13. The effect of seedling exposure on root conductance of 2-year-old Douglas-fir seedlings. Untreated control= no exposure, Exposed= Root and shoot exposed at 23°C for 60 min. Conductance was measured at a pressure=0.07 MPa, water temperature=15°C.

Final Growth Response

The exposure treatments significantly reduced most growth components. The R3 and RS3 treatments showed no difference for most growth components, except for RGR of biomass and root volume. Both RGR of biomass and root volume showed a significant interaction between transplanting dates and exposure treatments (Table II.5). All other growth components showed no interaction between exposure treatments and time of transplanting (Table II.4). Among transplanting dates, January and March seedlings performed to the same degree, while April transplants were more reduced. Seedlings that were transplanted later in the year (April) had reduced spring terminal growth and only a smaller percentage of seedlings showed lammas growth (Fig II.14). The April transplants had significantly higher R:S ratios, indicating a higher allocation to root biomass than to shoot biomass (Fig II.15). Both biomass RGR and stem diameter RGR were lower for April than for either January or March transplants (Fig II.16).

Table II.4. P-values from ANOVA for terminal and biomass growth as affected by transplanting dates and exposure treatments.

Source of variation	Spring terminal	Total length	Needle density	Shoot biomass	Root biomass	R:S
Transplanting date (L)	0.0002	0.001	0.04	0.18	0.98	0.009
Treatment (T)	0.0001	0.0001	0.005	0.0001	0.001	0.01
L x T	0.98	0.96	0.80	0.95	0.55	0.47

The terminal leader length and needle density were significantly affected by transplanting dates and exposure treatments, whereas root and shoot biomass was unaffected by the time of transplanting (Table II.4). Exposing the whole seedling for 3 hrs (RS3) had the same effect on spring leader growth as those seedlings with only roots exposed (R3). Untreated controls were longer than the stressed treatments by about 25-30% regardless of lift dates (Fig II.14). Terminal leader length decreased for seedlings from January to April by about 20%. The percentage of seedlings flushing a second time (lammas growth) decreased from January (40%) to April (20%) for the untreated controls and from < 20% to < 5% from January to April for the root stress treatments (Fig II.14). Final bud widths of the exposed seedlings were significantly ($p<0.005$) lower by 15% than the untreated controls. Relative biomass allocation to the roots increased from January to April and showed a similar trend between the untreated controls and the exposure treatments (Fig II.15).

Table II.5. P-values from ANOVA for final growth and relative growth rates of total biomass, stem diameter, and root volume as affected by transplanting dates and the exposure treatments.

Source of variation	Total biomass	RGR (biomass)	Stem diameter	RGR (Stem diameter)	Root volume	RGR (Root volume)
Transplanting date (L)	0.55	0.32	0.055	0.009	0.79	0.086
Treatment (T)	0.0001	0.0002	0.0001	0.0001	0.004	0.02
L x T	0.93	0.036	0.98	0.18	0.66	0.02

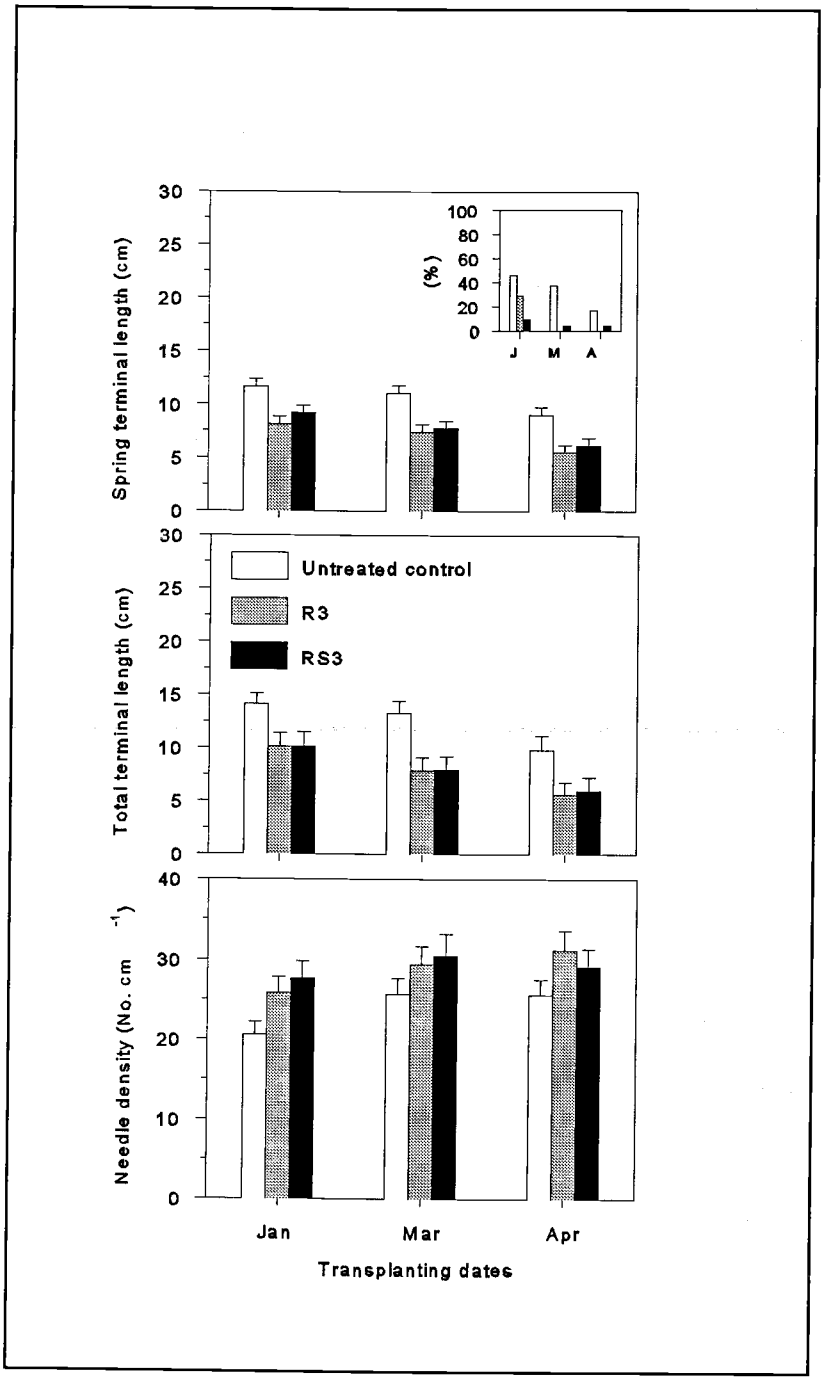

Figure II.14. The effect of transplanting dates and exposure stress on the components of terminal growth of 2-year-old Douglas-fir seedlings. Figure insert is the percentage of seedlings that had a summer flush. Vertical bars are one standard error of the mean. See Table II.4 for the level of significance of treatment effects.

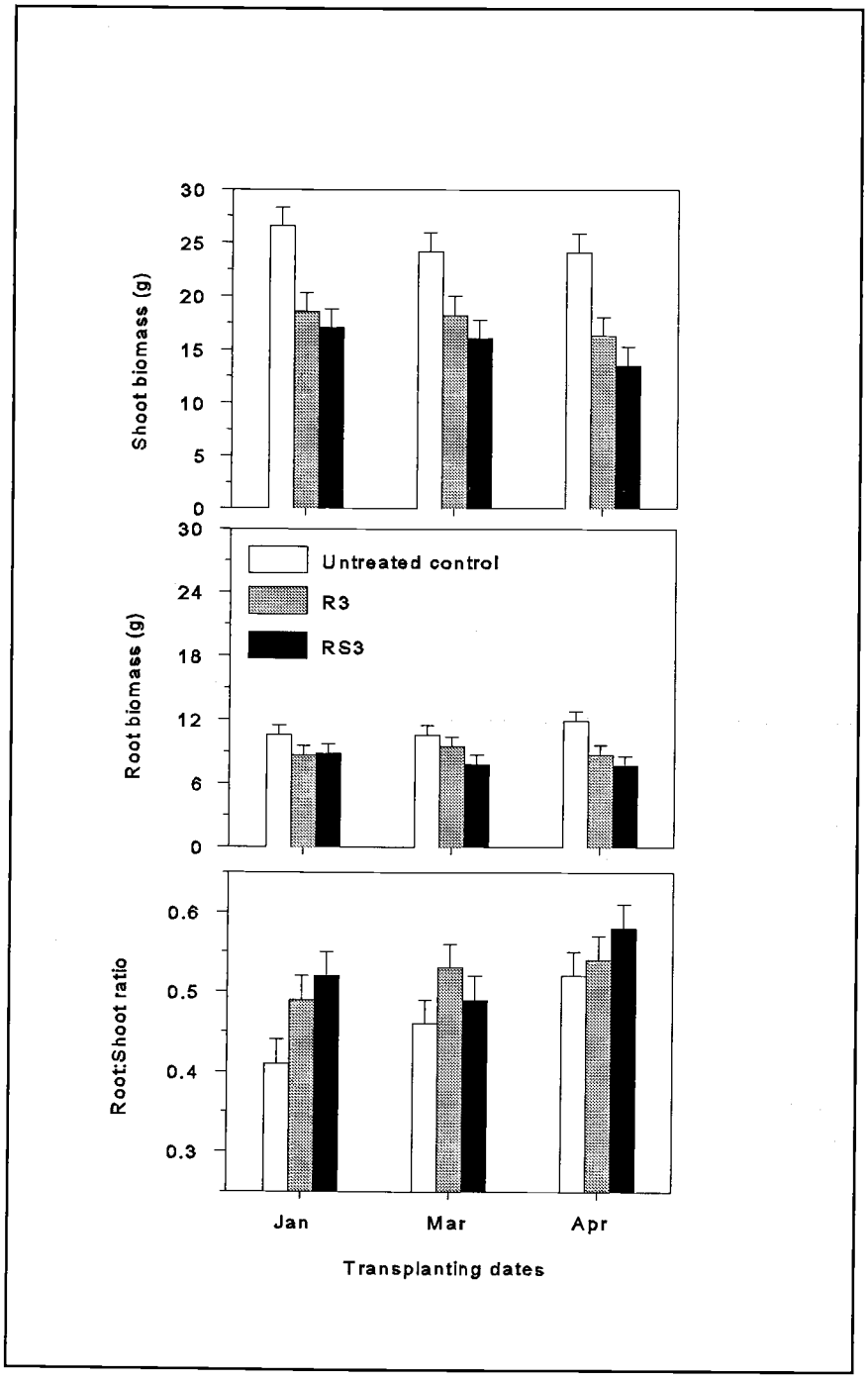

Figure II.15. The effect of transplanting dates and exposure stress on root and shoot biomass, and the ratio of root:shoot biomass of 2-year-old Douglas-fir seedlings. Vertical bars represent one standard error of the mean. See Table II.4 for the level of significance of treatment effects.

As mentioned earlier, at the time of planting, the biomass, stem diameter and root volume of April transplants were significantly greater than either January or March transplants. As a result, the final biomass, stem diameter or root volume showed no differences among the transplanting dates (Table II.5, Fig II.16). However, biomass RGR and root volume RGR showed a significant interaction between exposure treatments and transplanting dates (Table II.5).

The biomass RGR of R3 treated seedlings declined from January to April, whereas the RS3 treated seedlings increased from January to April (Fig II.16). The untreated controls showed the highest biomass RGR in March, but remained the same for April and January. Stem diameter RGR of untreated controls was unaffected by transplanting dates. Both exposure treatments reduced the stem diameter RGR by 30% of the untreated controls regardless of transplanting date. Root volume RGR of R3 was the same as the untreated controls in January, but was significantly reduced in March ($p<0.05$) and April ($p<0.05$) (Fig II.16). In contrast, the root volume RGR of the RS3 treatment was the same as the untreated controls for the April transplants, but was significantly reduced in March ($p<0.05$) and January ($p<0.05$). Root volume RGR of the R3 treatments increased from January to April, whereas the root volume RGR of the RS3 treatments did not change between transplanting dates.

The initial fresh weight of seedlings prior to the exposure treatments was the single most significant covariate contributing to the variation among final biomass and diameter of Douglas-fir seedlings (Table II.6).

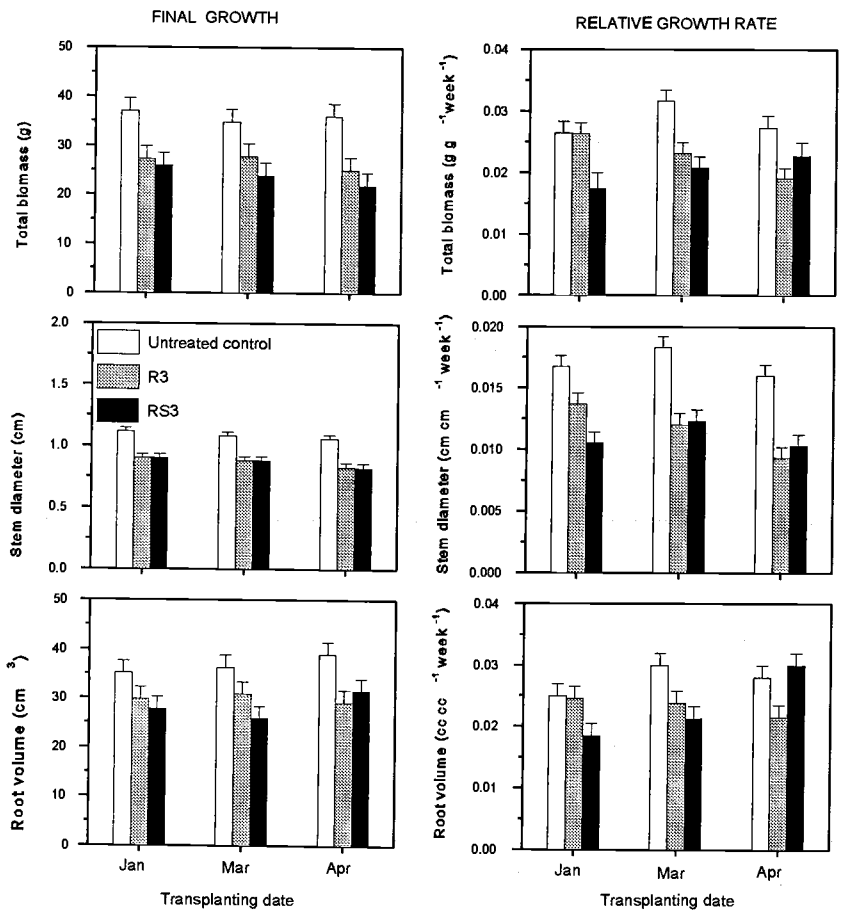

Figure II.16. The effect of transplanting dates and exposure stress on final biomass, stem diameter, and root volume and their relative growth rates (final-initial)/ initial/ days) of 2-year-old Douglas-fir seedlings. Vertical bars are one standard error of the mean. See Table II.5 for levels of significance of treatment effects.

This result suggests that the size of the seedlings at the time of transplanting significantly influences the response of seedlings to transplanting dates and differences in exposure treatments.

For none of the growth parameters analyzed did the covariates explain all the variation due to transplanting date or exposure treatments. The spring terminal length was significantly related to the initial budwidth, but was not related to the initial fresh weight. In addition to initial fresh weight, initial stem diameter was a significant covariate of final stem diameters.

Table II.6. P-values for significant covariates and treatment effects of some final growth measurements using ANCOVA.

	Final growth measurements		
Source of variation	Total biomass	Spring terminal	Stem diameter
Transplanting date	0.0001	0.0001	0.0001
Exposure treatment	0.0001	0.0001	0.0001
covariates:			
Fresh weight	0.0001	ns	0.0001
Stem diameter	ns	ns	0.007
Bud width	ns	0.0001	ns

Regardless of when seedlings were lifted, several initial morphological characteristics were significantly correlated with each other. The most inter-related were seedling fresh weight, stem diameter and root volume. Initial diameter was significantly ($p<0.0001$) correlated with fresh weight ($r^2=0.75$) and initial root volume

(r^2=0.57). Root volume was significantly ($p<0.0001$) correlated with fresh weight (r^2=0.81). Of the three variables physically characterizing a seedling at transplanting, fresh weight may be the simplest measurement, and also one that would effectively represent two other important traits, i.e., root volume and stem diameter.

Discussion

Exposing both roots and shoots to drying conditions prior to planting produced the same detrimental effect on growth and physiology of Douglas-fir seedlings as did exposing roots alone. This result demonstrates that roots are the most likely locus of damage during transplanting. Roots are also less tolerant than the shoots to exposure regardless of when seedlings were transplanted. The root system does not possess a cuticle or stomata like the leaves to regulate water loss. Roots are therefore vulnerable to water loss, particularly the younger roots with less suberization. Loss of water from the roots system may be primarily a function of the vapor pressure deficit (VPD), boundary layer resistance, and epidermal resistance. The variation in these factors may determine the extent to which seedlings may be damaged when exposed. The temperature and humidity (3°C, 85% RH) to which these seedlings were exposed, would have produced a very small VPD. The VPD can be calculated under these conditions by assuming that vapor pressure of the root tissue is at saturation with a corresponding vapor pressure of 6.45 mb. The ambient air is at a vapor pressure of 5.58 mb, the VPD would therefore be only 0.96 mb. This would provide a negligible gradient for water vapor loss from the tissue. On the other hand,

boundary layer resistance which is primarily a function of wind speed and root width should have been considerably reduced as a result of the wind generated in this exposure regime (Nobel 1991). Wind can potentially cause rapid drying of roots, consequently the need for extra care when handling and transplanting seedlings under windy conditions cannot be overemphasized (Cleary et al. 1978).

The degree of epidermal resistance of roots may be determined primarily by the extent of suberization. Since the degree of suberization increases with the relative age of the roots (Kramer and Kozlowski 1979), seedlings with more old roots than young roots may lose less water when exposed. Increased suberization of roots increases the hydraulic resistance to water absorption (Chung and Kramer 1975; Carlson 1986) and this may also increase the resistance to water loss when such roots are exposed. Unsuberized roots were not present on either the January or March lifted seedlings. However there were some new roots in the April transplants, which being less suberized than the old roots may have increased the evaporation of water. This loss of water may have caused the significantly lower ψ in April transplants that were root exposed. The presence of new roots in April coincides with a seasonal burst of new root growth prior to budbreak as soil temperature increases (Fielder and Owens 1988). Therefore, differences in the relative number of roots that are suberized at planting could contribute to the seasonal variation in tolerance to exposure.

Although warmer soils in spring may have increased new root growth over winter transplants, the difference in new root growth between the exposed and unexposed seedlings was more pronounced in spring than in winter. Soil temperature

in the beds in spring was around 12°C while in winter it was around 8°C. Root growth following planting depends on soil temperature (Stone et al. 1962; Lopushinsky and Kaufmann 1984; Tabbush 1986). Douglas-fir seedlings showed new root initiation after 10 days when the soil temperatures were above 8°C (Tabbush 1986). In another study, Douglas-fir showed the highest root activity in soils with temperatures >4°C (Dunsworth 1988a). Soil temperatures may play a more important role in new root initiation than preplanting root exposure when soil moisture is not limiting. Higher air temperatures combined with a lower relative humidity in spring may have increased water loss from the seedlings because of steeper vapor pressure gradients, resulting in larger water deficits. In the seedlings that were exposed to air prior to planting, such seasonal increases in the vapor pressure gradient would only increase their water stress, particularly, because root exposure decreases water uptake by decreasing root conductance and new root growth. This increased water stress in April (spring) exposed seedlings was evident in their low predawn ψ (Fig II.5). The resulting water deficit in seedlings could potentially have a negative feedback on new root growth even when soil temperature and moisture are optimum.

Exposing seedlings to dry air decreased stomatal conductance after planting, regardless of when seedlings were transplanted. Although lower conductance reduces water loss it also decreases the rate of carbon assimilation (Schulze and Hall 1982; Sandford and Jarvis 1986), which in turn can reduce the carbohydrates available for new root growth. In Pinus taeda seedlings, net photosynthesis dropped linearly with decreasing stomatal conductance regardless of the particular environmental condition

to which they were responding (Teskey et al. 1986). In Douglas-fir seedlings, new root growth depends on current photosynthate (Philipson 1988; van den Driessche 1991). New root growth in newly transplanted seedlings results in good root to soil contact (Sands 1984; Margolis and Brand 1990). Therefore, the decrease in stomatal conductance could exacerbate the process of seedling establishment and growth after transplanting.

For January and March transplants, the effect of root exposure on stomatal conductance did not appear to be modulated by ψ. This result is evident from the treatment differences between ψ and stomatal conductance. Seedlings show a smaller variation in predawn ψ, but a much larger variation in stomatal conductance between the exposure treatments. Root exposure could reduce water uptake by delaying new root growth and also by a concurrent loss in hydraulic conductivity (fig II.11), leading to lower plant ψ. Tissues under water stress accumulate abscisic acid (ABA) (Raschke 1982; Quarrie 1984). ABA modulates the drop in guard cell turgor, consequently reducing stomatal conductance (Blake and Ferrell 1977; Davies et al. 1986). This general mechanism, where stomatal closure is linked to decreasing internal ψ, could explain the low stomatal conductance measured for root-exposed April transplants. However, reduced stomatal conductance in the exposed winter transplants, where there was no parallel drop in ψ may be regulated by some other non-hydraulic, hormonally mediated effect on guard cell metabolism. One possible mechanism may involve the hormone cytokinin and root activity (Davies et al. 1986). Cytokinin, which is primarily synthesized in the root meristems (Short and Torrey

1972; Moore 1989) is transported to the shoot through the xylem (Doumas and Zaerr 1988). Cytokinin is known to increase the stomatal conductance in some species of plants (Letham et al. 1978; Davies et al. 1986; Incoll and Jewer 1987). A lack of meristematic activity of roots in January and March due to low soil temperatures combined with root damage due to exposure may lead to lower levels of cytokinins in the system which may reduce stomatal conductance, in spite of higher ψ. Coutts (1980) found that root damage to Sitka Spruce did not affect the needle ψ but reduced stomatal conductance, which suggested the effect on the stomata of some unknown chemical stimulus originating from the roots. In another study, cytokinin levels in Douglas-fir seedlings were low during winter and increased in spring (Morris 1978).

January transplanted seedlings that were exposed had recovered physiologically during the early stages of shoot elongation. However, the terminal shoot had already been affected, being shorter than the untreated controls. On the other hand, during this period, both untreated controls and exposed April transplants continued to show signs of physiological stress and reduced growth. The physiological recovery of the exposed January seedlings in spite of colder soil temperatures and lower initial stomatal conductance may be due to several factors. January transplants have a high root growth potential and a relatively longer period (90 days) in the soil before active shoot growth begins in spring allowing more time for establishing good root-to-soil contact. As a result, the number of new roots for January transplants during budbreak and new shoot growth may have been adequate to maintain a favorable

water status. Additionally, competition for carbohydrates by the roots may have been minimal, allowing a relatively higher allocation to shoot growth. Under normal conditions, new shoots can be a major sink for photosynthate (Krueger 1967; Webb 1977; Chung and Barnes 1980). However, if roots are disturbed or damaged, and are under stress, allocation to the roots may proceed at the cost of shoot allocation. Competition for limited resources may be accentuated in newly planted seedlings. This was demonstrated by a significant increase in terminal leader length of Douglas-fir when the lateral buds were removed before budbreak (unpublished data). On the other hand, seedlings planted in April have a short period (27 days) between planting and new shoot growth in which to grow adequate new roots to meet the growing shoot's water requirements. Secondly, new root growth in April may be a stronger sink for the limited carbohydrates than shoot growth. This reallocation of photosynthate to the roots may contribute to the observed stunted growth of terminals in April. The higher root:shoot ratio for April transplants supports this conclusion. More research to understand allocation patterns of photosynthates in newly transplanted seedlings would be necessary to confirm some of the observations reported here.

Cavitation-induced loss in stem conducting area appears to be a common phenomenon in transplanted seedlings. Because even the untreated controls had lost stem conducting area, the mere process of transplanting seedlings may have caused a sufficient drop in ψ to initiate cavitation. Root stresses imposed on seedlings while transplanting would aggravate the condition. For Douglas-fir, cavitation is initiated at

ψ lower than -2.0 MPa and at -5.0 MPa, there is a 100% loss in conductance (Cochard 1992). Cavitation proceeds from the early wood trachieds to late wood trachieds (Tyree et al. 1984; Dixon et al. 1984; Sperry and Tyree 1990). Most of the samples had no dye on the inner rings, suggesting that the previous years growth and the current early wood showed most of the cavitation. In western hemlock, xylem cavitation was initiated almost immediately following planting in moist soils (Kavanagh 1993). Cavitation in newly transplanted western hemlock seedlings may have been caused by increased water deficits, as a result of a higher ratio of shoot water loss to root water uptake. Western hemlock also starts to cavitate at higher ψs (<-1.5 MPa) (Kavanagh 1993) than Douglas-fir (<-2.0 MPa) (Cochard 1992).

Seedling exposure prior to transplanting reduced the area of stem conductance, probably due to cavitation during the first year's growth. Because cavitation in Douglas-fir occurs only at ψ below -2.0 MPa (Cochard 1992), exposed seedlings must have had periods of water stress after transplanting when ψ was <-2.0 MPa. Since cavitated tracheids may not refill under natural conditions in conifers (Sperry and Tyree 1990), cavitated conduits would tend to increase cumulatively when ψ decrease < -2.0 MPa. Therefore, any drop in ψ due to an increase in resistance to water flow through the soil-plant-atmosphere continuum (SPAC) would likely increase the possibility of cavitation.

Exposing roots may also induce cavitation of root xylem and cause a loss in the functional root conducting area. Root xylem may be equally or more vulnerable to cavitation than the shoots, although there are hardly any reports on the

vulnerability of roots to cavitation. Most of the work has focused on stem cavitation. Root xylary cavitation may occur at ψ that are higher than in the stems. For example, in Acer grandidentatum *Nutt.*, root xylem was more susceptible to embolism than stem xylem (Alder et al. 1996). Therefore, if root cavitation had occurred in seedlings that were exposed, then the subsequent increase in the resistance to water uptake would increase the possibility of ψ dropping below -2.0 MPa. This drop in ψ could potentially induce irreversible stem cavitation during establishment of seedlings. Research on the vulnerability of seedling roots to cavitation is warranted, since root damage appears to be the locus of transplanting stress.

Root-exposed seedlings planted in April had a higher loss in stem conducting area than those seedlings that were root exposed and planted in January. April transplants may have experienced <-2.0 MPa water potentials as a result of higher VPD and lower water uptake than January seedlings. April-exposed seedlings having lost more water through the roots than January-exposed seedlings may have damaged their root system reducing their water absorbing capacity.

Increasing the % loss in stem conducting area due to cavitation reduced terminal elongation. Because elongation is turgor dependent (Ray 1987), any drop in turgor would decrease elongation. When the resistance to water flow through the stem increases as a result of cavitation, a steeper potential gradient between the roots and the shoots is necessary in order to supply the needles with adequate water. Therefore, this lowering of ψ in the shoots may reduce the turgor pressure. The elongation of the terminal leader in freshly transplanted Douglas-fir seedlings may be

inhibited by an increase in stem hydraulic resistance possibly due to a drop in shoot ψ and a concomitant drop in turgor.

Seedling exposure reduced root hydraulic conductance immediately after exposure treatment. Exposure to desiccating conditions could cause cavitation of the xylem of fine roots, thus reducing the percent of the total root surface area available to conduct water. Coutts (1980) found that the fine roots of Sitka Spruce lost over 200% of their moisture (on dry weight basis) on exposure to desiccating conditions, while the woody roots lost <100%, suggesting that the fine root may undergo relatively more damage. It is also possible that the exposure treatment reduced root permeability. Kramer (1950) and Brix (1960) found that droughted plants had reduced permeability of living cells in the roots to water. If cells in the cortex dried faster than the rate of water supply from the stele, these cells could have plasmolyzed, thus increasing the resistance to symplastic radial water flow through these roots. Steudle and Jeschke (1983) have shown that symplastic water flow is significant, if not a major pathway for radial flow from the root surface to the endodermis. Therefore, the immediate drop in root conductance may be caused not only by a loss in effective root surface for conductance, but also due to a decrease in root permeability.

The reduction in root conductance after 30 days by the exposure treatments was probably due to a concurrent decrease in the number of new roots. Eastern white pine seedlings increase root conductance with increase in new root surface area (Johnsen et al., 1988). Similarly, Carlson (1986) observed that the potential for water

uptake is proportional to the number of new roots produced. Grossnickle and Russell (1990) also found root resistance to water movement in yellow-cedar seedlings and cuttings decreased with new root surface area. Therefore, any residual effect of root exposure on root conductance may due to reduced new root initiation and growth.

Physiological recovery in terms of increased net photosynthesis, higher ψ and stomatal conductance during elongation did not cause a proportional increase in leader elongation rates, although there was an increase in elongation rates. Final terminal lengths were more reflective of physiological conditions during the initial stages of growth than the latter stages. It appears that the growing shoot is more sensitive to stresses during the early phase of growth, before some hypothetical "critical phase" in growth. Leaf expansion in annuals is very sensitive to early changes in water supply (Bradford and Hsiao 1982). In other experiments that I conducted (data not shown), when Douglas-fir seedlings were exposed prior to budbreak and transplanted into well-watered beds at 90% RH, elongation was not inhibited, but when seedlings were exposed after budreak and transplanted into the same beds, elongation was inhibited, suggesting that a critical stage in the phenology exits, beyond which elongation is irreversibly inhibited.

The permanent effect on spring growth due to the initial stress may be due to hormone mediated changes in cell wall extensibility and the stage of growth. ABA is known to inhibit elongation of shoots and accumulates in response to reduced ψ (Davies et al. 1986; Saab et al. 1990). But ABA levels are also known to decrease once ψ are restored to normal (Blake and Ferrell 1977). ABA decreases cell wall

extensibility in bean (Van Volkenburgh and Davies 1983). However, it is not known whether the effect of ABA on cell wall extensibility is reversible. If all the cells required for elongation or expansion growth are laid down under conditions when ABA levels are temporarily high due to water stress, wall extensibility could be irreversibly reduced, such that reduced elongation of these cells would inhibit expansion of the whole organ. Further, induction of ABA accumulation in growing zones is more sensitive to changes in turgor than the induction which occurs in mature tissues (Creelman and Mullet 1991). In tobacco, the sensitivity of cell division may vary with the progress of leaf development. The leaf initiation on the shoot apex, primarily a result of cell division, is apparently more sensitive to water deficit than is cell division in the expanding leaf (Clough and Milthorpe 1975). Therefore ABA may tend to accumulate in high concentrations in the growing terminal of Douglas-fir seedlings in response to small changes in turgor.

Another reason for reduction in shoot growth could be the relatively higher allocation of available photosynthates to roots at the expense of the shoots (fig II.15). Moderate water stress generally increases root growth relative to shoot growth (Schulze 1986). Thus carbohydrates could become limiting to the shoot during active elongation. This allocation pattern may be due to an increase in the relative strength of the roots as sinks for carbohydrates over that of the shoots. This change in sink strength may be triggered by lowered ψ and modulated by hormones. Roots of maize continued to grow at low ψ that completely inhibited shoot growth, and this was shown to be related to increased ABA concentrations in the shoots (Saab et al. 1990).

In soybean seedlings, the variation in endogenous ABA levels, and differing sensitivity to ABA in shoot and roots can modulate root/shoot growth ratios (Creelman et al. 1990).

Exposing seedlings in April had the same effect on biomass, stem diameter and root volume (fig II.16) as those seedlings exposed and planted in January or March. April lifted Douglas-fir seedlings with a Dormancy Release Index (DRI) of 0.58 would be generally characterized as having poor physiological vigor and low tolerance for stresses associated with transplanting (Ritchie 1984). Additionally, with increasing ambient temperature, and declining relative humidity, April transplants would have had to adjust to a higher evapotranspirational demand far more rapidly than earlier transplants. In spite of these adverse conditions, final biomass attained by April transplants were the same as the January and March transplants. There are two possible reasons for this situation.

April seedlings were significantly larger than the other transplants at planting. Larger seedlings may be more tolerant of stress than smaller seedlings and apparently perform better than smaller seedlings on favorable sites (Howard and Newton 1984; Wagner and Radosevich 1991). This relative increase in tolerance may be due to larger food reserves and higher capacitance for water loss in larger seedlings. For example, larger seedlings with more coarse roots than smaller seedlings may not lose water at the same rate (Insley and Buckley 1985); therefore, they are probably able to recover more rapidly than smaller seedlings. In an experiment conducted in 1993, large Douglas-fir seedlings (fresh weight 32 g) when

exposed to air for 2 hr, showed a significantly smaller drop in ψ than smaller seedlings (fresh weight 15.5 g) (unpublished data, Joseph). In the current study, the covariate analysis also suggests that bigger seedlings may perform better if subjected to exposure or transplanted later in the season. Therefore, the larger size of April transplants may have compensated for the more adverse environmental conditions in April than in winter.

Secondly, the adequate soil moisture in the common garden plots may have minimized the otherwise potentially stressful conditions that seedlings face when planted in the field. Seedlings out planted to the field have to compete with adjacent vegetation for critical resources; in addition, poor root-soil contact, and summer drought would further exacerbate the stress of field-planted seedlings. Therefore, the relatively mild conditions in the beds, combined with rapid new root growth and higher allocation to root biomass by the April transplants, may have resulted in comparable performance to that of January or March transplants.

Symptoms of transplanting stress, typically characterized by shorter terminals and high density of needles, appear to be limited to the spring flush and disappear during the summer flush. The summer flush is thought to compensate for the reduced predetermined growth (Carlson et al. 1980) in Douglas-fir seedlings. Although April transplanted and root exposed seedlings had stunted spring terminals, and a reduced percentage of seedlings that flushed in summer, inter-needle length of the summer lammas shoot was large and not different across treatments. There was no seedling with any symptom of transplanting stress in the summer flush, although

absolute leader lengths varied depending on seedling size and treatment. It is most likely that the mechanism for this discrete appearance of Spring stressed and Summer unstressed needles in transplanted Douglas-fir is associated with its pattern of shoot morphogenesis. For instance, all the needle primordia and the embryonic shoot stem for the spring flush is laid down the previous season (Fielder and Owens 1988). Because elongation in spring involves the preformed cells, any turgor induced or hormone mediated loss in growth during the initial stages of elongation may affect all these cells and thus cause the stunting of the spring flush. Since the cells for the summer flush have not been formed, its growth is unaffected. If physiological conditions recover sufficiently prior to the laying down of summer terminal cells, the summer flush would probably show no signs of stress.

Summary

Exposing seedlings to air primarily damages the root system. Consequently, root conductance and new root growth are impaired. All transplanted seedlings in this study lost 20% of their stem conducting area, presumably to cavitation. Exposure of seedlings in April reduced the stem conducting area more than when exposed in January. Stomatal conductance and predawn ψ of exposed seedlings were lower than the untreated controls during the first 2 months after transplanting. Exposing seedlings caused a larger drop in g_s than in ψ. This result was particularly evident for the January and March transplants, which also showed the least amount of new root growth during this period. Possibly due to increasing

atmospheric vapor deficits in April and a subsequent increase in water demand, new root growth appears to be more critical for April than January or March transplants to maintain water balance.

Just after budbreak, exposed seedlings of the April transplants still showed low ψ, reduced g_s and A. On the other hand, the exposed seedlings of January transplants had recovered physiologically to the level of untreated controls. However, spring terminal elongation rates of exposed seedlings were still lower than untreated controls for both transplanting dates. Within 17 days of the first measurement, exposed April transplants had physiologically recovered to that of untreated controls, but showed no concomitant recovery in terminal elongation rates. Exposed seedlings, regardless of transplanting dates, recovered a favorable water balance before shoot elongation ceased; however, the effect of the initial water deficit was sufficient to cause a significant reduction in terminal elongation, an increase in needle density, and a substantial decrease in the % of terminals flushing twice.

Exposure of seedlings prior to transplanting reduced shoot biomass and stem diameter more than the root biomass irrespective of transplanting date. Allocation to the roots (R:S ratio) was always high when seedlings were exposed. Delayed spring (April) transplanting when compared with normal winter (January) planting decreased the terminal leader length and the % of terminals flushing twice, but did not affect the biomass or stem diameter attained. April transplants showed high R:S ratios and rapid new root growth; however, predawn ψ during the first 2 months after planting was lower than the other dates. April transplants took longer to

recover from internal water deficits and achieve net photosynthetic rates comparable to the January transplants.

In conclusion, physiological recovery during active elongation, defined in terms of a favorable water balance and a high net photosynthesis, may not result in enhanced terminal elongation rates. Spring terminal growth in transplanted Douglas-fir is apparently very sensitive to either early (pre-budbreak) internal water deficits, and/or root generated hormones. Characteristic symptoms of transplanting shock, such as densely packed needles, are limited only to the spring flush; seedlings that broke bud again showed no difference in needle densities among treatments. However, smaller budwidths of exposed seedlings at the end of the first year suggests that the absolute elongation of terminals in the second year may also be affected, although needle densities may be the same. The relationship of water stress induced by preplanting-exposure to first year growth in transplanted Douglas-fir is not entirely clear. However, minimizing such stresses will enhance the growth of transplanted seedlings.

CHAPTER III. EFFECT OF ROOT PRUNING AND TIME OF TRANSPLANTING ON WATER RELATIONS, GAS EXCHANGE AND GROWTH OF DOUGLAS-FIR SEEDLINGS

Introduction

During the process of lifting and transplanting, seedlings are damaged by exposure to air and may also lose roots. Exposure to dry air and root loss may be the two most important sources of stress to which seedlings are subjected prior to planting (Stoneham and Thoday 1985). Exposure reduces the functional root surface area by the desiccation of the smaller roots (Coutts 1981). Root loss also reduces root surface area. However, the effects of these stresses on the physiology and subsequent growth of transplanted seedlings during their establishment may differ. Therefore, it would be of interest to know the degree and nature of damage that Douglas-fir seedlings can tolerate. In addition, it may prove useful to determine the physiological mechanisms that link these pre-planting stresses with their subsequent growth.

Seedlings may lose their roots during the lifting process, or during processing, before being transplanted. Although the loss of roots during lifting is often kept to a minimum, it is hard to control the loss, especially with mechanized lifting. Some root loss during lifting may be inevitable. It is also difficult to quantify the loss of roots in order to assess the effect lifting may have on subsequent seedling performance. A major loss of roots after lifting also occurs when roots are pruned to a specified length in order to facilitate convenient packaging and planting

(Burdett and Simpson 1984). Surprisingly, there is no published information on the effect such root removal may have on the physiology and growth of Douglas-fir seedlings. Mullin (1973) has shown that Sitka Spruce seedlings had lower survival when they were root pruned at the time of planting. However, he did not quantify the amount of root loss, making it impossible to determine the threshold level of root loss that would be detrimental to seedling performance. In a study with Pinus radiata (D.Don) seedlings, root pruning to a specified length of 21 cm from the cotyledon scar resulted in a sharp increase in stomatal resistance and a concurrent drop in net photosynthesis, but seedlings recovered to only 60% of initial levels of photosynthesis by day 32 (Stupendick and Shepherd 1980). Because some amount of root loss is inevitable during transplanting, it was of interest to ascertain the level of root loss seedlings can sustain without affecting subsequent physiology and growth.

Another source of root loss prior to lifting Douglas-fir seedlings may be during undercutting and wrenching of seedlings in the nursery beds (Duryea 1984). Root pruning operations are done primarily to stimulate root growth and enhance fibrous root development of seedlings during their nursery phase (Duryea 1984). However, there are conflicting reports on the performance of wrenched vs. unwrenched seedlings after they are transplanted. Duryea and Lavender (1982) found no case in which root wrenching improved field growth of Douglas-fir seedlings. Actually, first-year growth was consistently greater for unwrenched than for wrenched seedlings under a number of planting-site conditions. On the other hand, Tanaka et

al. (1976) found wrenched seedlings to be superior in height and diameter after five years in the field. Among pines, the response to wrenching is also inconclusive, with reports of promotion (Rook 1971), inhibition (Mullin 1966) or no appreciable effect (Tanaka et al. 1976). In a long-term study on droughty sites in southwest Oregon, Hobbs et al. (1987) reported that both Douglas-fir and ponderosa pine seedlings showed no difference in growth between several undercut treatments and untreated controls after 4 years in the field. Overall, the response to this type of pruning may depend on several factors, such as the species, time of pruning, and the frequency and intensity of pruning.

The time of year that the seedlings are transplanted can be chosen to minimize the detrimental effects of root loss and other preplanting stresses. For Douglas-fir seedlings in the coastal ranges of the Pacific Northwest, lifting and transplanting between December and February appear to produce the best performance (Cleary et al. 1978; Dunsworth 1988b). Seedlings planted during the winter months are not only planted into favorable environments that are typically characterized by moist, cool conditions, but are also in deep dormancy and have maximum cold hardiness. Seedlings lifted when they are dormant and cold hardy are generally more tolerant of preplanting stresses and extended cold storage (Hermann 1967; Coutts 1982; Ritchie 1986; Deans et al. 1990). Very little is understood of the physiological mechanisms for this observed change in seasonal tolerance. None of these studies have addressed the fact that seedlings transplanted in winter have a relatively longer period in the soil than spring transplants before shoot growth begins

in late spring. The length of this period characterized by the number of root growth days (i.e., the number of days in which soil temperatures are > 4°C) may have a significant effect on shoot growth. Dunsworth (1988a) found that Douglas-fir seedlings had the highest root activity when soil temperature was >4°C. Therefore, fall transplants would have the longest period in the soil prior to resumption of shoot growth in spring, which may improve seedling performance.

In situations where cold soils or inclement winter weather can delay outplanting, Fall planting may offer a potentially feasible alternative. However, little is known on how seedlings would respond physiologically if transplanted in fall. Fall transplanting is done in some nurseries in the Northwest (Duryea 1984), but out-planting is rarely done in fall for reasons that are unclear. Comparing fall and spring transplanting, survival of fall-transplanted Scots pine (Pinus sylvestris L.), white spruce (Picea glauca [Moench] Voss), and Colorado blue spruce (Picea pungens Engelm.) showed tremendous variability; spring transplanting resulted in less variable survival and was most often better than transplanting in fall (Cram and Thompson 1981). Inconsistent survival of fall transplants could be due to the stock not being completely dormant. On the other hand, Duryea (1984), referring to two unpublished reports from the Northwest, found that there was no difference in performance between fall or spring transplanting of Douglas-fir seedlings. Zaerr and Lavender (1976) found that survival of Douglas-fir seedlings planted in November was higher than after winter or spring planting on either bare or grassy sites. It may be critical that fall transplanting be carried out after seedlings have become dormant which is

generally from October to December. For instance, in some Weyerhaeuser surveys, late October transplanted seedlings had a 91% transplant-bed survival while late September transplants had only 71% survival (Duryea 1984).

The objective of this experiment was to determine how the degree of root pruning and the time of transplanting affect the physiology and growth of Douglas-fir seedlings during the first growing season. The first hypothesis states that root pruning of Douglas-fir seedlings at the time of transplanting will significantly decrease new root growth, seedling biomass and stem elongation growth during the first season. The second hypothesis states that the negative effects of root pruning will be increasingly ameliorated for seedlings transplanted in November, January, and March respectively. The third hypothesis states that November and March transplants will show higher root growth due to warmer soils than January, but shoot ψ will be higher for January>November>March transplants during the first 2 months of establishment due to increasing VPD. The fourth hypothesis states that root pruning will decrease the water potential (ψ), stomatal conductance (g_s) and net photosynthesis (A) of seedlings.

Material and Methods

In order to study the effects of root pruning on the growth and physiology of 2-year-old Douglas-fir seedlings, seedlings had 30 or 50% of their root volume removed prior to planting (Fig III.1). Pruned seedlings were transplanted in fall, winter and spring to evaluate the effect that the time of transplanting may have

on seedling performance. Seedlings were transplanted into a common garden plot and their growth and physiology measured over one season. The experiment was conducted at the Forest Research Laboratory, Corvallis, Oregon from November 1990 to September 1991.

Seedlings

All seedlings used in this experiment were 2-year-old Douglas-fir from Oregon coastal sources, zones 072 and 062 (Oregon Tree Seed Zone Map), elevation 150-300 m, grown operationally as 2+0 stock type. All seedlings were grown at the International Paper Co. nursery at Kellogg, Oregon, before they were transplanted into raised nursery beds (common garden plot) at Corvallis, Oregon, where this experiment was conducted.

Preplanting Seedling Evaluation

Three hundred 2-year old Douglas-fir seedlings were lifted manually on November 15 (1990), January 15 and March 15 (1991), for a total of 900 seedlings. Prior to root pruning and subsequent transplanting, individual seedlings were weighed and measured. Measurements included total height, fresh weight, stem diameter at the root collar, root volume (Rose et al. 1991), terminal bud width and length. Bud width was measured at the widest section of the bud with a digital caliper to the nearest 0.01 mm. Measurements of cold hardiness, Root Growth Potential (RGP), and

days to budbreak (DBB) were made on a sub-sample of 15-20 seedlings to determine the over all physiological status of the seedlings at each transplanting date (Ritchie 1984). The DBB was converted to Dormancy Release Index (DRI= 10/DBB) (Ritchie 1984).

Root Pruning

Seedlings were pruned so that 30 or 50% of the original root volume was removed. Only small tertiary roots (mostly <2 mm in diameter) and thin secondary roots were removed. Roots were pruned under water with a pair of scissors. The root volume was determined by immersing seedling root systems in a container of water placed on a balance. The displaced water (measured in g) is equal to the volume (measured in cc) of the root system, since 1 g of water equals aprox. 1 cc at room temperature (Burdett 1979; Rose et al.1991). For each treatment, the resulting fresh weights, root volume, and percent root volume removed are summarized (Table III.1). Means, standard errors and the ranges were calculated for the different parameters.

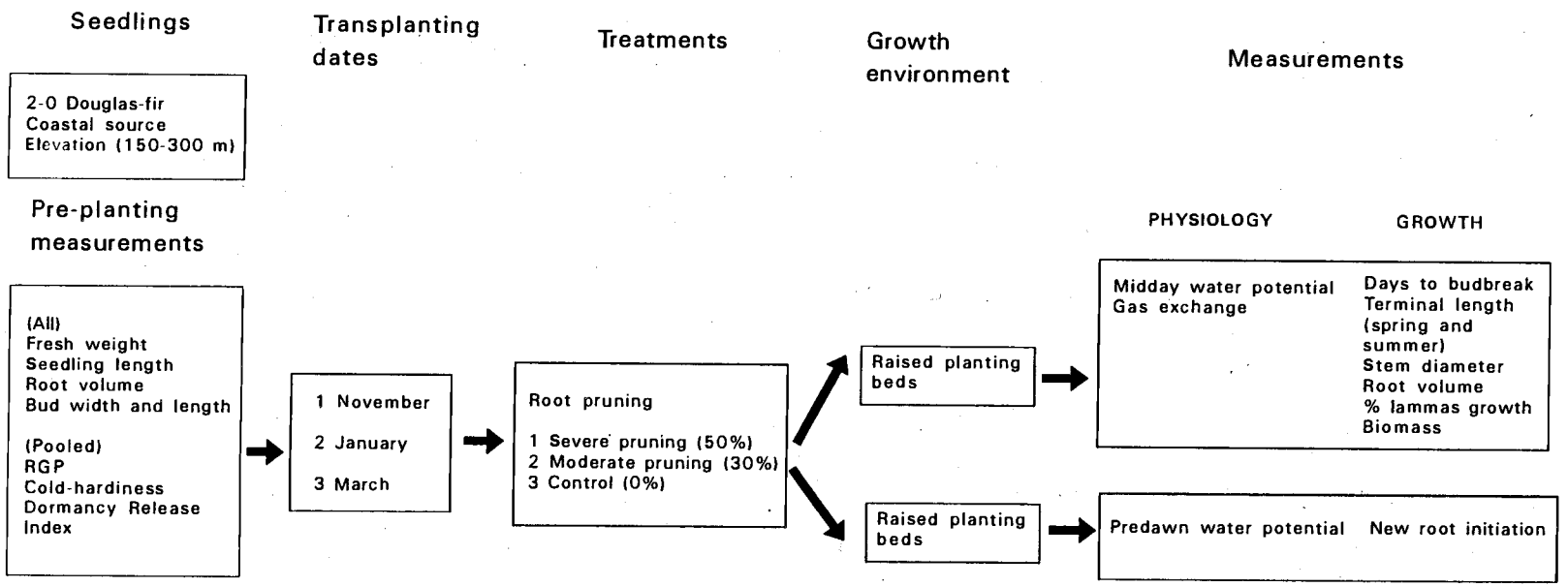

Figure III.1 Layout of the experiment outlining the different treatments, growth conditions and measurements taken.

Table III.1 Mean (± SE) initial fresh weight, root volume and percent root volume removed from 2-year-old Douglas-fir seedlings root pruned to different levels. Numbers within parentheses are the range of values for each treatment.

Root pruning	Fresh weight (g)	Root volume (ml)	Root volume removed (%)
Control (n=142)	41.79 ± 1.19 (21.1 - 89.4)	16.68 ± 0.50 (8.0 - 39.0)	0
Moderate (n=144)	29.15 ± 0.39 (11.8 - 77.6)	10.84 ± 0.39 (5.0 - 32.0)	31.45 ± 0.21 (12.5 - 50.0)
Severe (n=144)	22.36 ± 0.25 (9.2 - 54.5)	8.30 ± 0.25 (3.0 - 19.0)	47.31 ± 0.53 (28.6 - 64.3)

Planting

The experiment was a 3x3 factorial design (3 transplanting dates and 3 pruning levels) randomized over 8 blocks, with a total of 432 seedlings. Seedlings were planted a day after lifting in planting beds at the Forest Research Laboratory, Oregon State University, Corvallis. On each transplanting date, for every root pruning treatment, a total of 48 seedlings were planted. Each row contained 6 seedlings from a particular pruning treatment and transplanting date combination. The rows were randomized in each block. The inter-row spacing was 45 cm and the intra-row spacing was 20 cms. In order to measure plant ψ and root growth prior to budbreak, an extra 8-16 seedlings/root pruning treatment were planted at the same spacing at 2-4 seedlings/block at the end of 4 blocks on each transplanting date. The beds were maintained free of weeds and irrigated regularly to allow seedlings to grow

under uniform drought-free conditions. Soil analysis of the beds indicated that they had adequate nutrients, so no additional fertilizer was added.

Measurement of Growth and Physiology

Predawn ψ and Root Growth

Water potential measurements were made on a lateral twig using a pressure chamber (PMS Instrument Co., Corvallis OR)(Scholander et al. 1965). These seedlings were then harvested at 64 days for the November transplants, at 57 days for the January transplants, and at 60 days for the March transplants to measure new root growth. Total number of new roots were counted under two categories, those new roots over 1 cm in length and all new root initiates. Root numbers were expressed as the final number of new root initiates, and as a relative growth rate (RGR). The new root RGR expresses the new root growth per unit initial root volume per unit time.

New root RGR = # New roots/(initial root volume (ml) x days in soil)

Phenology and Growth

The percent budbreak (shoot emergence) was measured periodically by scoring all seedlings that had broken bud, both terminal and lateral, from the time that the first seedling broke bud until 100% budbreak. A seedling was considered to have broken bud if any of the buds showed green needles breaking through the

scales. Periodic measurements of leader elongation were made during active growth on April 30th, May 14th and June 4th. Growing leaders were measured from the base to the tip of the apex. On each date, a random sample of 16 seedlings/treatment and transplanting date were measured for leader elongation. Final growth measurements included fresh weight, terminal length, needle density, stem diameter, bud width, bud length, root volume, root dry weight and shoot dry weight. Most of the measurements presented in this paper are in terms of final or relative growth rates (RGR). The RGR is a measure of growth that is normalized for starting biomass (Hunt 1982; South 1995), or other growth parameter, therefore eliminating any initial variation in size or growth of seedlings.

$$\text{RGR (biomass,diameter,etc.)} = \frac{\log_e(w_n)-\log_e(w_0)}{(t_n-t_0)}$$

where w_n is the final weight (or other parameters), w_0 is the initial weight (or other parameters), t_n is the date when w_n is measured, and t_0 is the time when w_0 is measured. Needle density was measured by counting all the needles in a 1 cm segment located at the middle of the leader, which is a modification of the stem-unit length (Cannell et al.1976). Root and shoot dry weight were determined gravimetrically after drying at 70°C for 72 hrs.

Gas Exchange and Midday ψ

On April 30th, during shoot elongation, gas exchange and ψ measurements were made between 10 am and 2 pm on a random sub-sample of 4

seedlings/treatment and transplanting date. Weather conditions during the measurements were: air temperature 23°C, RH 34% (VPD 18.5 mb), photosynthetic photon flux density (PPFD) 1100 μmoles m^{-2} s^{-1}. Gas exchange was measured with a portable infrared gas analyzer (LICOR 6250, Lincoln, Nebraska). Net photosynthesis (A), and stomatal conductance (g_s) were computed according to von Caemmerer and Farquhar (1981). Measurements were made on 1-year-old needles on a lateral branch on the first whorl. Duplicate measurements were made on the same sample. Projected needle areas were measured with a leaf area meter (LICOR 3100, Lincoln, Nebraska).

Statistical analysis

The root measurements were rank transformed and examined by ANOVA. Ranking of data was necessary to meet the homogeneity of variance assumption (Conover and Iman 1976). Fisher's protected least significant difference (FPLSD) was used to separate means at $p<0.05$.

Percent budbreak scores were also ranked and examined by ANOVA. Differences between means were separated by the FPLSD at $p<0.05$. The periodic leader elongation was analyzed as a split-plot design with time (Steel and Torrie 1980), and significant differences between means were separated by the FPLSD at $p<0.05$. All other data were analyzed using a 2-way factorial ANOVA and means were separated using FPLSD at the 0.05 level. A covariate analysis (ANCOVA) was made to determine which of the initial morphological seedling characteristics

were significant covariates of final growth parameters, with transplanting date as the main effect. The root volume of individual seedlings was included as a covariate, and to eliminate any confounding, the pruning treatment was dropped from the model. Total biomass, shoot biomass, root biomass, spring terminal growth, and stem diameter at the end of the growing season were the dependent variables of interest. Type III mean sum of squares, corresponding F-ratios and associated p-values are reported for the significant variables ($p<0.05$) of the covariate model.

Results

Preplanting Seedling Characteristics

On the different transplanting dates, Douglas-fir seedlings reflected wide variation in their physiological status. January transplanted seedlings showed 50% mortality at -21.5°C (LT_{50}), while those transplanted in November and March had LT_{50}'s of -7.5 and -9 respectively. November transplanted seedlings took 205 days (DRI = 0.05) to break bud under a forcing environment, while January and March transplanted seedlings took 49 (DRI=0.20) and 17 days (DRI=0.58) respectively. According to Ritchie (1986), seedlings with a DRI of 0-0.25 are below peak physiological vigor and hardiness, 0.25-0.40 are in peak quality, while anything >0.40 are deteriorating. An average of 24 new roots were recorded for the January planted seedlings in the RGP test, while the November and March planted seedlings had only 17 and 14 respectively.

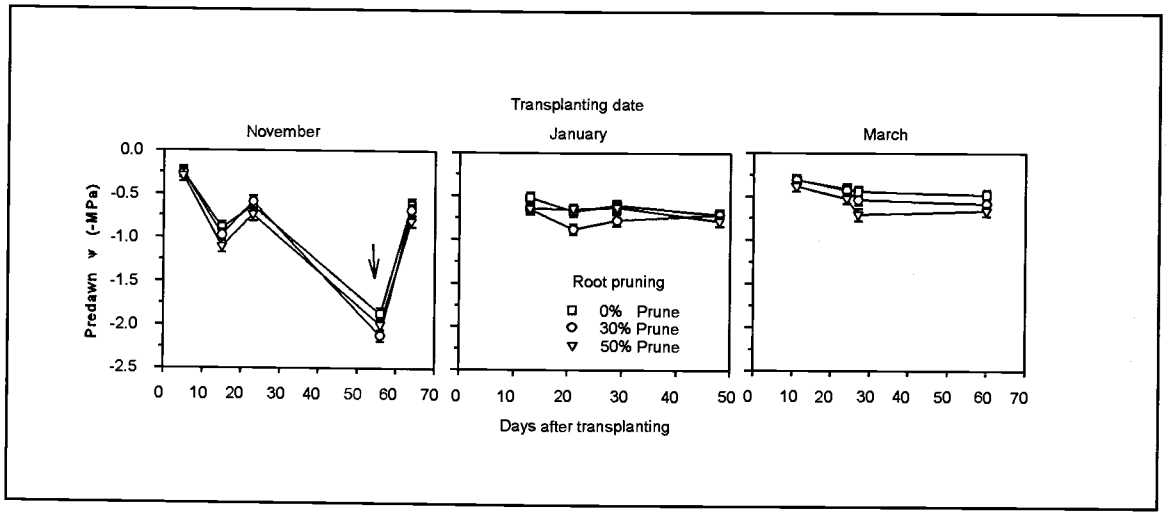

Figure III.2 The effect of root pruning on the predawn ψ during the first 70 days after transplanting 2-year-old Douglas-fir seedlings on different dates. Arrow indicates date when soils froze. Standard error bars are smaller than the size of the symbol.

Two-month predawn water potentials and new root growth

During the first 2 months after transplanting, the pruning treatments had no effect on the predawn ψ of seedlings on any of the dates (Fig. III.2). However, the mean predawn ψ of March transplanted seedlings was slightly > January >November. The November transplants were unexpectedly subjected to below freezing temperatures 57 days after they were planted, and the predawn ψ decreased to <-2.0 MPa. However, the pruned treatments did not show a lower ψ as expected. All the November transplants recovered within the next week to a predawn ψ that was present prior to the cold snap.

Transplanting dates had a significant effect on new root growth, characterized as the number of new root initiates or as the RGR of new root initiates (Table III.2). On the other hand, the pruning treatments had a significant effect only on the number of new root initiates and showed no effect on the RGR of new root initiates. Seedlings transplanted in November had <10% of the number of new roots as the January transplants and <4% of March transplants (Fig III.3). The trend was almost similar for the RGR of new root initiates. The number of new root initiates of the severely pruned treatment was 50% of the controls and 70% of the moderately pruned treatments. However, the number of new root initiates RGR was unaffected by the degree of pruning.

Table III.2. P-values for ANOVA of different transplanting dates and treatment effects for the number of new root initiates and their RGR. Both parameters were Rank transformed prior to analysis.

Source of variation	No. of new root initiates	No. of new root initiates (RGR)
Transplanting date (L)	0.0001	0.0001
Treatment (T)	0.0003	0.24
L x T	0.45	0.30

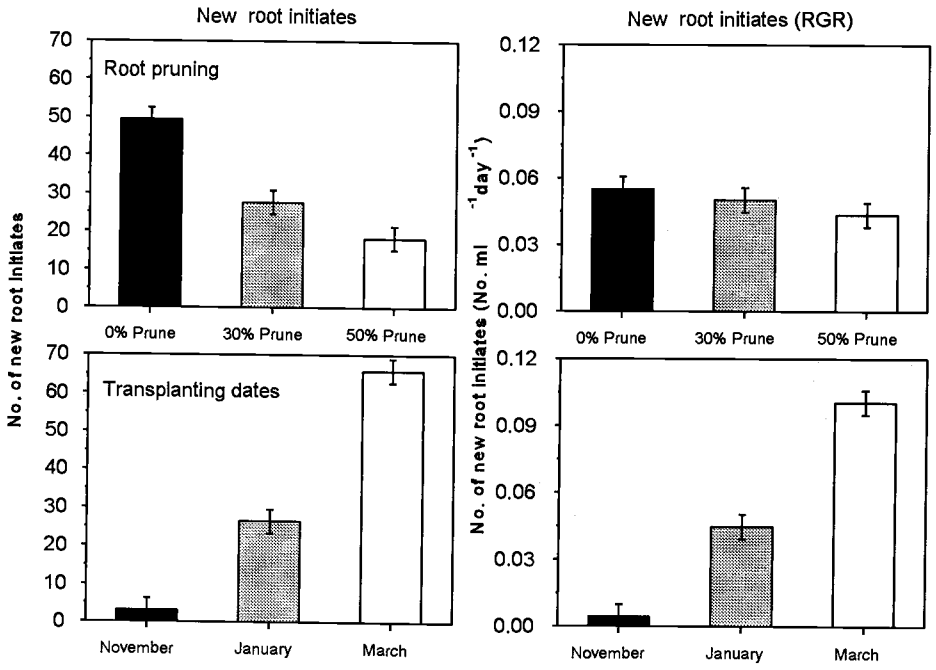

Figure III.3. The number of new root initiates and the number of new root initiates RGR at the end 57-64 days for root pruned seedlings transplanted on different dates. Vertical bars represent one standard error. See Table III.2 for the level of significance of treatment effects.

Phenology and Leader Growth

The effect of root pruning and the time of transplanting on the rate of bud break (terminal and lateral buds) was significantly influenced by the observation date. (Table III.3, Fig. III.4). This significant interaction between observation date and treatments is generally due to either a lack of difference in the % budbreak at the beginning or at the end of the observations, and a relatively large difference in % budbreak in between these observation dates. The time of transplanting affected the rate of budbreak of both terminals and laterals more than did the pruning treatments. For instance at 50% budbreak, the difference between seedlings planted in November (slowest emerger) and in March (fastest emerger) was 11-13 days, while the difference between controls and severely (50% prune) pruned seedlings was only

Table III.3. P-values for ANOVA of different transplanting dates and treatment effects for percent budbreak and terminal length. Budbreak scores were Rank transformed prior to ANOVA.

Source of variation	Terminal budbreak	Lateral budbreak	Terminal length
Day (D)	0.0001	0.0001	0.0001
Transplanting date (L)	0.0001	0.0001	0.007
Treatment (T)	0.03	0.08	0.01
L x T	0.55	0.077	0.75
L x D	0.0001	0.0001	0.0001
T x D	0.007	0.04	0.04
L x T x D	0.90	0.19	0.78

4-5 days. Among the transplanting dates, the seedlings planted in November took significantly longer for shoots to emerge than either of the other dates (on 4/27, for terminals $p<0.001$, laterals $p<0.001$). Seedlings planted in March and January showed no difference in rates of emergence for lateral shoots, whereas terminal shoots for seedlings planted in March emerged significantly faster (on 4/27 $p<0.001$) than did seedlings planted in January. Rates of shoot emergence for both terminals and laterals decreased with increasing levels of pruning, but this effect, though significant (for terminals on 4/27 $p<0.01$, for laterals on 4/15 $p<0.001$), was minimal. On average, lateral shoots emerged 15 days earlier than the terminals.

The significant interaction effect of pruning and observation date (Table III.3) on terminal length is primarily due to the lack of any difference between treatments on April 30 whereas there are large differences between treatments on the other dates (Fig III.5). However, the significant interaction effect of transplanting date and observation date (Table III.3) is primarily due to differences in terminal growth rates between March and November transplants after May 14 (Fig III.5). Leader length for November planted seedlings lagged behind ($p<0.001$) March planted seedlings until May 14 but by June 4 they had outgrown them ($p<0.001$). January and March transplants grew at a similar rate during the first phase of elongation, but the March transplants were unable to maintain the same growth rates after May 14. Elongation rate decreased proportionately with increasing root pruning ($p<0.05$).

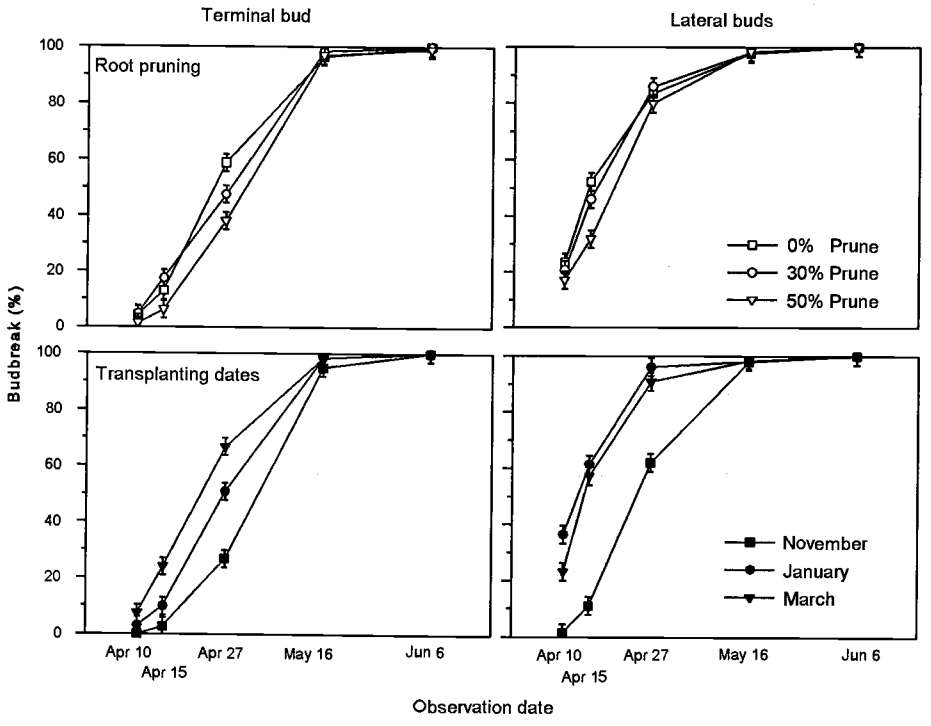

Figure III.4. Effect of transplanting dates and root pruning on the terminal and lateral budbreak for 2-year-old Douglas-fir seedlings. Vertical bars represent one standard error. See Table III.3. for the level of significance of treatment effects.

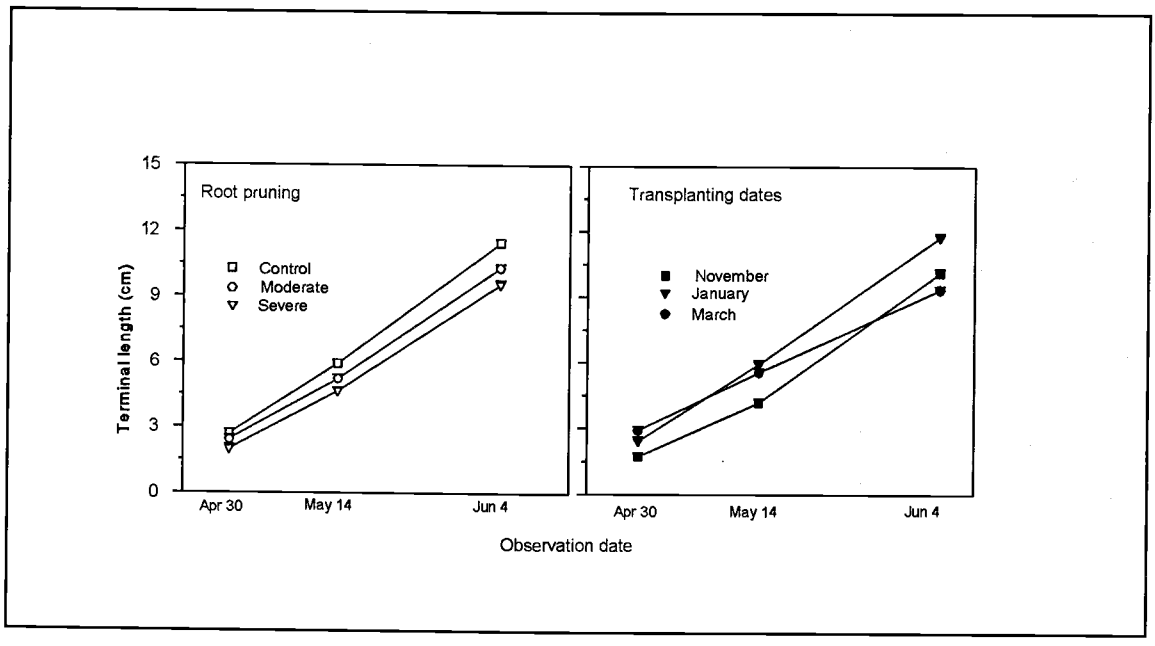

Figure III.5. Patterns of terminal spring growth during active elongation of 2-year-old Douglas-fir seedlings in response to different transplanting dates and levels of root pruning. Standard error bars smaller than size of symbol. See table III.3 for the level of significance of treatment effects.

Water relations and net photosynthesis after budreak

Both date of transplanting and degree of root pruning significantly affected midday ψ, g_s, and A (Table III.4). Although, there was no significant interaction ($p<0.11$) between transplanting dates and pruning levels on midday ψ, the differences between the pruning treatments decreased from November to March (Fig III.6). For instance, the ψ of controls were the same as the pruning treatments in March; however, in November, the ψ of controls was higher than the root pruning treatments by 50%. Mean midday ψ significantly decreased with increasing pruning intensity.

Table III.4. P-values for ANOVA of different transplanting dates and treatment effects for midday ψ, g_s, and A.

Source of variation	Midday ψ	g_s	A
Transplanting date (L)	0.03	0.035	0.0006
Treatment (T)	0.002	0.05	0.0006
L x T	0.11	0.64	0.36

Mean g_s was significantly higher for the controls than for either root pruned treatments on all transplanting dates. The g_s of March transplants was significantly lower than either November or January transplants. The difference in g_s between the control and pruning treatments was again much smaller for the January and March transplants than the November transplants. Seedlings planted in November and January had almost twice the rates of photosynthesis of seedlings planted in March (Fig. III.6). Controls had 70% faster rates of photosynthesis than did either of the pruning treatments. There were no differences in rates of photosynthesis between the severe and moderate pruning treatments on any of the transplanting dates. Seedlings planted in January showed the least difference (25%) in rates of photosynthesis between controls and pruned seedlings, while the difference between the two treatments for November and March was 50%.

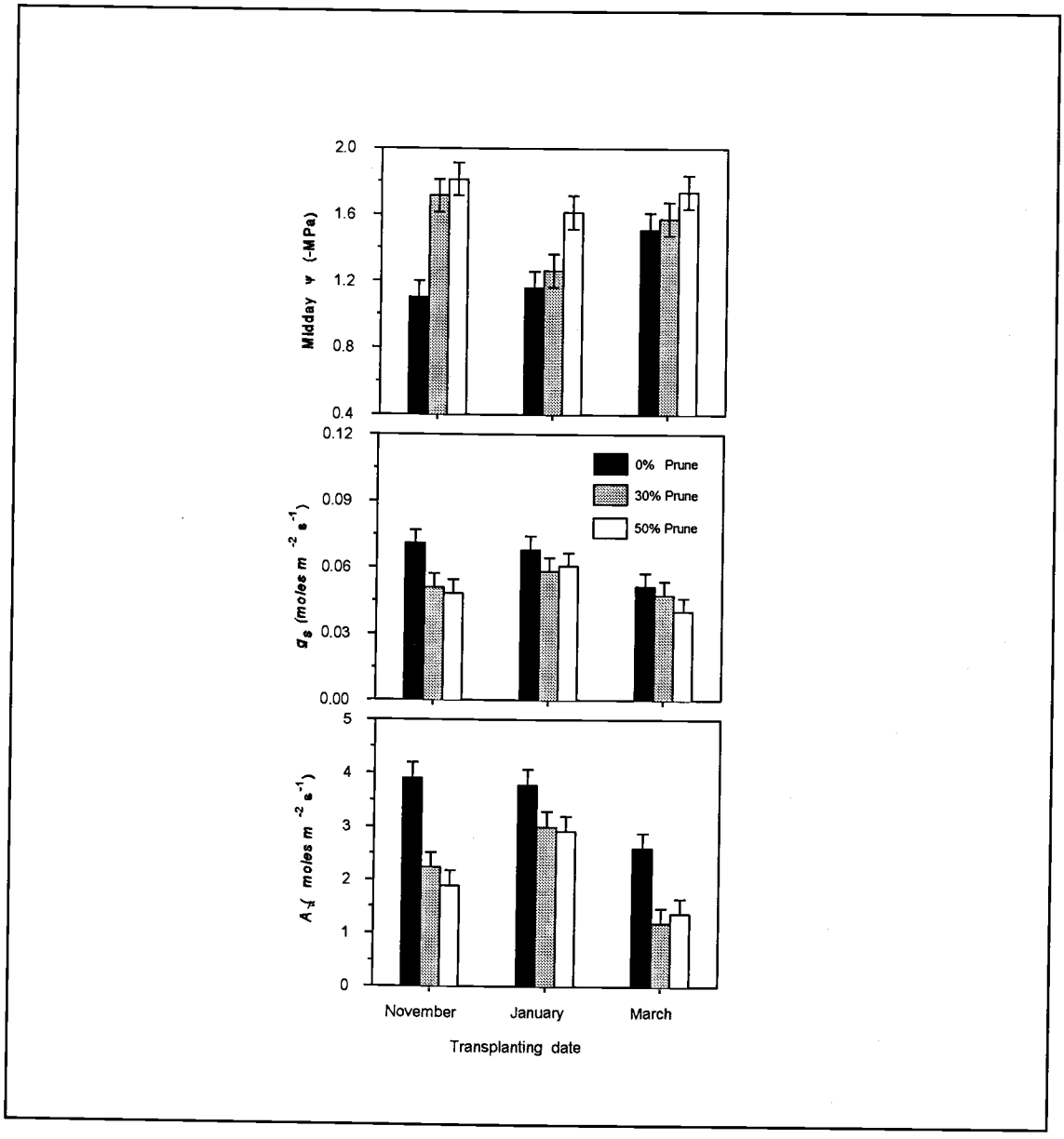

Figure III.6. Midday water potential, stomatal conductance, and net photosynthesis of 2-year-old Douglas-fir seedlings transplanted on different dates and subjected to differing levels of root pruning. Measurements were made between 10 am and 2 pm on April 30, 1991. Vertical bars represent one standard error of the mean. See table III.4 for significance of treatment effects.

Net photosynthesis of seedlings was significantly correlated with g_s and ψ. Stomatal conductance was positively correlated with A ($r=0.70$, $p<0.0001$). On the other hand, the ψ was negatively correlated with A ($r=-0.70$, $p<0.0001$), and negatively correlated with g_s ($r=-0.41$, $p<0.02$).

Final growth response

Date of transplanting and level of root pruning significantly affected most of the terminal growth components and the biomass of seedlings at the end of the growing season (Table III.5). The summer terminal length was unaffected by transplanting date or the pruning treatments. Root biomass and the R:S ratio showed a significant interaction effect between the pruning treatments and transplanting dates.

Table III.5. P-values for ANOVA of different transplanting date and treatment effects for terminal and biomass growth.

Source of variation	Spring terminal	Summer terminal	Bud width	Needle density	Shoot biomass	Root biomass	R:S
Transplanting date (L)	0.0001	0.45	0.001	0.0001	0.0001	0.0001	0.0001
Treatment (T)	0.0001	0.46	0.0007	0.0001	0.0001	0.0001	0.62
L x T	0.75	0.98	0.36	0.30	0.12	0.078	0.03

Transplanting dates significantly affected ($p<0.01$) the % of seedlings flushing a second time in summer (Fig III.7). The effect of pruning levels was

significant only at $p<0.07$. Percentage of seedlings flushing in summer ranged from 31% for March transplanted seedlings to 45 and 56 % for November and January planted seedlings, respectively. On the other hand, the length of summer flush did not significantly differ between treatments, with a range from 6.2-8.7 cm. Needle densities (number of needles/cm) on the spring growth of the terminals were significantly (November $p<0.001$, $r = -0.69$, January $p<0.001$, $r=-0.44$, March $p<0.001$, $r=-0.52$) correlated with spring terminal shoot length. Longer terminals had less dense needles (larger interneedle lengths) whereas shorter terminals had more dense needles (smaller interneedle lengths). Terminal bud widths were significantly reduced by 5% of controls for the pruning treatments ($p<0.05$) and by 5% of fall and winter transplants for the spring transplants ($p<0.05$).

Both root and shoot biomass were significantly affected by transplanting dates ($p<0.001$) and level of root pruning ($p<0.001$) (Fig III.8). Seedlings planted in January or November had no difference in root or shoot biomass, but were significantly larger ($p<0.001$) than the March transplants. Among pruning levels, root and shoot weights significantly decreased ($p<0.01$) with increasing levels of root pruning. However, the difference between the pruning levels for root or shoot biomass was largest for seedlings planted in January while there were no differences (or they were very small) for seedlings planted in November or March.

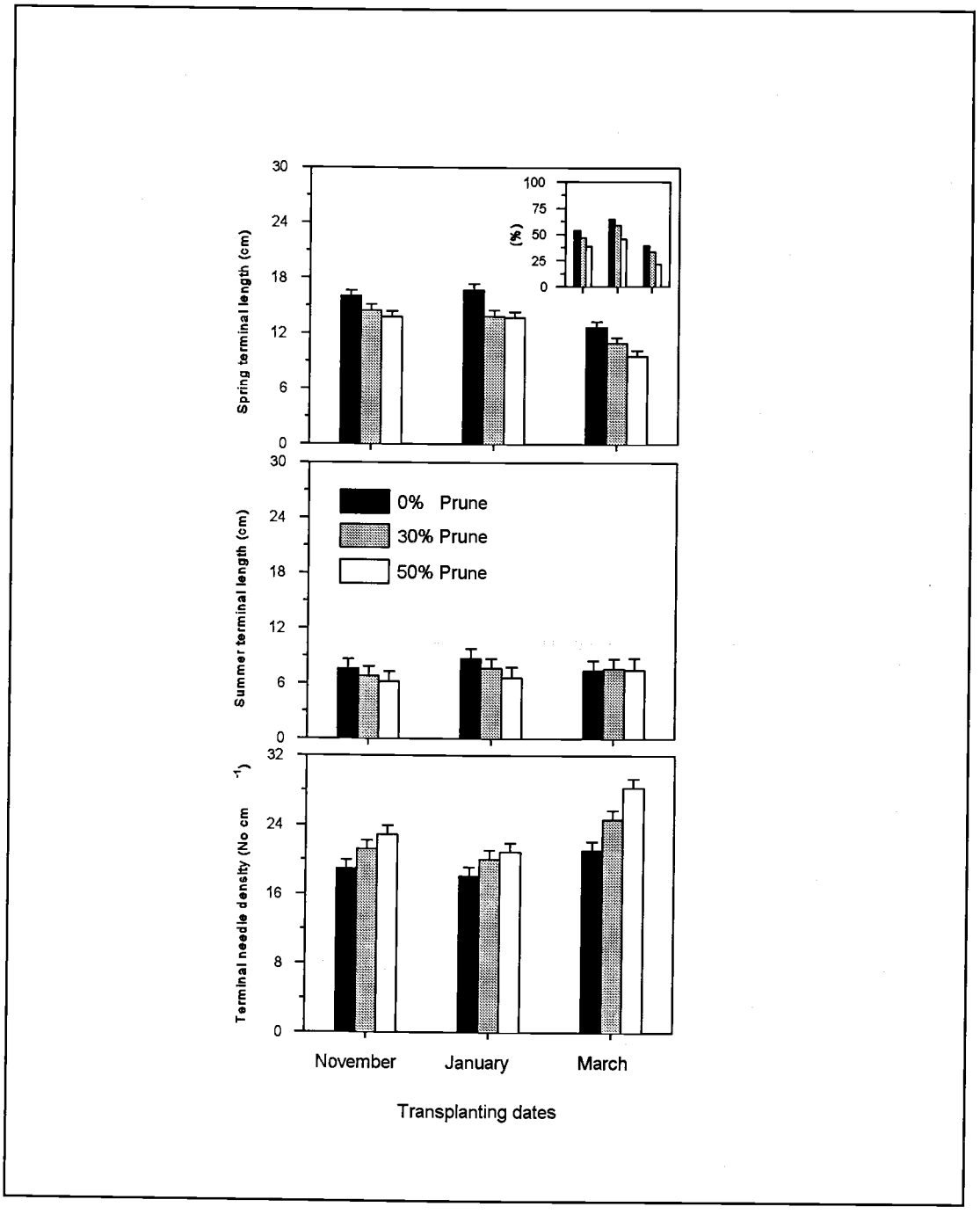

Figure III.7. The effect of transplanting dates and root pruning on the components of terminal growth of 2-year-old Douglas-fir seedlings. Figure insert is the percentage of seedlings that had a summer flush. Vertical bars represent one standard error of the mean. See table III.5 for significance of treatment effects.

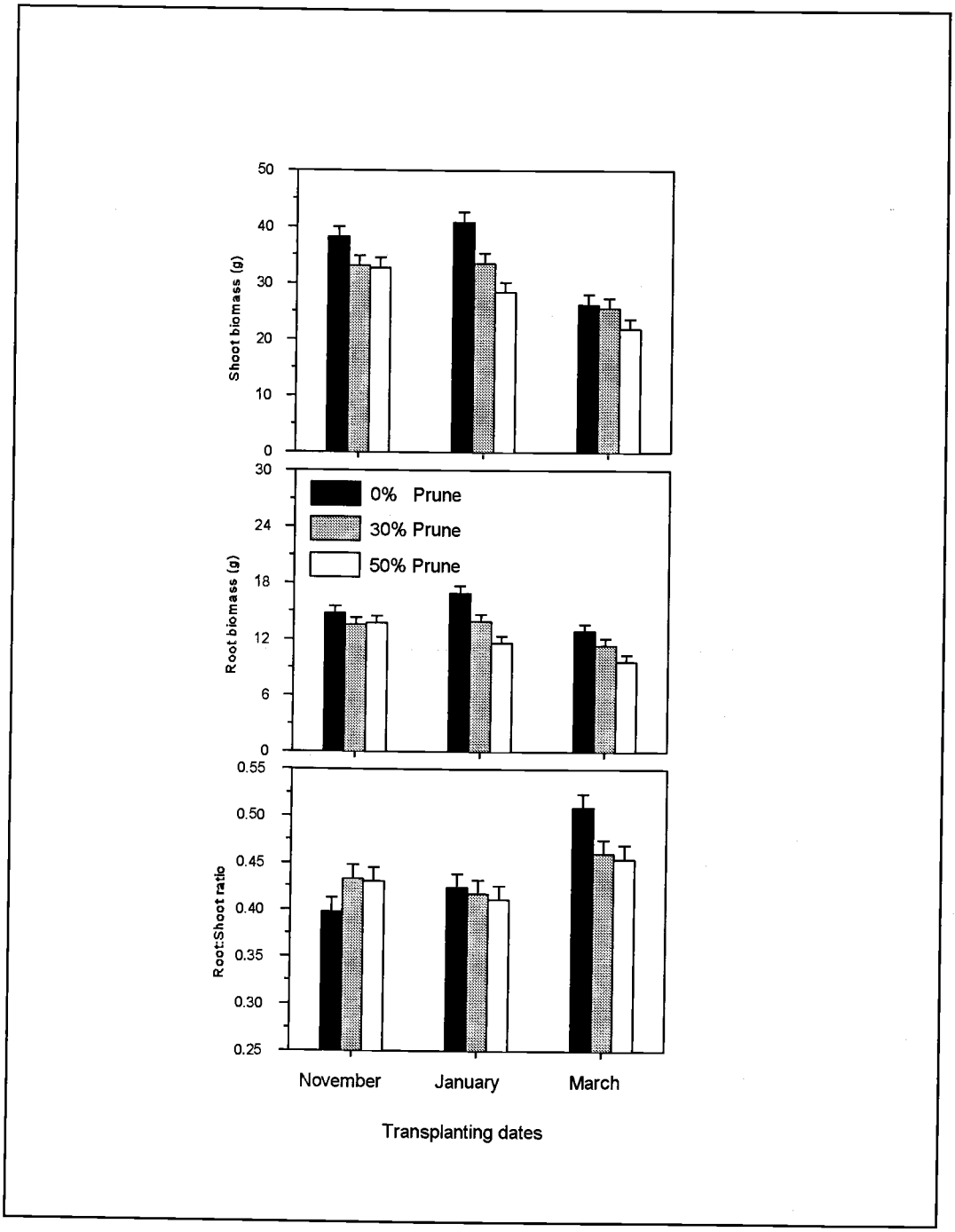

Figure III.8. The effect of transplanting dates and root pruning on root and shoot biomass, and the ratio of root:shoot biomass of 2-year-old Douglas-fir seedlings. Vertical bars represent one standard error of the mean. See table III.5 for significance of treatment effects.

March transplanted seedlings had significantly higher ($p<0.001$) R:S ratios than either January or November transplanted seedlings regardless of pruning level (Fig III.8). Such root: shoot allocation ratios were unaffected by pruning levels for the January transplants. However, for the March and November transplants the R:S ratios between the pruned and control seedlings were reversed. For the November transplants the controls had lower R:S ratios ($p<0.05$) than the pruned seedlings, whereas for the March transplants the controls had higher R:S ratios ($p<0.005$) than the pruned seedlings.

Table III.6. P-values and ANOVA for final growth and relative growth rates of total biomass. Stem diameter, and root volume as affected by transplanting dates and pruning treatments.

Source of variation	Total biomass	RGR (biomass)	Stem diameter	RGR (stem diameter)	Root volume	RGR (root volume)
Transplanting date (L)	0.0001	0.0001	0.0001	0.0001	0.003	0.0001
Treatment (T)	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
L x T	0.12	0.19	0.056	0.03	0.37	0.37

Root pruning treatments and transplanting dates significantly affected the final and relative growth rates of total biomass, stem diameter, and root volume (Table III.6). Stem diameter RGR was the only growth parameter that had a significant interaction effect between pruning treatments and the time of transplanting. This interaction is primarily due to the lack of any difference between controls and

moderately pruned seedlings in March, whereas on the other dates the moderately pruned seedlings had stem diameter RGRs that were the same as the severely pruned seedlings.

Final biomass of March transplants was significantly lower than the other transplants, whereas the biomass RGR of November transplants was significantly lower than either March or January transplants (Fig III.9). Seedlings with no root pruning generally attained a higher biomass than either of the root pruning treatments, whereas biomass RGR was lower for the unpruned controls than the pruning treatments. Severely root pruned seedlings had a significantly lower biomass than the moderately root pruned seedlings only for the January transplants. For March and November transplants there was no difference in biomass between the two pruning levels. Biomass RGR increased from November to March regardless of pruning treatment. Final stem diameter and stem diameter RGR were generally lower for both the pruned treatments than the controls in November and January (Fig III.9). However, in March, the moderately pruned seedlings were the same as the controls. Final stem diameter of the March transplants was lower than that of November or January transplants. However, the stem diameter RGR increased from November to March. Final root volume was lower for the March transplants than that of November or January transplants. The root volume RGR, on the other hand was highest for the March transplants.

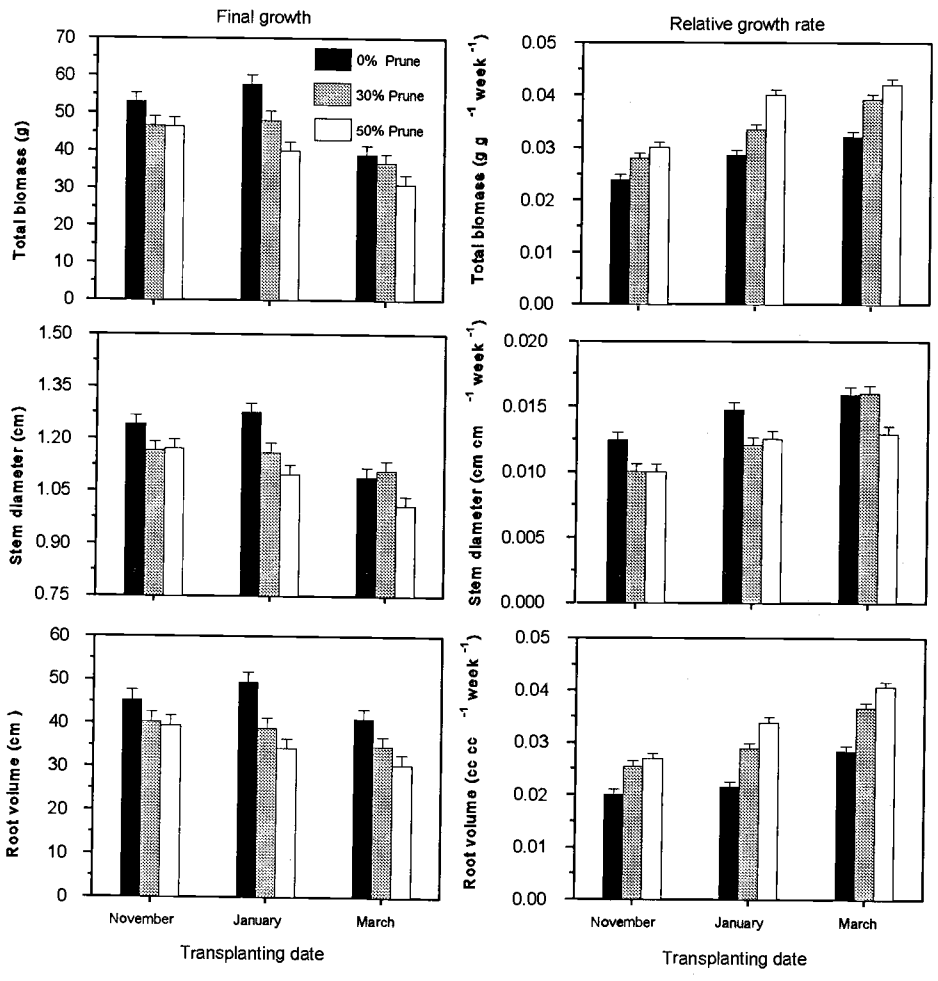

Figure III.9. The effect of transplanting dates and root pruning on final biomass, stem diameter, and root volume and their relative growth rates (final-initial) / initial / days) of 2-year-old Douglas-fir seedlings. Vertical bars represent one standard error of the mean. See table III.6 for significance of treatment effects.

Initial fresh weight and root volume after pruning were the most significant covariates contributing to the variation in the final biomass of Douglas-fir seedlings (Table III.7). Transplanting date was a significant factor for all parameters of growth, except summer terminal length. The spring terminal length was significantly related to the initial bud width, but did not covary with the initial fresh weights. It is interesting that seedlings with longer spring terminals also had longer summer terminals.

Table III.7. P-values for significant covariates and treatment effects of some final growth measurements using ANCOVA.

Source of variation	Total biomass	Spring terminal	Summer terminal	Stem diameter
Transplanting date	0.0001	0.0001	ns	0.0001
Covariates:				
Pruned root volume	0.0001	0.0001	ns	0.0001
Fresh weight	0.0001	ns	ns	ns
Stem diameter	ns	ns	ns	0.0001
Bud width	ns	0.0003	0.05	ns
Spring terminal length	-	-	0.0001	-

Discussion

Root pruning >30% of the original root volume decreased both the biomass and elongation growth of Douglas-fir seedlings. There was little difference in growth or physiology between seedlings that had 30% or 50% of their roots removed. Mortality of seedlings subjected to any of the pruning treatments was <1%. Transplanting seedlings in fall (November) and in spring (March) ameliorated the negative effects of root pruning better than transplanting in winter (January). This is evident in the smaller differences in biomass and growth between controls and the pruned treatments for fall and spring transplants than the winter transplants. However, the average biomass attained by the January and November transplants was significantly greater for all the treatments than the March transplants. Severe root pruning (50% of root volume) reduced average terminal elongation by approximately 10% of the unpruned controls, and reduced total biomass by approximately 20%. Root pruning decreased the number of new root initiates at the end of 2 months in the soil. However, the number of new root initiates per unit volume of root was unaffected by the root pruning treatments. The number of new root initiates at the end of 2 months increased almost exponentially from November to March. Predawn ψ of seedlings during the same period was unaffected by root pruning treatments or transplanting dates. However, after budbreak, midday ψ of pruned seedlings was lower than the controls. Stomatal conductance followed the same trend as the ψ; however, differences between treatments and transplanting dates were not as

pronounced. Net photosynthesis was lower for the pruned seedlings than the controls from all transplanting dates, but it was substantially lower for the November and March transplants.

Surprisingly, removing 50% of the original root volume caused negligible mortality, reduced biomass growth by <20% and elongation growth by <10% of the controls. The detrimental effect of root pruning on seedlings lasted only through the spring flush, with complete recovery in the summer growth. These results demonstrate that Douglas-fir seedlings are generally tolerant of root pruning. Hobbs et al. (1987) found that several undercutting treatments in the nursery had a more pronounced effect on the morphology of ponderosa pine than Douglas-fir seedlings at the time of lifting. However, no treatment effects were detectable for either species 4 years after planting on droughty sites. Root pruning (0-75%) of white spruce (Picea glauca [Moench]) immediately prior to transplanting did not have any detectable advantage nor negatively affect either cold-stored or March lifted seedlings (Blake 1983). Nevertheless, the relatively mild effect of severe root pruning observed in the present study may in part be due to the favorable moisture regime maintained throughout the growing period. Negative effects of root pruning may have increased considerably, if the seedlings were under any degree of soil moisture stress. For instance, Stoneham and Thoday (1985) found that effects of preplanting exposure and postplanting drought on shoot growth of birch were additive. After pruning Douglas-fir seedlings, the residual root system and new root growth were adequate to meet the water demand of the shoots, at least during the period prior to budbreak.

The relative new root growth of seedlings during the first 2 months was unaltered by root pruning, suggesting that allocation of resources to maintain new root growth was unaffected by root pruning. However, there was a reduction in absolute root initiation, as pruning decreases the root surface area available for new root initiation. Root pruning reduces the number of root tips that elongate on being planted (Stone et al. 1962). For instance, Deans et al. (1990) found that most of the new roots of Sitka spruce (Picea sitchensis) that grew after 14 days in the soil were due to the elongation of pre-existing root apices. Therefore, the root pruning treatments in the current study may have reduced the number of root apices, therefore reducing the number of new roots that elongated from pre-existing apices. On the other hand, the relative new root initiation rates were unaffected by root pruning due to several possible factors. High predawn water potentials of pruned seedlings on all transplanting dates suggests that seedlings were not under water stress, therefore maintaining high root initiation rates. For instance, root regeneration did not occur in transplanted Atlantic cedar (Cedrus atlantica Manetti) and Corsican pines (Pinus nigra Arn.ssp. laricio Poiret var.corsicana) below predawn ψ of -1.8 MPa and -1.5 MPa respectively (Aussenac and El Nour 1986; Kaushal and Aussenac 1989). In the prior experiment (chapter II), new root growth of Douglas-fir decreased with decreasing predawn ψ. New root growth was also apparently more critical to the water status of transplants in April than in January, when potential evapotranspirational demand is generally higher. Similarly, Nambiar et al. (1979) also found that the plant ψ of transplanted Pinus radiata D. Don. seedlings was

limited by new root growth. Root pruning the seedlings in the current study did not decrease relative new root initiation nor did it lower the plant water status. However, the possible mechanisms that link new root growth to the plant water status under different transplanting dates may be more complicated, as discussed below.

March transplants were able to allocate sufficient resources to new root initiation and growth in spite of potential competition from flushed shoots during this initial period of establishment. In Douglas-fir, new shoots are a strong sink for new and stored carbohydrates (Webb 1977; Krueger 1967). However, in transplanted Douglas-fir, the relative sink strength between roots and shoots may be altered in favor of the roots. This is evident in the higher R:S ratios for March transplants than for the other transplant dates, and also in the substantially greater new root initiation rates in the warmer March-April soils. Increases in relative allocation to below-ground growth have been reported in naturally growing conifers on poor sites (Keyes and Grier 1981), and in plants subjected to artificial drought (Bongarten and Teskey 1987; Nguyen and Lamant 1989). Tung et al. (1986) showed that conifers drastically increased their root:shoot ratios during the first growing season after planting. Webb (1977) found that Douglas-fir seedlings surprisingly showed relatively high carbon translocation to the roots during periods of shoot activity in May. Also, the relative allocation of carbohydrates appears to be related to relative sink strengths of various organs (Kozlowski 1992), and in the current study, the roots of transplanted seedlings may have had a greater sink strength than other tissues.

The enhanced allocation to roots in March transplants occurred regardless of higher midday water deficits, indicating that carbohydrate translocation and new root growth may not be sensitive to diurnal water deficits when soil moisture is high. Translocation is generally insensitive to water stress (Dale and Sutcliffe 1986), and can proceed under conditions that inhibit leaf expansion. Restricted foliar sink activity during the day favors photosynthate accumulation in leaf storage, as well as sucrose translocation to the roots (Luxmoore et al. 1995). Midday water deficits that develop in the shoots on hot sunny days take relatively longer to reach the roots (Kozlowski et al. 1991). For example, midday root ψ in Sitka spruce was much higher than the shoots progressively up the tree (Hellkvist et al. 1974). In drying soils, root growth is maintained longer than shoot growth in several herbaceous plants (Sharp and Davies 1979; Molyneaux and Davies 1983), and in loblolly and Scots pine seedlings (Kaufmann 1968). There is, however, little known about the effect of midday transitory water deficits on root growth, which deserves more study (Kozlowski et al. 1991).

Predawn ψ of seedlings during the first 2 months after transplanting was surprisingly unaffected by either transplanting date or pruning level. Actually, ψ were slightly higher for the March transplants than either the November or January transplants during this period (Fig III.2). It was expected that those seedlings transplanted in March would be under some degree of stress during the first few weeks after planting, because temperatures are naturally high and VPD is gradually increasing. To further increase the potential for severe stress in seedlings

transplanted in March, seedlings had all flushed and terminals had elongated an average of 5-6 cm within the first 2 months of planting. Flushing and elongation would increase the transpirational surface area and also compete as a sink for photosynthates. However, March transplanted seedlings may have compensated for these potentially stressful conditions by expanding their water absorption capacities by rapidly growing more roots in favorable warm soils (as discussed below). The consistently greater R:S ratios for the March transplants also reflects a higher allocation to the roots than shoots.

Rates of new root initiation during the first 2 months after transplanting were substantially higher for March transplanted seedlings than for the other dates. This is most probably the result of higher soil temperatures in March than in January or November. For Douglas-fir seedlings, low soil temperatures (<10°C) delayed root initiation and decreased root growth (Lopushinsky and Kaufmann 1984). Low soil temperatures increased the viscosity of water, increased root resistance (Kaufmann 1975; Grossnickle and Blake 1985), and decreased stomatal conductance and net photosynthesis (Running and Reid 1980; Delucia 1986). The substantially lower new root growth for the November transplants than either March or January may have been due to the episode of soil-freezing a few weeks after planting. This low temperature may have killed freshly initiated root tips in the November transplants, and further delayed root initiation.

Although the November and January transplanted seedlings were planted in colder soils, seedlings were not under any water stress. Further, the November

transplants showed decreased ψ (-2.0 MPa) 56 days after they were transplanted as a result of freezing temperatures, but recovered to pre-freezing levels within 10 days. This freezing event may have caused emboli in the shoot and root xylem (Robson et al. 1988; Sperry and Tyree 1990); however, it did not affect the water status during this period. These seedlings were able to maintain a favorable water balance inspite of such unfavorable conditions, for the following probable reasons: 1) low ambient temperatures and high relative humidities reduced the VPD and therefore reduced the overall demand for water (Cleary et al. 1978; Nobel 1991), 2) reduced metabolic activity of seedlings, and increased winter hardiness during this predormant and quiescent stage of seedlings could also decrease the demand for (or the loss of) water (Havranek and Tranquillini 1995). Continuous low but above-freezing temperatures also decrease stomatal conductance (Teskey et al. 1984; Strand and Oquist 1988), which would limit the water loss of seedlings.

The amelioration of the negative effects of root pruning was evident for both November and March transplants. Seedlings transplanted in November or March showed relatively little difference in net biomass between the levels of root pruning, unlike seedlings planted in January. The moderate ameliorating effect of transplanting in November and March is probably due to a different set of factors as discussed below. Amelioration of November transplants may result from a long period in the soil prior to budbreak, which compensates for lower physiological vigor of these transplants. Root biomass recovered to that of controls. Seedlings lifted in November had a DRI of 0.05 and a cold hardiness of -7.9 °C which is generally

indicative of low stress resistance (Ritchie 1986). Fall transplanted seedlings have a longer time in which to grow new roots before bud break in spring when growth resumes. On the other hand, March transplants had a very short period in the soil prior to budbreak, and their physiological vigor measured by their degree of cold hardiness (-9°C) and DRI (0.58) was also low. The March transplants were able to exploit the generally warmer soils, and showed high root growth during the time of budbreak. The R:S ratios were consistently higher for the March transplants than either November or January.

Enhanced stress resistance of seedlings planted in January with a DRI of 0.20 and a cold hardiness of -21.5°C was not as pronounced as observed in other studies with Douglas-fir or Sitka spruce (Ritchie 1986; Deans et al. 1990). For instance, severely root-pruned seedlings in January showed the same biomass RGR as the March transplants (Fig III.9). In chapter II, seedlings exposed to dry air and transplanted in January or March showed no difference in performance. Hermann (1967) observed a similar response with Douglas-fir seedlings exposed to dry air and transplanted in January and March. It is apparent that the stress resistance during the dormancy cycle of Douglas-fir seedlings is quite complex and variable. It may differ with the type and intensity of the stress, the relative climate under which seedlings are lifted and planted, and the soil growing conditions after planting. Interpreting experiments that include different lifting dates is somewhat difficult because of concurrent seasonal changes and physiological changes in planting stock, unless seedlings are grown in a uniform environment (Zaerr and Lavender 1976).

After budbreak, root pruned seedlings showed larger midday water deficits than the controls. These deficits apparently developed during the day and the seedlings probably recovered over night. This is particularly evident for the March transplants, because predawn ψ was relatively high and was unaffected by pruning treatments. The increase in midday water deficits suggests that the root pruned seedlings are unable to take up sufficient water from the soil. Warmer soils in April may maintain rapid root growth; however, the root surface area attained was insufficient to meet evapo-transpirational demand during this period. The demand for water would be enhanced by the expansion of new shoot growth, therefore leading to a net water deficit during the day in such seedlings.

Water deficits were large enough to reduce midday photosynthesis. Water deficits can directly reduce A by decreasing mesophyll conductance (Bunce 1977; Teskey et al. 1986), or by reducing electron transport in photophosphorylation (Conroy et al. 1986). Water deficits indirectly reduce A primarily through its effect on stomatal conductance. For example, a linear relationship between g_s and A, that is normally consistent with respect to slope, has been shown for several conifer species including Douglas-fir (Teskey et al. 1995; Chapter II). Among transplanting dates, a relatively large reduction in A is not always associated with either a parallel reduction in g_s or ψ. Net photosynthesis of November and January transplants was apparently limited by higher stomatal resistance (Fig III.6). However, in the March transplants, the net photosynthesis of pruned seedlings was considerably lower than could be accounted for solely by a decrease in midday ψ or g_s. It is possible that high light

intensity combined with lower ψ may have caused a photo-inhibitory effect on photosynthetic rates of these seedlings (Teskey et al. 1995). Nevertheless, the mechanisms that reduce A as a result of root pruning, in the absence of either decreased g_s or ψ, are still unclear.

March transplants not only showed the lowest mean midday ψ, but also the lowest g_s and A among the three dates. This indicates that the March transplants had still not recovered physiologically from transplanting. Although March transplants were able to rehydrate overnight (high predawn ψ), the inability of these seedlings to meet midday water demand may be the combination of a short period (46 days) prior to active resumption of spring growth, and increasing ambient VPD. The period prior to spring growth was relatively short in which to grow adequate new roots to meet the increasing water demand of these seedlings. In addition, under increasing VPD, rapid expansion of needles would increase the transpirational surface area (increase the water demand) and aggravate the physiological stress of these seedlings.

Rapid rates of budbreak are assumed to reflect enhanced vigor of transplanted seedlings (van den Driesshe 1985; Thompson 1983; Gleason et al. 1990). This observation may not always be true. For instance, the November transplants in the current study took significantly longer to break bud than seedlings transplanted in January or March, but final terminal growth or biomass were not correspondingly lower. Douglas-fir seedlings generally require aprox. 2000 hrs of chilling (temperatures at or below <5°C) before buds can break in response to higher

temperatures and increasing photoperiod (Lavender and Hermann 1970, Campbell and Sugano 1975). It is possible that the induction and progress of dormancy for fall transplanted seedlings was disrupted by the stress of transplanting. Therefore, November seedlings may have not accumulated the necessary hours of chilling for early budbreak to occur in spring. Exposing Douglas-fir seedlings to air prior to planting also produced a delay in budbreak and a subsequent reduction in growth (Chapter II). Soil temperatures may also affect bud activity (Lavender et al. 1973). Therefore, days-to-budbreak in transplanted seedlings may not always be a good indication of overall first season growth, and has to be used with caution. Under certain conditions, it may be advantageous to delay bud break until seedlings are well established, i.e., have sufficient root surface area. Bud break can be conveniently delayed by placing seedlings lifted in winter in freezer storage (Ritchie 1984).

Depending on the way the growth measurements were expressed, the response of seedlings to pruning were dramatically different. The effect of pruning levels on biomass and root volume were reversed for absolute growth and relative growth rate, while such an effect was not found between transplanting dates (Fig III.9). Seedlings were more efficient at converting resources to biomass in the following order of pruning 50% > 30% > controls, regardless of the transplanting dates. Relative growth rates of smaller Douglas-fir seedlings were greater than those of larger seedlings, and the relative biomass differences decreased with time (van den Driessche 1992). It is hard to explain why those seedlings in the current study that

showed high relative growth rates did not attain comparable final biomass at the end of the first growing season. Pruned seedlings with higher RGR may eventually equal or out grow the controls, although this was not evident the first year. For example, Hobbs et al. (1987), found that several nursery undercutting treatments reduced seedling top growth after one growing season in the nursery; however, there were no detectable treatment effects 4 years after out-planting. It must, however, be pointed out that in the current study, the final biomass and root volume of pruned seedlings attained at the end of the first year were lower than the controls by only <20%. Pruning seedlings would substantially decrease the immediate R:S ratio, which would increase the ratio of photosynthetic area to that for water absorption. Because these seedlings were planted under well watered conditions, the relative decrease of water absorptive area may have not been limiting to seedling growth. Secondly, control seedlings with higher overall initial biomass than the pruned seedlings would also have increased respiration requirements. For example, in mature trees it has been suggested the total respiration as a percent of fixed carbon increases with age/size due to the relative increase in non-photosynthetic tissue (stem wood) (Waring and Schlesinger 1985). Interestingly, the relative growth rates of stem diameter among pruning treatments was similar to the absolute diameter growth (Fig III.9). This suggests that varying the shoot:root ratios of these seedlings does not affect the relative allocation of resources to the stem.

Bud width measured at planting was a significant covariate of both terminal spring and lammas growth. Correlation coefficients of this relationship

indicated a positive association between bud width and shoot growth. This supports the results for several conifer species, where spring leader length is related to bud size and primordia development (Clements 1970; Kozlowski et al. 1973; Kremer and Larson 1982; Graham and Hobbs 1994). Bud width is apparently related to terminal length even when seedlings were pruned or transplanted at different times. It was surprising to observe that lammas growth was also related to bud width at the time of planting. Although the relationship was poor, it is significant that bud width may influence the lammas growth of newly transplanted Douglas-fir seedlings. In contrast, lammas growth in black spruce Picea mariana (Mill) seedlings was unaffected by bud size (Colombo 1986). However, in Douglas-fir, determining and varying the nursery practices to increase bud size may enhance its outplanting performance (Burdett 1983; Graham and Hobbs 1994).

Smaller buds of Douglas-fir (Graham and Hobbs 1994) and white fir (Macey and Arnott 1986) apparently compensate for shorter spring growth by growing longer lammas growth. However, in the current study, lammas growth increased with spring terminal growth. There was no compensatory lammas growth. It is also important that neither the pruning treatments nor transplanting dates had a significant effect on lammas growth. Apparently, lammas growth in the first year is dependent on the relative degree of recovery from transplanting stress. The effect of transplanting stress is primarily on the spring growth, and on the percent of seedlings that showed lammas growth (Fig III.7). However, seedlings that recovered rapidly

and showed longer fixed growth were able to produce longer lammas growth. This effect is relatively small but significant in the first year.

Summary

Root pruning >30% of the original root volume decreased both the biomass and elongation growth of Douglas-fir seedlings. There was little difference in growth or physiology between seedlings that had 30% or 50% of their roots removed. Severe root pruning (50% of root volume) reduced average terminal elongation by approximately 10% of the unpruned controls, and reduced total biomass by approximately <20%. Mortality of seedlings subjected to any of the pruning treatments was <1%. Transplanting seedlings in November or March, rather than in January, moderately ameliorated the negative effect of root pruning. On the other hand, January and November transplants attained a larger mean biomass than the March transplants. Root pruning decreased the number of new root initiates. However, the number of new root initiates per unit volume of root was unaffected by the root pruning treatments. The number of new root initiates increased exponentially from November to March. Predawn ψ of seedlings during the same period was unaffected by root pruning treatments or transplanting dates. However, after budbreak, midday ψ of pruned seedlings was lower than the controls. Stomatal conductance followed the same trend as ψ, however the differences in g_s between treatments and transplanting dates were not as pronounced as ψ. Net photosynthesis

was lower for the pruned seedlings than for controls from all transplanting dates, but it was substantially lower for November and March transplants.

In conclusion, root pruning seedlings up to 50% of original root volume did not cause substantial reduction in growth of Douglas-fir seedlings, nor did it affect first year mortality. It appears that Douglas-fir seedlings are generally tolerant of such severe root removal during transplanting. However, one must extrapolate these results to the field with caution. Seedlings were well watered throughout the period of the experiment; therefore they were not subject to the natural summer drought. Seedlings with 50% of their roots removed may not be able to meet evapotranspirational demand during the dry summer months in the field, which can potentially cause considerable mortality. Under well watered conditions, such as generally found from January-April in the coastal ranges of the Pacific Northwest, considerable water deficits may not develop during the first 2 months after transplanting. Midday water deficits may develop in root pruned seedlings as the season progresses, and as seedlings flush and expand their transpirational surface area. This water deficit during the day can reduce the net carbon balance of seedlings, and consequently reduce growth. Pruning roots at the time of transplanting to facilitate easy handling may not increase mortality, but may reduce growth, although extensive field trials are necessary to confirm the results reported here.

CHAPTER IV. EFFECT OF SEEDLING EXPOSURE AND ROOT PRUNING ON THE ONTOGENETIC AND SEASONAL CHANGES IN TISSUE WATER RELATIONS AND GAS EXCHANGE OF DOUGLAS-FIR SEEDLINGS

Introduction

Transplanted seedlings invariably go through a period of stress and recovery from the damage associated with the process of transplanting (Rietveld 1989). This period of stress and recovery can be prolonged and aggravated by an inhospitable planting environment. Symptoms of stress can range from reduced growth to seedling death during the first season. Frequently, if seedlings survive the first year they may show reduced growth for several years. In Douglas-fir seedlings, particularly in the coast range of the Pacific Northwest, the typical visible symptoms of transplanting stress are densely packed needles with reduced shoot elongation. Seedlings that are relatively tolerant of stresses associated with transplanting or have recovered develop a long leader with larger stem units and long needles.

The physiological mechanisms underlying the symptoms of transplanting stress are not well understood. The work described in the earlier chapters determined the magnitude of effects of stresses such as seedling exposure and root loss, and examined possible physiological mechanisms that mediate post-transplanting effects. Exposure to air reduced root hydraulic conductance and new root growth (chapter II). Exposure also increased the stem resistance to water flow as a result of cavitation of xylem trachieds. During the first few weeks after transplanting, stomata were more sensitive to root damage than were changes in the internal water status of

seedlings. Lower stomatal conductance reduced carbon assimilation, potentially limiting photosynthates available for root growth and establishment. Exposed seedlings showed signs of physiological stress during the early stages of shoot elongation, but recovered midway during elongation. Spring growth was unaffected by this physiological recovery and showed a permanent reduction in elongation. However, the summer lammas shoot with longer needles and stem-units showed signs of recovery.

Pruning loss of > 30% of fine roots prior to transplanting reduced shoot elongation, although this root loss did not reduce plant ψ sufficiently to have inhibited root growth during the first 2 months after transplanting (chapter III). Stomatal conductance was not measured during this period but was probably reduced as in the exposed seedlings. New root initiation was significantly reduced for pruning levels above 30%, although the rate of new root initiation per initial root volume was unaffected by pruning. This suggests that the removal of a even a large proportion of the root system did not alter the allocation of photosynthates to the roots nor the water balance during the first 2 months after transplanting. However, pruned seedlings had lower ψ and concurrently lower stomatal conductance and net photosynthesis after budbreak. To what extent this decrease in carbon acquisition and impaired water balance modified the first season growth is difficult to determine.

Differences in the observed shoot elongation between stressed and untreated Douglas-fir seedlings may be associated with a varying rate of cell wall hardening (Ritchie and Shula 1984). Generally, cell walls remain viscoelastic during shoot

elongation, but continue to harden as the shoot matures (Ray 1987). This viscoelastic nature of immature cell walls permits irreversible elongation in response to a positive growth-active turgor (Lokhart 1965; Cosgrove 1986; Ray 1987). If cell walls were to harden at a faster rate in the stressed seedlings than the controls, I hypothesized that elongation growth would not recover even if turgor were to increase sufficiently during the elongation process. Several reports have indicated that inhibition of elongation growth in response to water stress can occur without long-term reductions in the turgor pressures of expanding cells (Hsiao and Jing 1987; Nonami and Boyer 1990a; Serpe and Mathews 1992), suggesting that inhibition of cell expansion could be associated with the hardening process. Direct measurements of cell wall extensibility in growing tissues of intact plants have recently confirmed evidence of rapid cell wall hardening in soybean and maize in response to water deficits (Nonami and Boyer 1990b; Chazen and Neumann 1994).

In mature cells, the modulus of tissue elasticity measures the reversible extensibility of the cell wall (Nonami and Boyer 1990b). A higher cell wall elasticity (low modulus of elasticity) indicates better turgor maintenance under water deficits, because an elastic tissue will sustain a smaller decrease in turgor (p) as a given volume of water is lost, than will a more rigid tissue (Joly and Zaerr 1987). However, in expanding immature tissue, the changes in the modulus of tissue elasticity could be indicative of cell wall hardening as well as the capacity to maintain turgor. The modulus of tissue elasticity in such tissue may therefore influence turgor mediated processes such as elongation growth (Hsiao et al. 1976).

In growing tissue, cell wall hardening is a function of both the plastic and elastic components of extensibility (Nonami and Boyer 1990b). Irreversible plastic extensibility of the cell wall of immature cells in elongating tissue of soybean and maize plants decreased in response to water deficits within a few minutes to hours after a mild water stress (Nonami and Boyer 1990a; Chazen and Neumann 1994). Nonami and Boyer (1990b) also measured the elastic component and found that it did not change in the elongating tissue of maize stems during 3 days of mild water stress. For whole Douglas-fir seedlings, modulus of tissue elasticity (measured by PV curve analysis) increased during the spring flush period, suggesting that a decrease in cell wall elasticity is associated with ontogenetic cell wall hardening (Ritchie and Shula 1984). Similarly Tyree et al. (1978) found that the tissue modulus of elasticity increased with leaf age in sugar maple (Acer saccharum) and poplar species (Populus spp).

There are few detailed studies of the ontogenetic changes in tissue-water relations of expanding new growth. Ritchie and Shula (1984) measured the tissue-water parameters of Douglas-firs seedlings monthly over the entire year. However, they did not separate the new growth from the older growth, therefore confounding the independent effects of tissue age on the tissue-water relations. They also did not correct for the initial "plateau" in the PV curves because of possible over saturation, which could considerably affect the magnitude of some of the parameters measured (Kubiske and Abrams 1991). A similar study with the same deficiencies in PV curve analysis was reported for western hemlock and western red cedar by Grossnickle

(1993). However, Gross and Koch (1991) working with Picea abies provide evidence that suggests naturally present xylary water in the stem may contribute to the observed "plateau effect" in whole shoots. In the present study, I corrected for the initial "plateau affect" as suggested by Kubiske and Abrams (1991) and determined the tissue-water relations on current and 1-year-old shoots separately. In addition, this study characterizes the ontogenetic and seasonal development of water relations of Douglas-fir seedlings that were stressed prior to transplanting, in order to understand the stress and recovery associated with the transplanting process.

This experiment was specifically designed to test the hypothesis that Douglas-fir seedlings stressed prior to transplanting lose tissue elasticity of elongating spring shoots faster than unstressed controls, and that this increase in the rate of cell wall hardening is associated with a lower measurable turgor pressure in the elongating shoots of Douglas-fir. The second hypothesis states that osmotic adjustment in stressed newly transplanted Douglas-fir seedlings would account for some degree of turgor maintenance in alleviating internal water deficit. Finally, measurement of gas exchange parameters of mature and expanding needles in stressed and unstressed controls would aid in characterizing carbon acquisition, and stomatal regulation of water loss, during early establishment of transplanted seedlings.

Material and Methods

Two-year-old Douglas-fir seedlings were either root pruned or exposed to dry air and subsequently grown under well-watered conditions in pots (Fig IV.1). At periodic intervals during active terminal leader elongation, the water relations of new and 1-year-old shoots were characterized using the PV-curve analysis. The gas exchange of new and 1-year-old shoots were also measured.

Seedlings

Douglas-fir seedlings were obtained as 2+0 stock type from the US Forest Service Nursery at Wind River, Washington. They were from a 915-1372 m

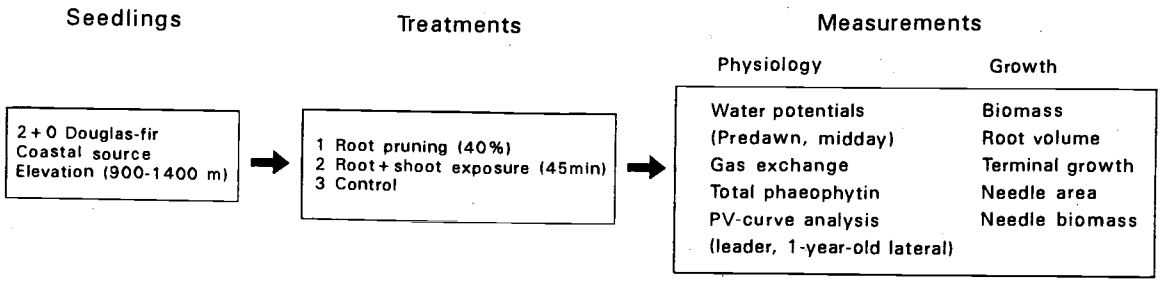

Figure IV.1. A general layout of the experiment. Seedlings were grown under shade in a cold frame and brought into a controlled environment where periodic measurements were made.

elevation, coastal Oregon seed source. Seedlings were carefully uprooted from the nursery on February 13, 1992, and transported to the Forest Research Laboratory, Corvallis, Oregon, where they were stored at 4°C till the start of the experiment on July 4, 1992

Exposure and Pruning Treatments

Seedlings were removed from cold storage on March 12, washed free of soil and placed back in the cold room. On July 4, 180 seedlings were randomly selected from the cold stored lot and divided into three sets of 60 seedlings for each of the 3 treatments. Exposure treatments involved exposing the seedlings in a growth room on a slotted rack for 45 min. The growth room was maintained at 25°C, 35-40% RH and 30 μmoles m^{-2} s^{-1} photosynthetically active radiation (PAR). Root pruned seedlings had 40% of their fine roots removed. Tertiary and secondary root tips were clipped and the initial and final root volumes were measured by the volume displacement method (Burdett 1979). Prior to root pruning, the average root volume of seedlings was 10.5 ml. Seedlings were immediately planted in 3.6 liter pots with a sand:peat:vermiculite mixture (2:1:1 v/v) and watered to saturation.

Seedling Culture

Potted seedlings were grown under shade in a cold frame. Seedlings were watered regularly and fertilized twice with Miracle Gro (15:30:15) at the rate of

45g/m^2. PAR in the cold frames was 150-200 μmoles m^{-2} s^{-1}, day temperature was 15-28°C, and vapor pressure deficit (VPD) was 3-12 mb over the growing season. Seedlings were misted frequently during the first few days after transplanting because temperature and VPD were very high. Seedlings were transferred to growth rooms in the evening of the day before gas exchange measurements were made and watered to saturation. Growth rooms were held at a day \ night temperature of 25 \ 15°C, 150-200 μmoles m^{-2} s^{-1} PAR and 16 hr photoperiod.

Sampling Period

Periodic measurements of gas exchange, growth and water relations were made on 6 dates: July 22, July 29, August 7, August 19, September 2, and October 21. Measurements on these dates were made on a different pool of randomly selected seedlings from the same population. The above dates correspond to 18, 25, 34, 46, 60 and 109 days after transplanting.

Growth

The growth response of the treated and untreated seedlings was characterized by days to budbreak, terminal leader elongation, number of roots tips, root volume, root dry weight, stem diameter, and lateral needle biomass and area (Fig IV.1). The date of terminal budbreak was recorded for each seedling and the number of days from planting to terminal budbreak (DBB) was calculated. When seedlings were

removed for gas exchange and pressure-volume analysis (PV analysis), the length of the terminal new shoot was measured to the nearest mm. After gas exchange and PV analysis measurements were made, the seedling was removed from the pot, washed free of soil, and stored in the cooler at 4°C till the other growth measurements were made. The number of new root tips was estimated to the nearest 10 tips. Root volume was measured by the root displacement method (Burdett 1979) to the nearest 0.01 ml. The roots were dried at 70°C for 48 hrs and weighed. Stem diameter was measured at the root collar to the nearest 0.1 mm with a digital caliper. Specific leaf area (SLA, $cm^2\ g^{-1}$) of the lateral needles of the first whorl was calculated by dividing the needle area by its biomass. Specific leaf area was calculated for current and 1-year-old needles separately. The projected leaf area was measured with an leaf area meter (LICOR 3100) and multiplied by 2 before SLA calculation. Dry weight of the lateral twig was measured gravimetrically at 70°C for 48 hrs.

Pressure-Volume Curve Derivation

General

Pressure-volume analysis was done on the expanding terminal leader and on 1-year-old lateral twigs of the first whorl. The pressure-volume curve (PV-curve) was derived from a set of data points that were generated by simultaneously measuring the drop in relative water content (RWC) and the water potential of a twig as the pressure in the chamber was increased. The PV-curve was generated using the sap-

expression method (Cheung et al. 1975; Ritchie and Shula 1984; Joly 1984). The procedure described below is a slight modification of that used by Joly (1984). The pressure volume technique was developed by Scholander et al. (1964, 1965) and Tyree and Hammel (1972) to study the tissue water relations of higher plants. The PV-curve can be used to estimate several useful measures of tissue water status, including osmotic potential (π) and turgor pressure potential (p) as a function of water content (Table IV.1).

Table IV.1. Symbols and descriptions of tissue water-relation parameters derived from the pressure-volume analysis of shoots of Douglas-fir that were either exposed or root pruned prior to transplanting.

Symbols	Description	Units
ψ	Water potential	- MPa
π	Osmotic potential	- MPa
p	Turgor pressure	+ MPa
π_{100}	Osmotic potential at full turgor	- MPa
π_0	Osmotic potential at zero turgor	- MPa
RWC	Relative water content	%
RWC_0	Relative water content at zero turgor	%
TWDWT	Turgid weight / dry weight	g/g
ϵ^{max}	Maximum tissue modulus of elasticity	+ MPa
p^{max}	Maximum mean turgor pressure	+ MPa
AP_v	Apoplastic fraction	%

Twig Preparation

Twigs from current and 1-year-old shoots were cut and immediately transported to the lab in a cooler. Twigs were recut under distilled water. The terminal leader was recut such that 5 cm of the last-year's stem was attached to facilitate easy measurements. Needles were carefully removed from the 5 cm segment and the bark peeled back for 2 cm. The twig was then weighed to the nearest 0.001 g to determine its fresh weight in order to calculate the RWC at the time of harvest.

Rehydration

The portion of the twig that had needles was wrapped in clear plastic and the whole twig was placed with its base submerged in approximately 2 cm of distilled water in a beaker; the foliage was held above the water. The beaker was placed in another beaker with a small quantity of water and the top was covered with a plastic bag which was secured with a rubber band. This dual-beaker setup was then placed in a water bath at room temperature (25°C) overnight to rehydrate. The rehydration period extended from 12-15 hr. This setup ensured 100% humidity for adequate rehydration of the twigs.

Pre-measurement Sample Preparation

The next day the twig was removed from the beaker-setup, surface dried between paper towels and weighed to determine the rehydrated fresh weight (W_s) to

the nearest 0.0001 g. It was then quickly placed inside a clear plastic sheath along with a small piece of moist paper towel. The stem was forced through a small hole at one end of the sheath, which provided a firm seal. The opposite end of the sheath was tied with a rubber band, leaving 2-3 cm of the stem protruding from this enclosure. Two pressure chambers (PMS Instruments, Corvallis, Oregon) were used for developing PV data. Each was fitted with a head modified to accommodate three samples. After a set of samples had been inserted into a rubber stopper, the protruding stem section was wrapped with parafilm^{R} to prevent any evaporative losses. The chamber was lined with moistened paper towels to further minimize tissue water loss and leaf temperature fluctuations during pressure changes (Wenkert et al. 1978).

Data collection

The flow of nitrogen gas into the chamber was set at approximately 0.004-0.006 MPa s^{-1} until fluid appeared on the cut surface of the stem. This initial leaf ψ (balance pressure) was recorded for each stem; then the chamber pressure was reduced to slightly below the lowest of the three balance points. Each of the stems was quickly fitted with a pre-weighed sap collection apparatus. The sap collection apparatus consisted of a 1.5 ml Eppendorf^{R} microtube with its tip cut off so the stems could be inserted. Since the base of these tubes were conical, the size of the opening could be varied to fit the stem snugly and prevent any evaporation losses of water. The tubes were stuffed loosely with strips of absorbent paper towel (Kimwipes^{R}).

After recording the initial leaf ψ, the chamber was again slowly pressurized to a predetermined over pressure of 0.2-0.5 MPa above the lowest balance point. This resulted in exudation of fluid from the cut surface and its absorption by the paper strips. The elevated pressure was maintained for 10-12 min; after this exchange time, chamber pressure was slowly reduced to 0.1-0.2 MPa below the anticipated new balance pressure. The sap collection apparatus was removed and weighed to the nearest 0.0001 g. The chamber was slowly repressurized until fluid was just visible on the cut surface. This new equilibrium pressure was recorded. Balance pressures could be determined to within ± 0.005 MPa by examining the cut surface under 20X magnification. A new pre-weighed sap collection apparatus was placed over each stem and this procedure repeated 14-18 times. Typically 6-9 data points were in the range of positive turgor and 8-11 data points were in the region of zero turgor. All data collection was done in an air-conditioned room where temperature was maintained at 25-26°C.

After the final balance pressure was determined, the sample was removed from the chamber, and its residual fresh weight (W_f) was quickly measured. Dry weight (W_d) was determined after drying the sample in an oven at 70°C for 48 hrs.

Analysis

A typical PV-curve describes the relationship between the reciprocal balance pressure and 1-RWC (Fig IV.2). The linear region of the curve is where the turgor is zero and extrapolation to the ordinate gives an estimate of $1/\pi_{100}$. Extrapolation to

the abscissa gives an estimate of the relative apoplastic fraction (AP_v) (Relative symplastic volume = 1-AP_v). The point at which the linear and non-linear regions of the PV curve intersect is where the bulk turgor potential first reaches zero, or the "zero turgor point". Values for turgor pressure (p) were estimated from differences between reciprocal points on the curvilinear portion of the curve and the corresponding reciprocal extrapolated π values. The RWC_0 can be estimated by

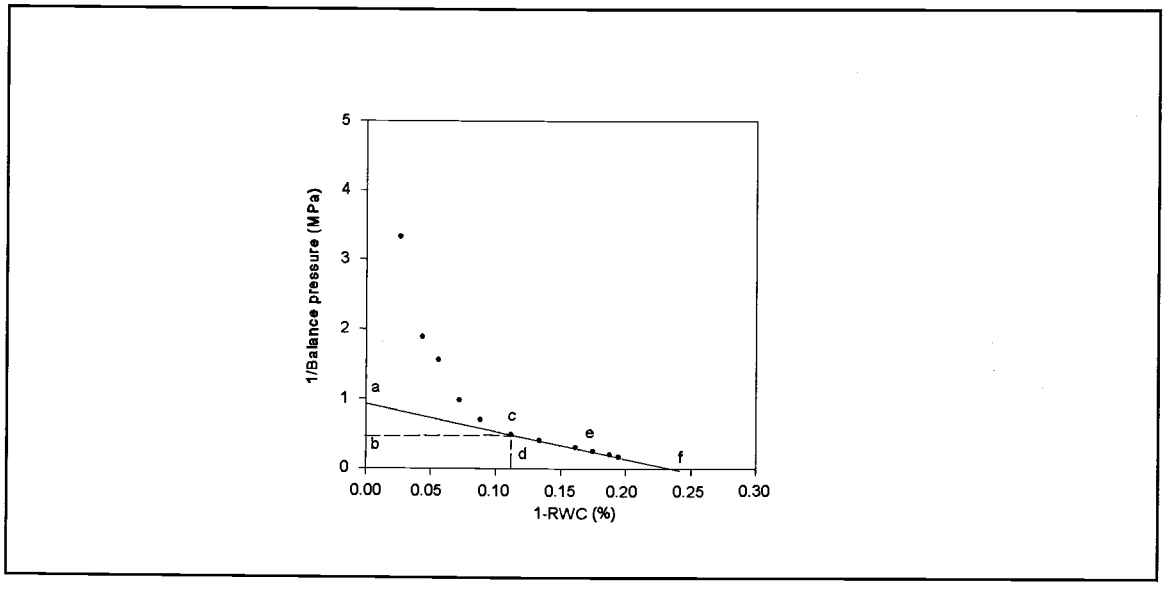

Figure IV.2 A sample pressure-volume curve and the derived parameters of tissue-water relations of a Douglas-fir shoot. a) π_{100}, Osmotic potential at full turgor, b) π_0, Osmotic potential at zero turgor, c) Turgor pressure (p) = 0, d) 1-Relative water content at zero turgor (RWC_o), e) Water potential (ψ) = Osmotic potential (π), f) Apoplastic fraction (AP_v).

dropping a perpendicular to the abscissa from the point on the curve where turgor is zero. The PV data were analyzed using a computer program developed by Davie et al. (1993).

The collected data had to be corrected for two technical artifacts prior to analysis. One was to correct for over saturation of tissues (Kubiske and Abrams 1991), and the other was to correct for the water lost from the system through evaporation into the chamber (Ritchie and Shula 1984). Artificial rehydration, depending on the tissue and duration of rehydration, may cause a "plateau" effect near the region of full turgor (Kubiske and Abrams 1991). Because one of the assumptions of the PV-curve analysis is constant apoplastic volume, this extra free water in the apoplasm would cause a deviation from the assumption and cause erroneous shifts in the PV curve (Fig IV.3), consequently changing the magnitude of the parameters estimated. Most of the samples in this experiment exhibited a plateau effect (see sample Fig. IV.4). In order to correct for this over saturation, the turgid weight at full turgor has to be re-estimated and the reciprocal RWC recalculated. The correct turgid weight at full turgor can be estimated by extrapolation of the first three or five points of the PV-curve (Kubiske and Abrams 1991; Davie et al. 1993). The first 3 to 5 points were selected for a best fit least-square regression and the intercept on the fresh weight axis was the correct turgid weight. This was done using the computer program developed by Davie et al. (1993).

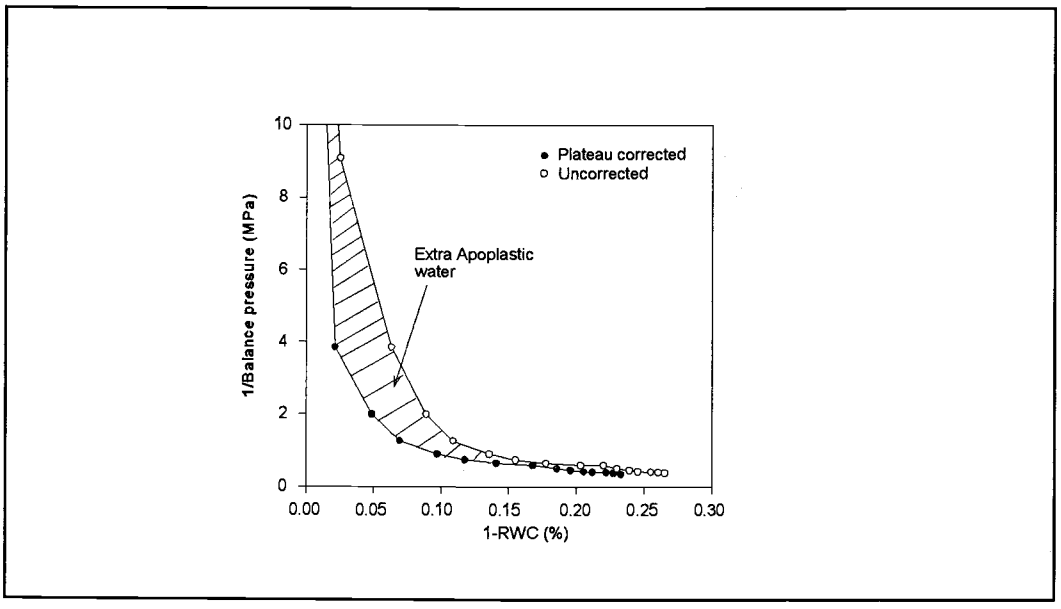

Figure IV.3. A typical pressure-volume curve with and without plateau correction showing a substantial shift in the PV-curve due to extra apoplastic water.

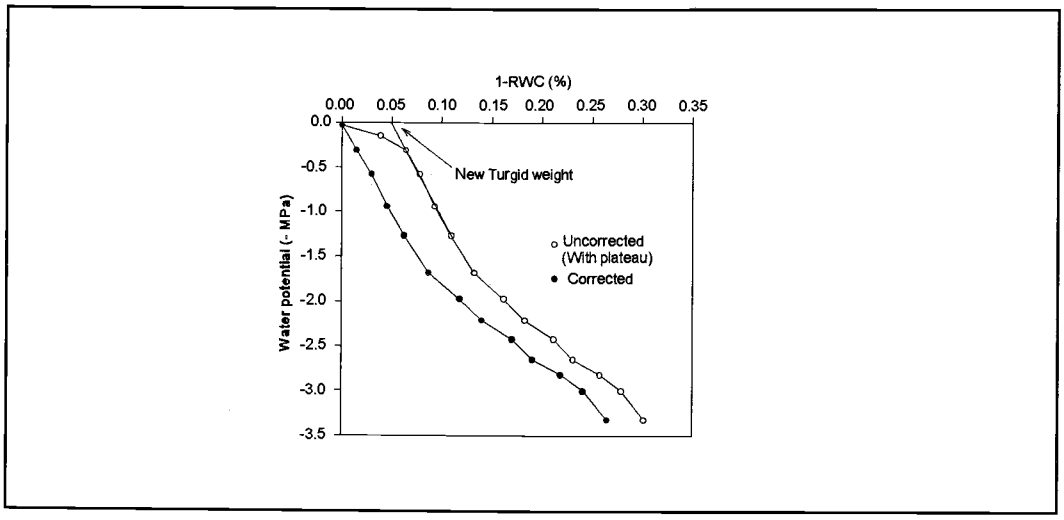

Figure IV.4. A modified PV curve (1-RWC versus water potential) showing the plateau region at high water potentials. New turgid weights were calculated from extrapolation of the linear section of the curve to the x-axis and corrected curves replotted.

The corrected turgid weight (W_t) was then substituted in the equation (2) and the PV-curve was replotted. Figure IV.3, shows the PV-curve before and after turgid weights were corrected.

A correction factor C was estimated to correct for the water lost from the system through evaporation into the chamber:

$$C = \{(W_s-W_f)-S_i\}/N \qquad (1)$$

Where W_s is the initial saturated weight (plateau uncorrected), W_f is the final residual fresh weight of the sample, S_i is the total weight of xylem sap collected through the test, and N is the number of balance pressures applied during the measurement. This method of correction assumes that evaporation into the chamber occurs at a constant rate throughout the analysis.

To produce the PV-curve, reciprocal balance pressures were calculated and plotted against 1-RWC at that pressure using:

$$1\text{-RWC} = 1-\{(W_i-W_d)/(W_t-W_d)\} \qquad (2)$$

$$W_i = W_t-(S_i+C) \qquad (3)$$

where W_i is the fresh weight of the sample at that balance pressure, W_d is the oven dry weight of the sample, W_t is the recalculated turgid weight, S_i is the weight of the sap collected up to that pressure, and C is the correction term for evaporation loss into the chamber. A representative PV-curve is plotted for a current and 1-year-old twig of Douglas-fir (Fig. IV.5).

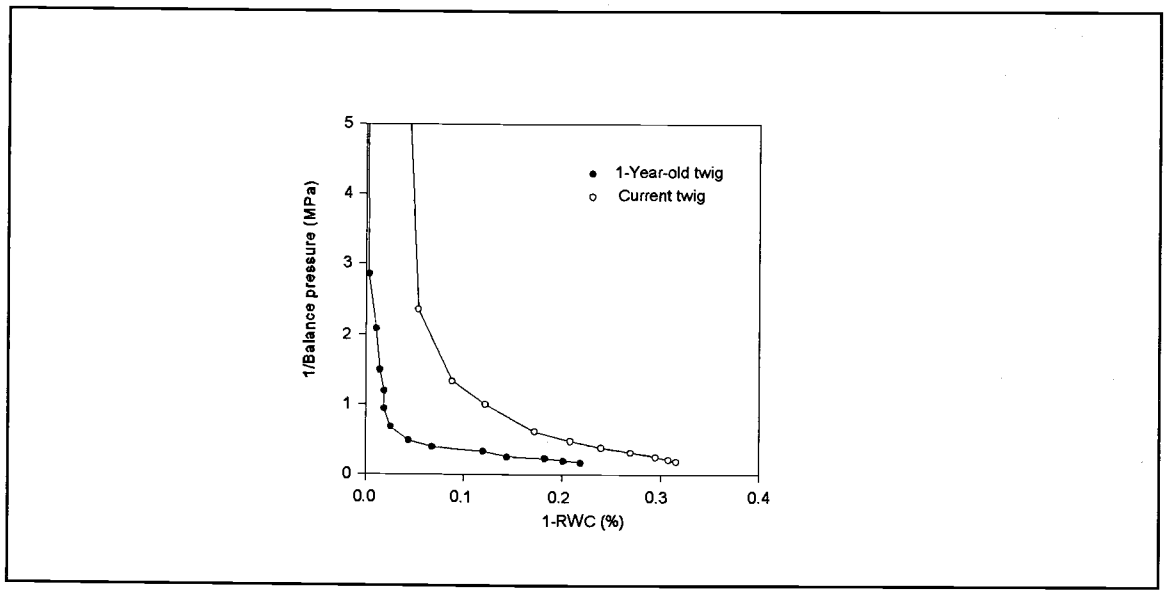

Figure IV.5. A PV curve of a typical current and 1-year-old twig of Douglas-fir.

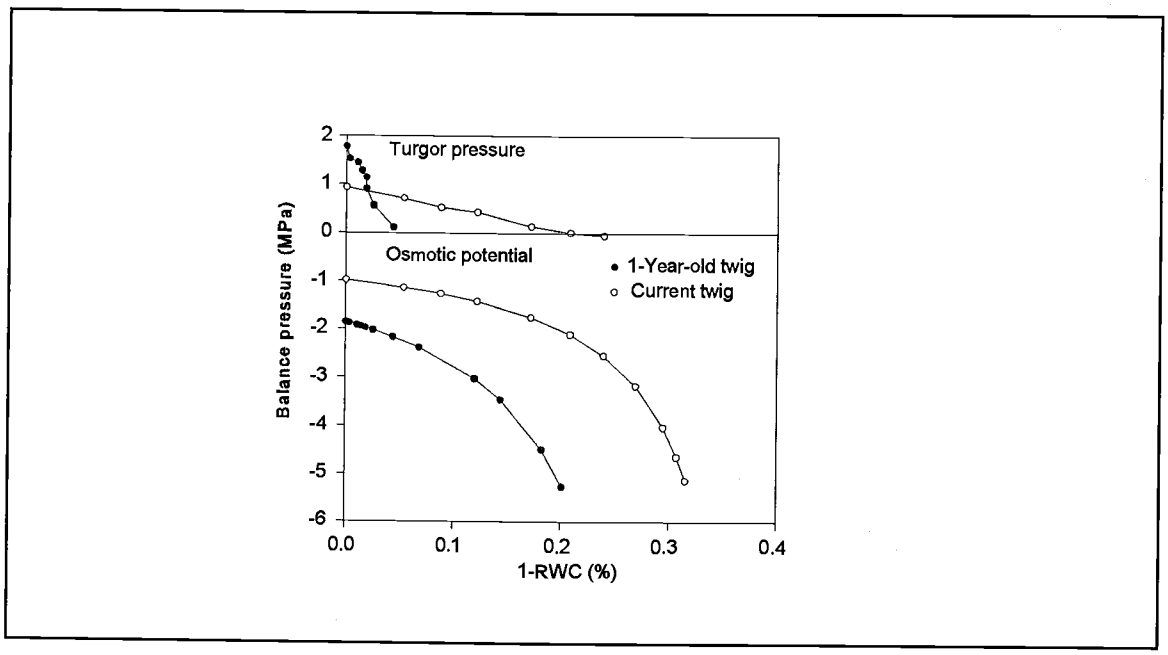

Figure IV.6. A modified Hofler diagram with p and π as a function of 1-RWC for current and 1-year-old twigs of Douglas-fir. The curve for water potential is not shown.

A Hofler diagram was generated showing π and p as a function of RWC (Fig IV.6) for a representative current year twig and a 1-year-old twig. In order to simplify the diagram , ψ was not included.

The bulk modulus of elasticity is defined as the change in tissue turgor pressure for a given fractional change in symplastic water (Steudle et al. 1977). As the apoplastic water content is assumed to be constant, this fractional change can be approximated by the RWC, and a bulk weight averaged elasticity ϵ can be calculated. Following Robichaux (1984), ϵ is given by:

$$\epsilon = (\Delta P/\Delta RWC) \times (RWC_x) \qquad (4)$$

where RWC_x is the mean RWC. Since ϵ is turgor and volume dependent (Pallardy et al. 1990), ϵ is calculated as a function of p. The linear slope of the first 4 points of the p vs RWC relationship was calculated, RWC_x is the mean RWC of these points. This was repeated for points 2-5, 3-6, etc.; viz. each successive set of 4 points (Davie et al. 1993). The mean turgor of each of these 4 points was recorded along with ϵ. To standardize estimates of ϵ among the samples, I selected the maximum bulk tissue modulus of elasticity (ϵ^{max}) and the corresponding value of the mean turgor pressure (p^{max}), and these were used for comparisons.

Water Potential and Gas Exchange

Predawn and midday ψ were measured on 6 seedlings/treatment on each sampling date. Midday ψ were taken 6-7 hrs after lights were switched on. Water potentials were measured on a lateral twig with a pressure chamber (PMS Instruments

Co, Corvallis, Oregon). Predawn π and p were estimated from the PV curve at the measured value of predawn ψ , since $\psi = -\pi + p$. Gas exchange was measured with a portable infrared gas analyzer (LICOR 6250, Lincoln, Nebraska) on the same 6 seedlings/treatment. During sampling days, measurements were taken between 4 and 6 hrs after lights were switched on. Separate measurements were made on 1-year-old needles and current needles on a lateral branch on the first whorl. Duplicate measurements were made on the same sample. Net photosynthesis (A) was measured directly, but stomatal conductance (g_s) was calculated using equations from von Caemmerer and Farquhar (1981). Projected needle areas were measured with a leaf area meter (LICOR 3100, Lincoln, Nebraska).

Phaeophytin Determination

Phaeophytin is the acidic derivative of chlorophyll (Scheer 1991). When tissue samples are acidified prior to analysis, there is 1:1 correspondence between the concentrations of chlorophyll and phaeophytin (Moran 1982). Chloropyll pigments deteriorate to their corresponding demetalated (- Mg) phaeophytins in exposure to light and extreme temperatures (Inskeep and Bloom 1985). I did not estimate chlorophyll immediately and had to store sample tissues for several months in a freezer at -20°C. Because freezing and thawing could also cause substantial conversion to phaeophytin, I decided to convert all the existing chlorophyll to phaeophytin and determine the relative concentration of phaeophytin.

A sample of the lateral needles that was used for gas exchange measurements was also used for phaeophytin determination. Relative concentrations of phaeophytin were determined using a method after Moran (1982), with slight modifications. 50-100 mg of fresh needles were weighed into 15 ml glass test tubes. After adding 5 ml of N,N-Dimethylformamide (DMF) to each sample, 10µl of 0.5N HCl was pipetted into each test tube. The test tubes were covered with an aluminum foil and placed in the dark in a cooler at 4°C for 3-5 days. After incubation, samples were brought to room temperature and the % absorbance measured in a spectrophotometer (DU-40, Beckman Instruments Inc, CA) at the 666 and 654 nm wavelengths. The spectrophotometer had a path length of 1 cm with a 1 ml cuvette. The relative concentration of phaeophytin was calculated based on equation (5) from Moran (1982):

$$\text{Phaeophytin } (\mu g/ml) = 7.53\ A_{666} + 30.19\ A_{654} \qquad (5)$$

Where A_{666}=Maximum absorbance at 666 nm, and A_{654}=Maximum absorbance at 654 nm. The concentrations were converted to mg g^{-1} fresh weight.

Statistical Analysis

The growth and water potential measurements were analyzed as a completely randomized 2-factorial design, with date and treatment as the two factors. The PV-curve derived data were analyzed in two separate ways, because measurements on 1-year-old needles were not done for the third sampling period. Separate analysis with and without the 1-year-old needles allowed for a more robust treatment of the data

than using a single analysis for both tissues. The differences between treatments and dates for the current tissue were analyzed as a completely randomized design with treatment and dates as the 2 main factors. To determine differences in gas exchange and water relations among treatments, dates and tissues, the data were analyzed as a split-plot design. Treatment and date were main plots and tissue was the sub-plot. Data were checked for normality and homogeneity of variance, and $\log_e$ transformed when necessary. All statistical analysis was done using SAS (SAS Institute Inc. 1989). The p-values from ANOVA for the different treatment effects are included in the text in the form of a table. Means and population standard errors are graphically presented in the results.

Results

Growth

Exposure and root pruning had a significant effect on all the growth variables except specific leaf area (SLA) (Table IV.2). However, all these variables changed significantly with time. Growth differences between the treatments, sampling periods, and differences between tissues, and their interactions are discussed below.

Untreated controls flushed 5 days earlier ($p<0.001$) than both root pruned or root exposed seedlings. Untreated controls took 16 days to flush whereas the stress treated seedlings took 21 days to flush. Seedlings from the different treatments

Table IV.2. P-values associated with the different treatment effects for the growth variables.

Source of variation	Growth variables						
	Days to bud-break	Terminal length	Root weight	Root volume	Lateral needle weight	Lateral needle area	Lateral SLA
Treatment (T)	0.0001	0.0001	0.0001	0.0001	0.0001	0.0002	0.22
Tissue (Ti)	-	-	-	-	-	-	0.0001
Date (D)	0.02	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
T x Ti	-	-	-	-	-	-	0.04
T x D	0.44	0.60	0.88	0.96	0.29	0.63	0.51
Ti x D	-	-	-	-	-	-	0.0001
T x Ti x D	-	-	-	-	-	-	0.45

were phenologically similar only for the first two sampling dates (Fig IV.7). Thereafter, the untreated controls were always 5-10 days ahead phenologically than both the stress treated seedlings. Therefore, while making comparisons of treatment effects on any given sampling date, the phenological difference between treatments should be taken into consideration.

There was no difference between the two stress treatments for terminal length (Fig IV.8); however, stress treatments produced terminals that were shorter ($p<0.0001$) than the controls. Elongation rate of the terminal leader for all treatments was maximum during the first 34 days, after which it decreased. Root dry weight and root volume differed significantly ($p<0.05$) among the 3 treatments: control > exposure > pruning. Root mass and volume continued to increase over the duration

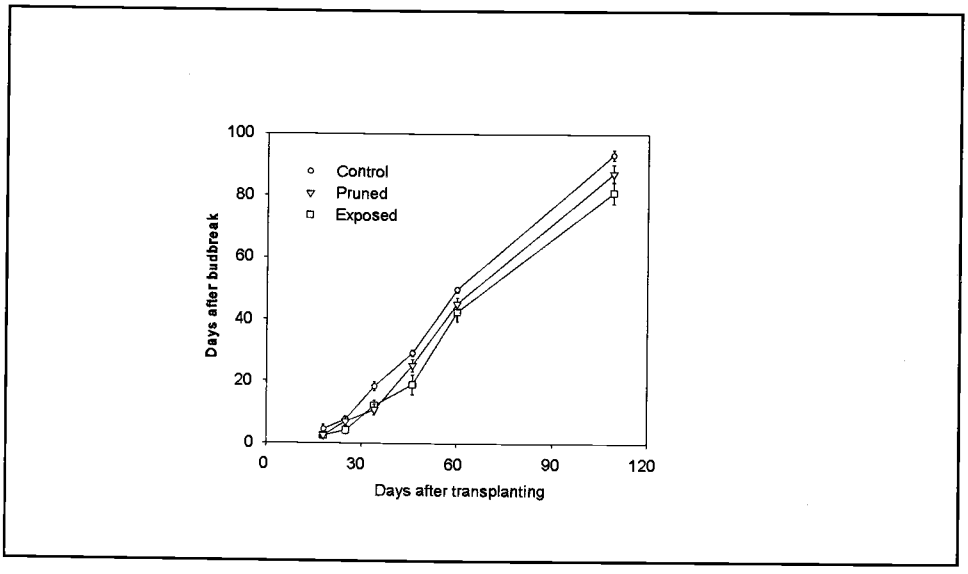

Figure IV.7. The days after budbreak for each treatment corresponding to the days after transplanting (n=6). Bars are one standard error of the mean. See table IV.2 for the level of significance of treatment effects.

of the experiment (109 days). The number of new root tips increased from an average of 10, 18 days after transplanting, to >200 at 109 days. There was no measurable change in diameter growth (data not shown) over the duration of the measurements. Stem diameters ranged from 4.6 - 5.0 mm for the different treatments.

The dry weight accumulation and expansion of the current lateral needles were the same for both root exposed and pruned seedlings, although they were lower than the untreated controls (Fig IV.9). A large difference between the stressed and untreated control seedlings was apparent only after the first 34 days of rapid expansion.

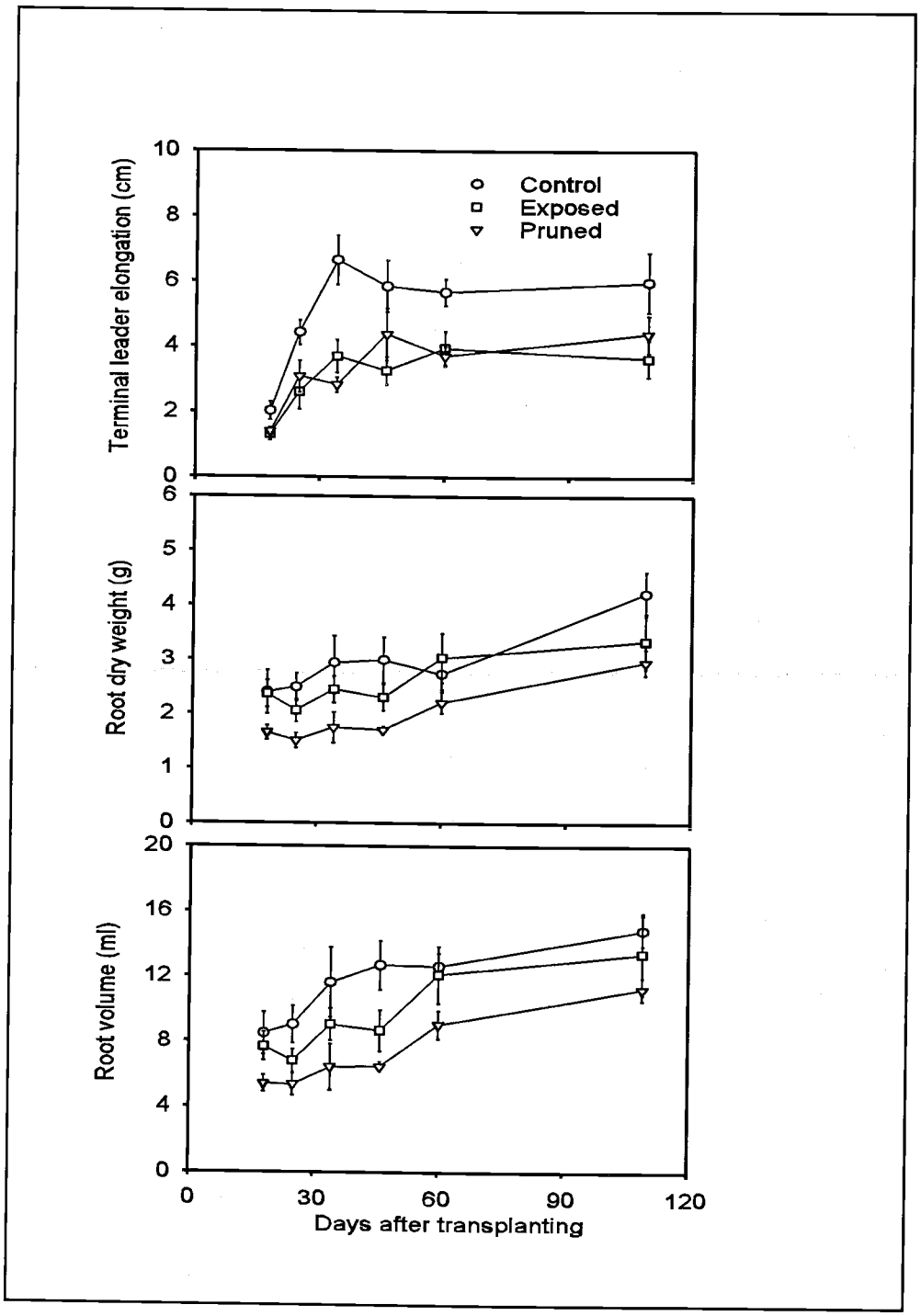

Figure IV.8. Effect of exposure and pruning on terminal leader growth, root dry weight, and root volume of 2-year-old Douglas-fir seedlings (n=6). Bars are one standard error of the mean. See table IV.2 for the level of significance of treatment effects.

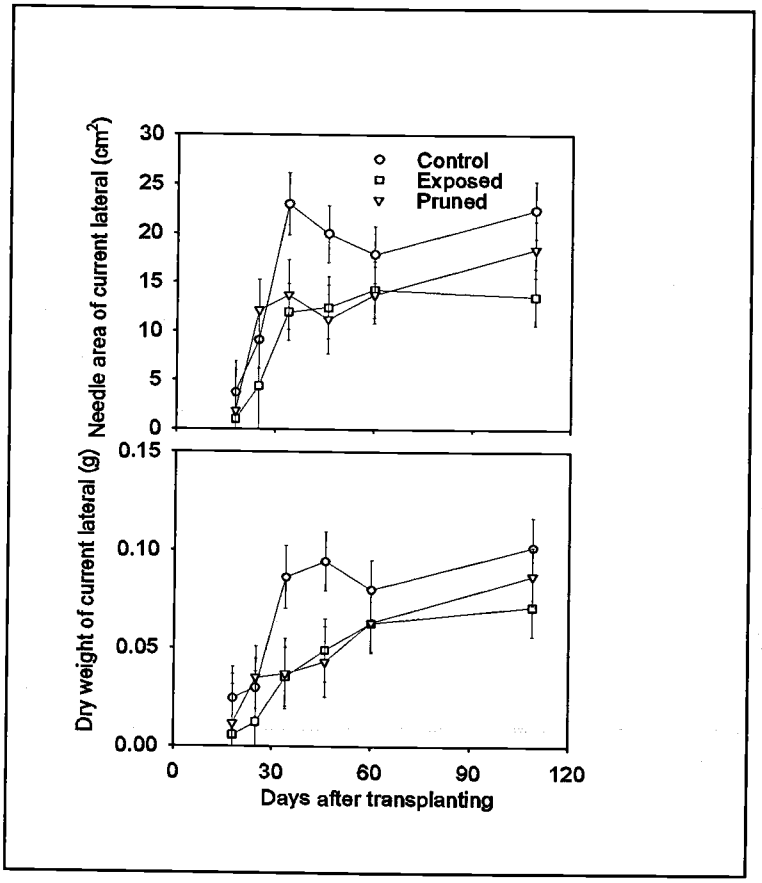

Figure IV.9. Changes in the leaf area and biomass of expanding first-order lateral needles of Douglas-fir that were either exposed or root pruned prior to transplanting (n=6). Bars are one standard error of the mean. See table IV.2 for the level of significance of treatment effects.

SLA varied more with tissue age than with treatment (Fig IV.10). The 1-year-old tissue had a mean SLA of 160 cm^2/g over the entire growth period. The SLA for the current needles doubled from 160 to 320 cm^2/g from 18 to 25 days after transplanting. SLA decreased after 25 days, but still remained 40% higher than initial values. Mean SLA of current needles over the growth period was significantly ($p<0.01$) higher for the stress treatments than the controls (Fig. IV.11).

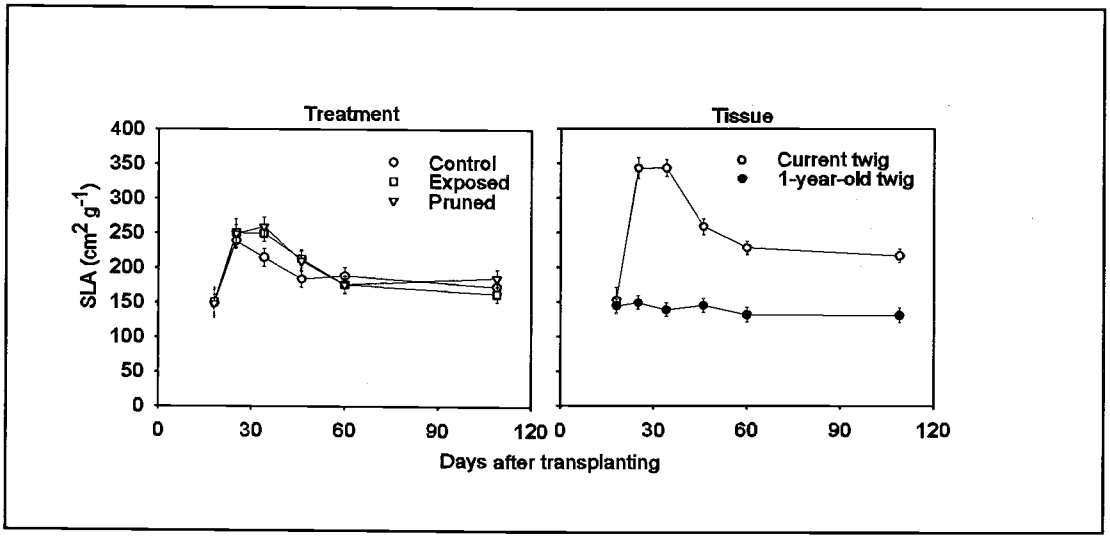

Figure IV.10. Changes in specific leaf area (SLA) of current and 1-year-old needles of Douglas-fir that were either exposed or root pruned prior to transplanting (n=6). Bars are one standard error of the mean. See table IV.2 for the level of significance of treatment effects.

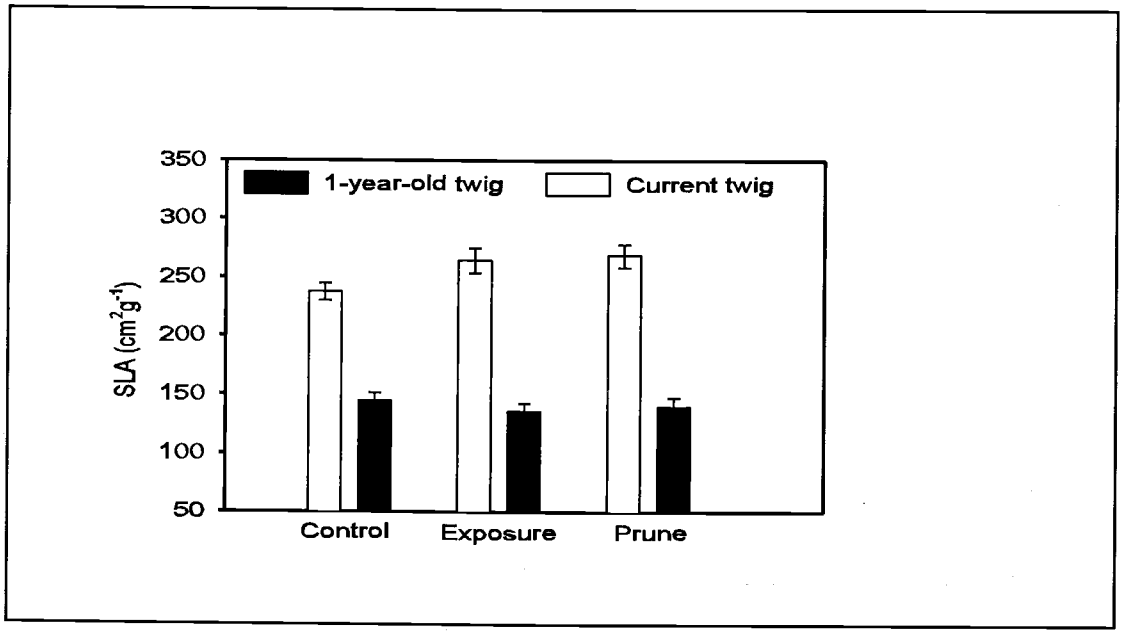

Figure IV.11. Changes in the mean SLA of exposure and root pruning on the mean SLA of current and 1-year-old needles of Douglas-fir (n=36). Bars are one standard error of the mean. See table IV.2 for the level of significance of treatment effects.

Water Relations

The treatments had a significant effect on all water relation parameters except predawn π (Table IV.3). There was a significant date x treatment interaction for predawn ψ, predawn *p*, and % saturation turgor. For predawn π, predawn *p*, and % saturation turgor differences between tissue age changed with time. Specific effects of the different factors and their interactions on these parameters are presented below.

Table IV.3. P-values associated with the different treatment effects for water relation parameters such as ψ, π and *p*.

Source of variation	Water relation parameters				
	Predawn ψ	Midday ψ	Predawn π	Predawn *p*	% saturation turgor
Treatment (T)	0.0001	0.04	0.66	0.02	0.0004
Tissue (Ti)	-	-	0.0001	0.0001	0.0001
Date (D)	0.0001	0.08	0.0001	0.0001	0.0001
T x Ti	-	-	0.13	0.54	0.12
T x D	0.0001	0.65	0.17	0.04	0.002
Ti x D	-	-	0.0001	0.0001	0.0001
T x Ti x D	-	-	0.6	0.39	0.05

Predawn ψ was significantly ($p<0.0001$) lower for both stress treatments than the controls 18 days after transplanting (Fig. IV.12). Among the stress treatments,

seedlings that were exposed had significantly ($p<0.0001$) lower predawn ψ on day 18 than seedlings that were root pruned. There were no differences between the stress treatments after day 18. Although the predawn ψ of controls remained high (low water stress) through the duration of the experiment, the predawn ψ of stress treatments were the same as the controls (>-0.4 MPa) 46 days after transplanting. On the other hand, midday potentials that were measured on day 25 through day 60 showed no significant interaction between date and treatments. Stressed treatments had lower midday potentials than controls during all but the 60th day, when they were the same (Fig IV.12). From day 34 to day 60, while predawn ψ increased, midday ψ decreased for all the treatments.

Predawn turgor and π of Douglas-fir seedlings show a larger difference between needle age than among the stress treatments (Fig IV.13). Both parameters showed a significant phenological change. Immature tissue, just after budbreak, showed the lowest p and most negative π. Sixty days after transplanting (about 45 days after budbreak), the value of these parameters in immature needles equaled that of the mature year-old needles. The π of the mature needles significantly increased ($P<0.002$) from -2.05 MPa on day 18 to day -1.73 MPa on day 25 and then did not change much for the remaining period. There was no significant treatment effect on predawn π of current shoots. However, predawn p of current shoots was higher for the controls until 60 days after transplanting, after which differences between treatments were indistinguishable.

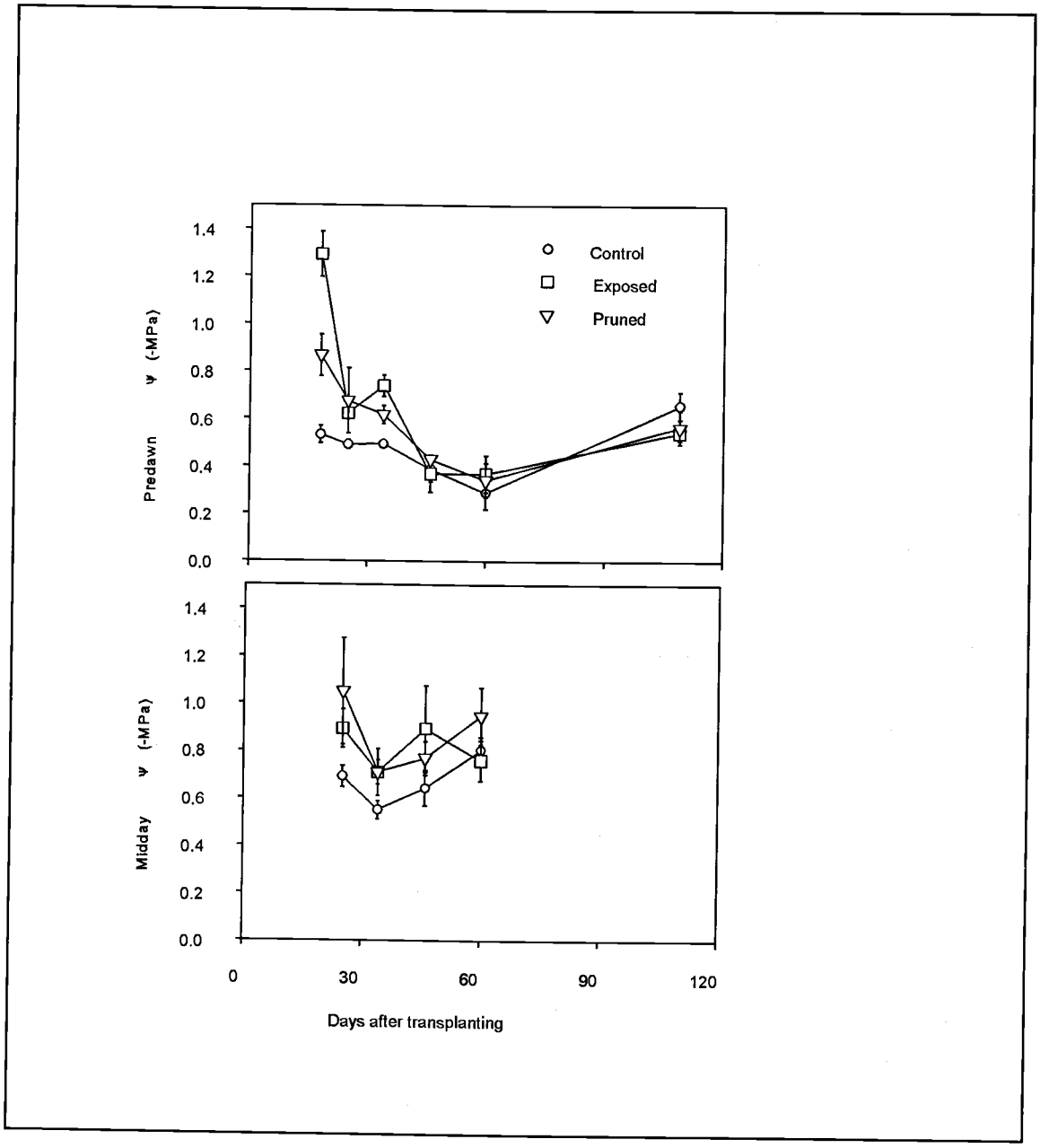

Figure IV.12. Predawn and midday water potential (ψ) of 2-year-old Douglas-fir seedlings that were either exposed or root pruned prior to transplanting (n=6). Bars are one standard error of the mean. See table IV.3 for the level of significance of treatment effects..

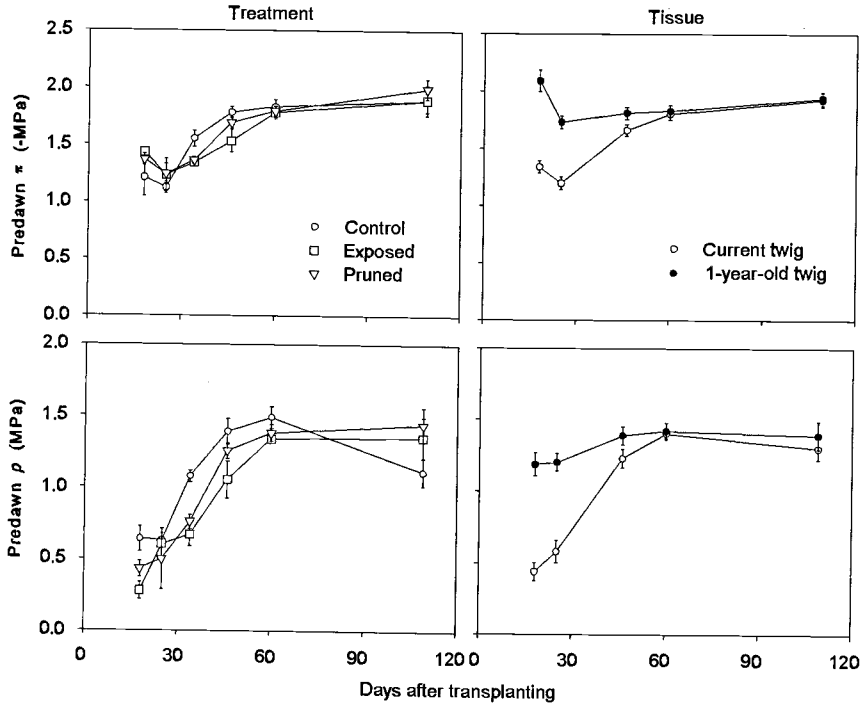

Figure IV.13. Predawn osmotic potential (π) and turgor pressure (p) of current and 1-year-old twigs of 2-year-old Douglas-fir seedlings that were either exposed or root pruned (n=4-6). Bars are one standard error of the mean. See table IV.3 for the level of significance of treatment effects.

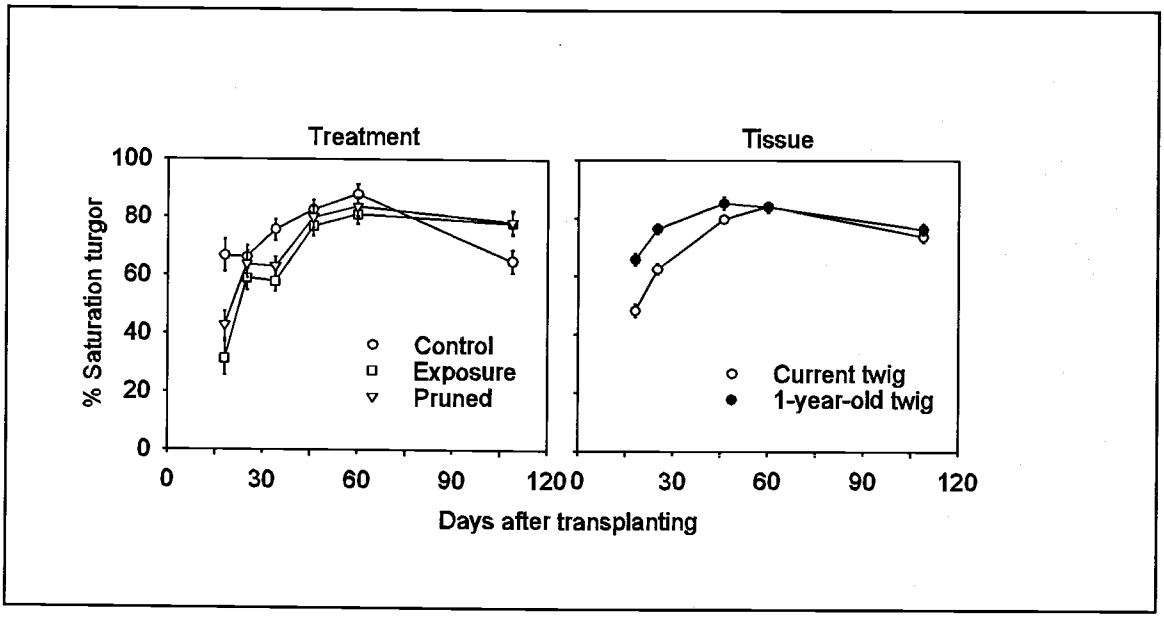

Figure IV.14. Changes in % saturation turgor (100 x predawn turgor pressure / turgor pressure at full saturation) of 2-year-old seedlings that were either exposed or root pruned prior to transplanting (n=4-6). Bars are one standard error of the mean. See table IV.3 for the level of significance of treatment effects.

The % of saturation turgor is a convenient parameter to estimate the turgor status of the shoot relative to its turgor at a potential maximum (full saturation). It is calculated as the ratio of predawn turgor to the turgor at saturation times 100. Turgor at saturation = π_{100} - ψ_{100}. The ontogenetic change in % of saturation turgor varied significantly among treatments ($p<0.005$), and between the different tissues ($p<0.0001$) (Fig IV.14). Although % of saturation turgor in the current shoot of control seedlings was relatively low on day 18, it was still 2x the value of the stress treatments. The % saturation turgor increased to around 85% on day 60 for both the control and stressed seedlings. On day 109, the % saturation turgor

dropped to 64% for the controls, and was significantly lower ($p<0.05$) than the stressed seedlings.

Of all the derived parameters of tissue water relations, only π_0 was significantly affected by the treatments (Table IV. 4). Most of the parameters were significantly affected by tissue age and date. Tissue x date interactions were also significant for most of the variables. Osmotic potential at full turgor (π_{100}), and ϵ^{max} measure of cell wall elasticity were both unaffected by treatments, but varied significantly with tissue and date.

Table IV.4. P-values associated with the different treatment effects for some water relation parameters derived from the PV-curve analysis.

Source of variation	Water relation parameters							
	RWC_m	RWC_0	TWDT	π_0	π_{100}	ϵ^{max}	p^{max}	Ap_v
Treatment (T)	0.07	0.2	0.55	0.05	0.17	0.19	0.95	0.55
Tissue (Ti)	0.0006	0.26	0.0001	0.0001	0.0001	0.0004	0.61	0.02
Date (D)	0.61	0.18	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
T x Ti	0.36	0.05	0.85	0.01	0.18	0.63	0.37	0.04
T x D	0.003	0.2	0.42	0.006	0.24	0.77	0.33	0.52
Ti x D	0.03	0.44	0.0009	0.0008	0.0001	0.0001	0.0001	0.25
T x Ti x D	0.11	0.65	0.72	0.21	0.62	0.71	0.35	0.28

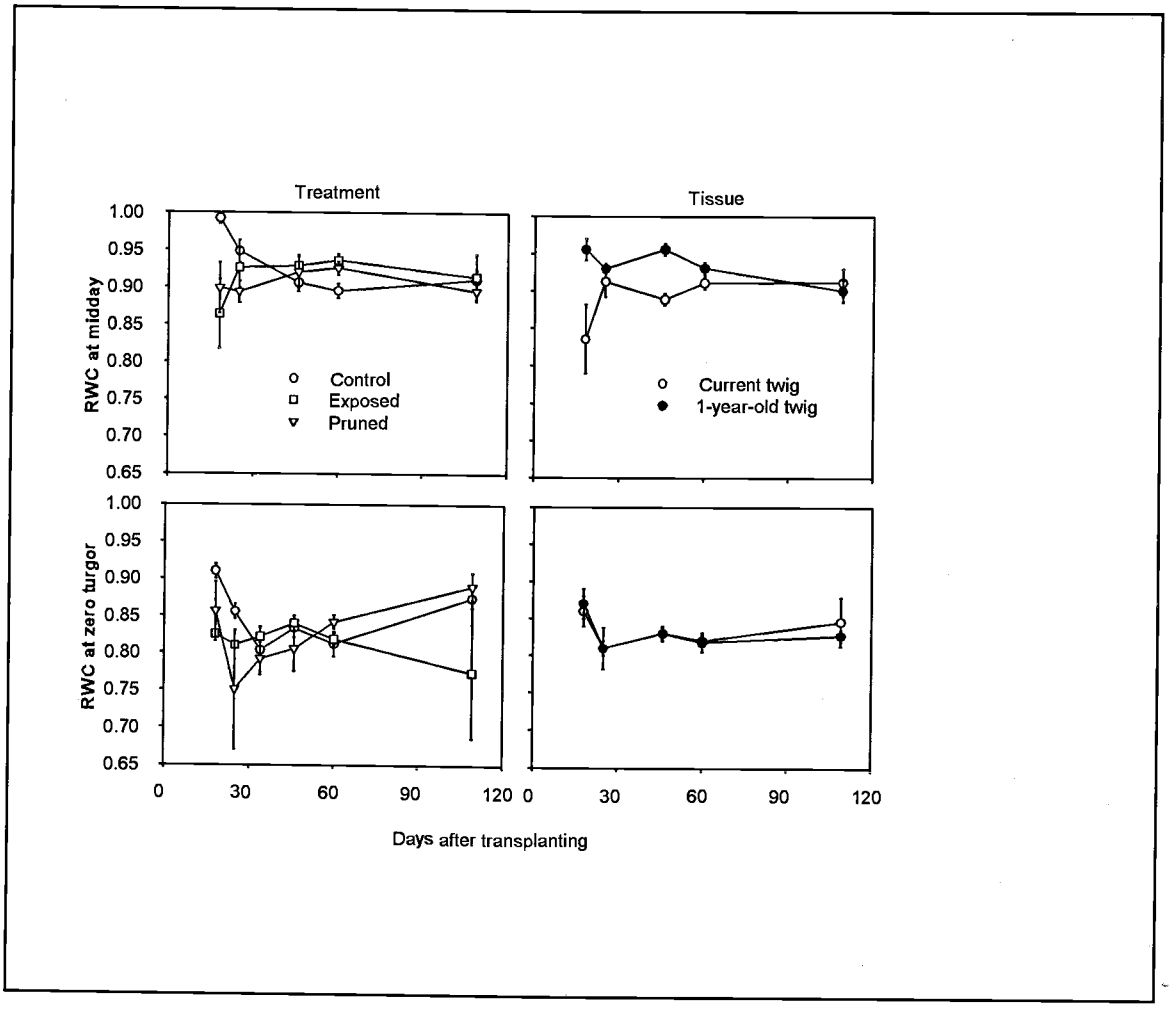

Figure IV.15. Changes in relative water content (RWC) at the time of gas exchange measurements (midday) and RWC at zero turgor for different tissues and treatments of 2-year-old Douglas-fir seedlings (n=4-6). Bars are one standard of the mean. See table IV.4 for the level of significance of treatment effects.

The relative water content (RWC) at the time of gas exchange measurements showed a significant date x treatment interaction. The RWC of control seedlings was near saturation 18 days after transplanting, while the exposure treatment showed a water deficit of 15% and the deficit of pruned treatments was 10% (Fig. IV.15). After 34 days the RWC of all the treatments stabilized at approximately 93%. Over the course

of the sampling period, the controls reached a minimum RWC at 90%, 60 days after transplanting. RWC_0 did not show any significant ontogenetic response to either treatment or tissue age. Mean RWC_0 for both tissues decreased during the first 25 days from 0.88 to 0.80 and remained the same through the rest of the dates.

The turgid weight:dry weight ratio (TWDWT) was unaffected by treatments but significantly differed between the tissues ($p<0.001$) for most of the growing season (Fig IV.16). The TWDWT of current needles gradually declined over the growing period, but was significantly higher than the 1-year-old needles on all dates except on day 109.

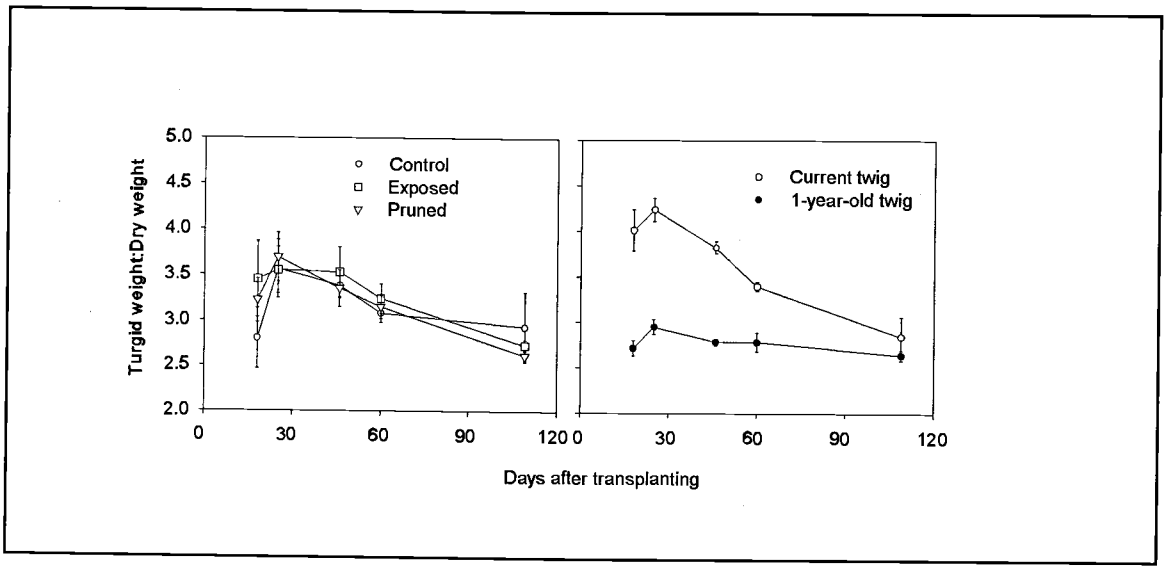

Figure IV.16. Turgid weight: dry weight ratio (TWDWT) of different tissues and treatments for 2-year-old Douglas-fir seedlings (n=4-6). Bars are one standard error of the mean. See table IV.4 for the level of significance of treatment effects.

Osmotic potential at zero turgor (π_0) was significantly affected by the stress treatments over time ($p<0.01$). However, no meaningful pattern was discernable between the treatments. (Fig IV.17). Osmotic potential at zero turgor was significantly ($p<0.01$) lower in the 1-year-old needles than the current needles, but this tissue difference decreased with time (Fig IV.17). The difference in π_0 between tissues for the controls and the exposed treatments was -0.6 MPa, whereas the difference was only -0.15 MPa for the pruned seedlings (Table IV.5). Osmotic potential at full turgor (π_{100}) changed significantly with time ($p<0.0001$), and tissue ($p<0.0001$), but was unaffected by the treatments (Fig IV.17). Osmotic potential at full turgor for 1-year-old needles remained constant at -1.77 MPa through out the period of sampling. However, for current needles π_{100} decreased from -1.00 MPa on day 18 to -1.70 MPa on day 60 after transplanting.

Maximum tissue modulus of elasticity (ϵ^{max}) and associated maximum mean turgor pressure (p^{max}) responded more to time of sampling and tissue age than to treatments (Fig. IV.18). The maximum tissue modulus of elasticity of current needles increased from a minimum of ca. 2.0 MPa to a maximum of ca. 8.5 MPa over the course of the sampling period. Controls increased to the maximum ϵ^{max} on day 34, while the two stress treatments reached the maximum levels only on day 60. The maximum tissue modulus of elasticity of 1-year-old needles decreased from a high of 12.0 MPa on day 18 to a constant level of 8.0 MPa through the rest of the sampling period. For current needles, the turgor pressure at which ϵ is maximum

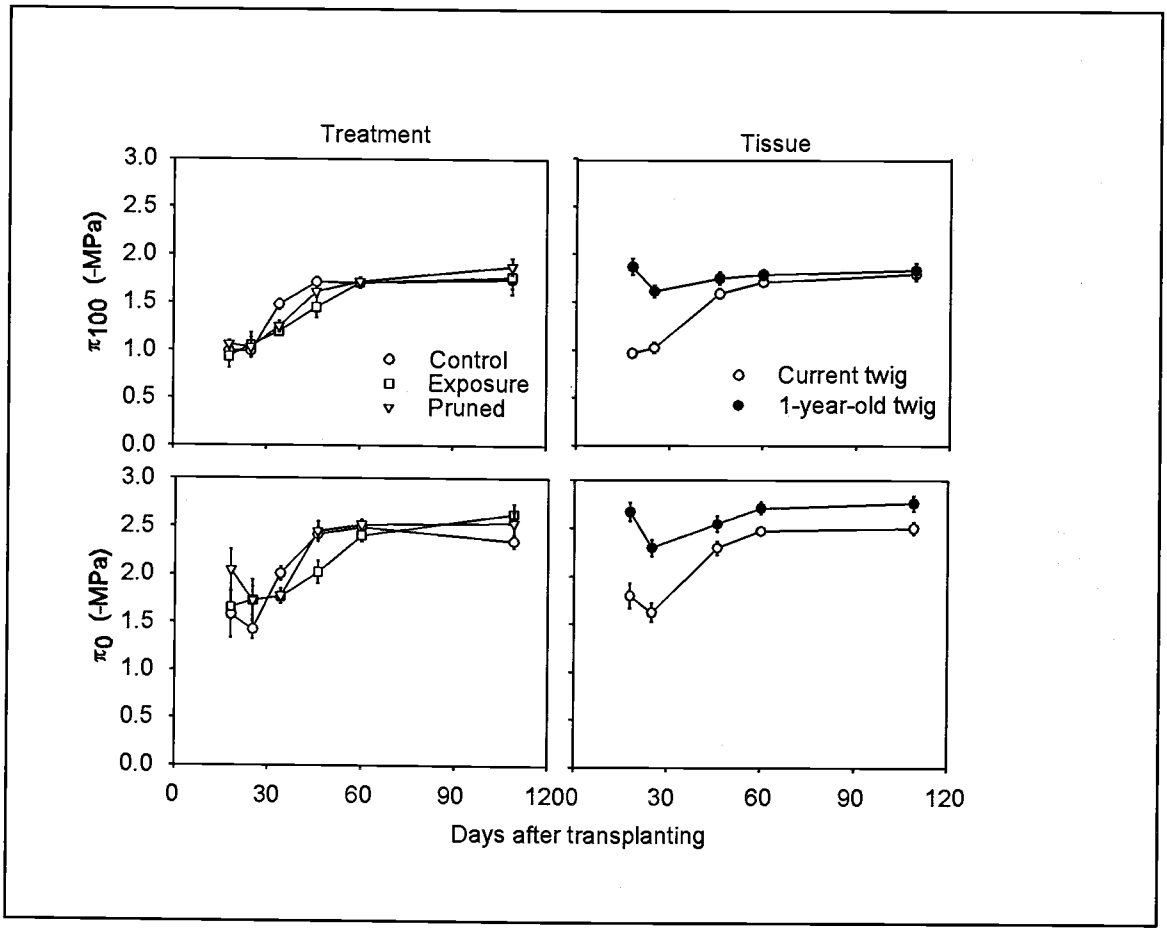

Figure IV.17. Changes in osmotic potential at zero turgor (π_0) and osmotic potential at saturation turgor (π_{100}) for different tissues and treatments of 2-year-old Douglas-fir seedlings (n=4-6). Bars are one standard error of the mean. See table IV.4 for the level of significance of treatment effects.

Table IV.5. Effect of stress treatment and tissue age on the osmotic potential at full turgor (π_{100}), the osmotic potential at zero turgor (π_0), RWC at zero turgor (RWC_0), Maximum modulus of elasticity (ϵ^{max}), and the apoplastic fraction (AP_v) of transplanted 2-year-old Douglas-fir seedlings (Mean ± SE).

Water relation parameters	Tissue age[1]	Treatments[2]			Significant levels (p)[3]
		Control	Exposed	Pruned	
π_{100}	C	1.43 ± 0.08	1.36 ± 0.08	1.46 ± 0.08	0.18
	L	1.77 ± 0.04	1.83 ± 0.04	1.72 ± 0.07	
π_0	C	2.07 ± 0.10	2.09 ± 0.09	2.25 ± 0.08	0.01
	L	2.60 ± 0.07	2.70 ± 0.07	2.49 ± 0.07	
RWC_0	C	0.85 ± 0.01	0.82 ± 0.02	0.83 ± 0.02	0.05
	L	0.82 ± 0.009	0.83 ± 0.01	0.84 ± 0.01	
ϵ^{max}	C	6.99 ± 0.65	6.36 ± 0.63	6.11 ± 0.60	0.63
	L	7.25 ± 0.64	8.72 ± 1.80	8.07 ± 1.19	
AP_v	C	0.56 ± 0.03	0.48 ± 0.03	0.52 ± 0.03	0.04
	L	0.40 ± 0.03	0.47 ± 0.03	0.51 ± 0.04	

1 C=Current year terminal leader; L=1-year-old lateral.

2 Control=No stress; Exposed=Seedlings were exposed in a growth chamber for 45 mins under the following conditions, 25°C, RH 38%, PAR 30μmoles m^{-2} s^{-1}. Root pruned seedlings had on average 40% of it's secondary roots clipped and removed.

3 Level of significance for the 2-way interaction effect between tissue age and treatment. For those parameters that had no significant interaction effect, tissue age alone was significant while treatment was not significant.

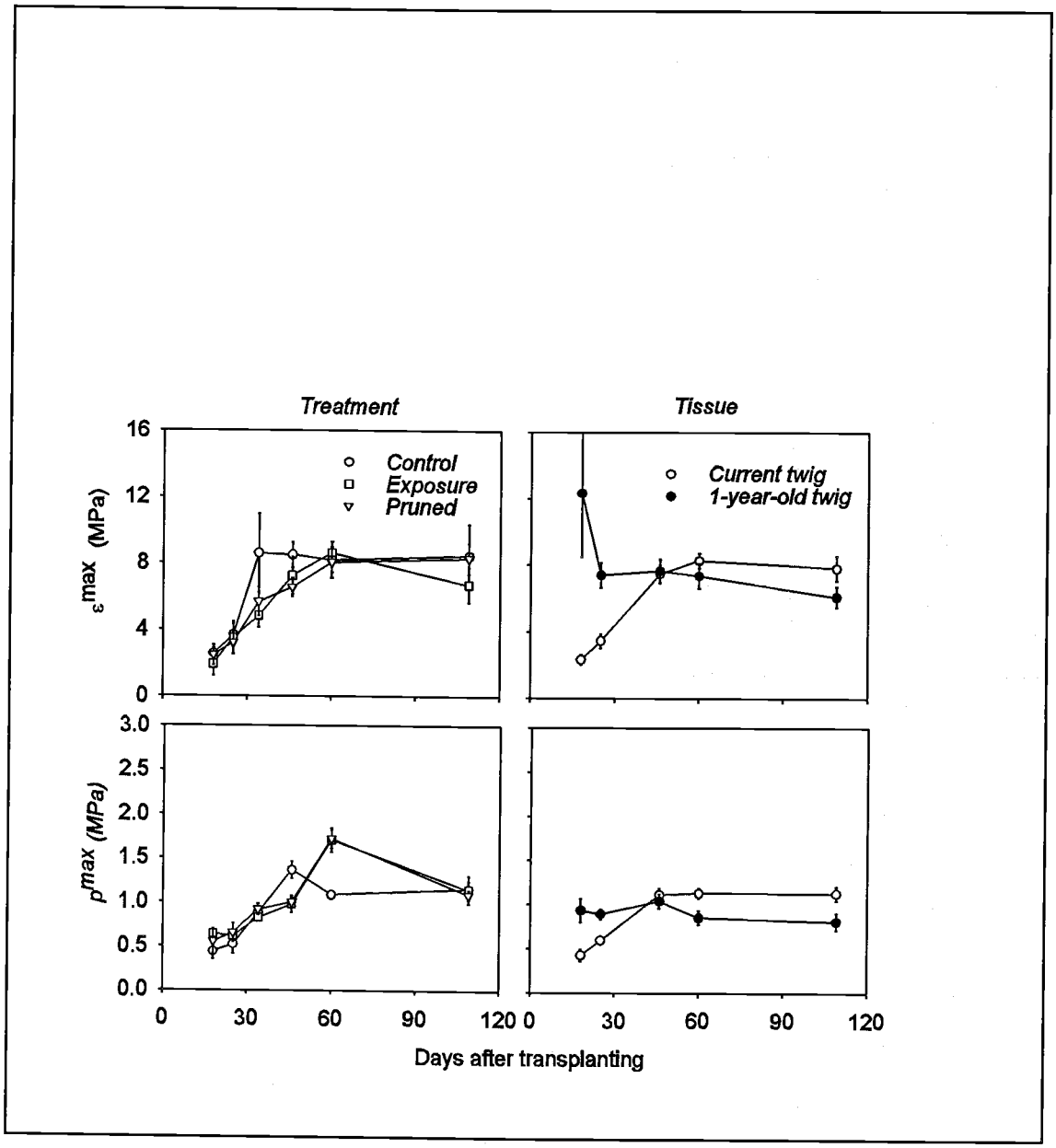

Figure IV.18. Changes in modulus of tissue elasticity (ϵ^{max}) and maximum mean turgor pressure (p^{max}) for different tissues and treatments of 2-year-old Douglas-fir seedlings (n=4-6). Bars are one standard error of the mean. See table IV.4 for the level of significance of treatment effects.

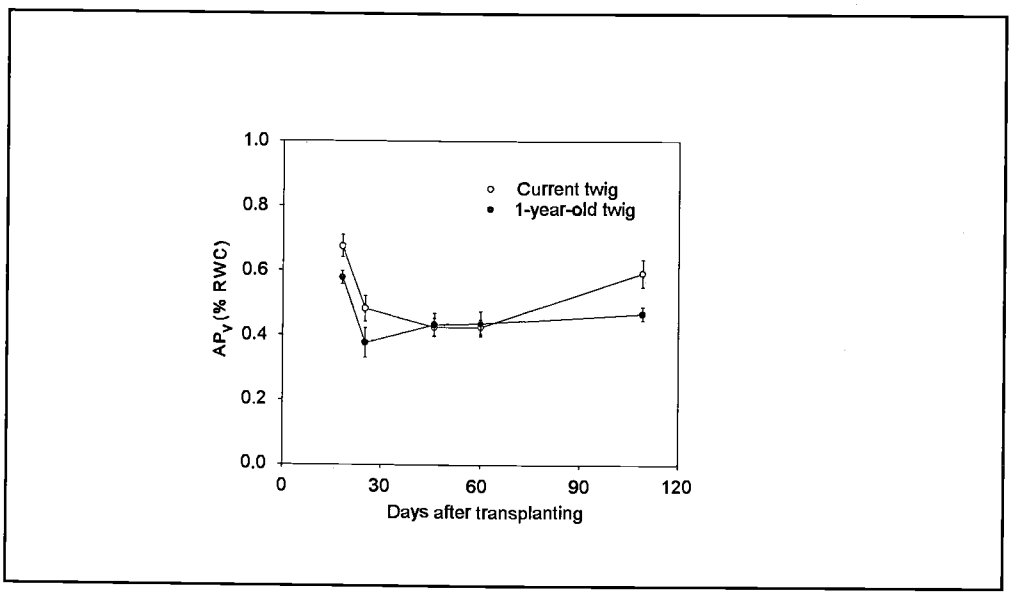

Figure IV.19. Changes in the apoplastic fraction (AP_v) of current and 1-year-old twigs of transplanted 2-year-old Douglas-fir seedlings (n=4-6). Bars are one standard error of the mean. See table IV.4 for the level of significance of treatment effects.

(p^{max}) increased over time in a pattern similar to ϵ^{max}. Maximum mean turgor for the controls showed a high of 1.4 MPa while the stressed seedlings showed a high of 1.75 MPa. On day 109 the P^{max} for the different treatments were similar at 1.25 MPa.

The ontogenetic changes in the apoplastic fraction (AP_v) of water in the shoots was unaffected by the treatments. The AP_v drops rapidly for both current and 1-year-old needles from day 18 to day 25 (Fig IV.19). The apoplastic fraction of current needles was larger by 10-15% for the first 25 days after transplanting, and then showed no difference till day 109, when AP_v in current shoots increased again

by 15%. The current shoots of controls had a 16% higher mean AP_v than 1-year-old shoots, but the stressed treatments did not show any difference in mean AP_v between the tissues (Table IV.5).

Gas Exchange

Net photosynthesis and stomatal conductance both showed a significant treatment x date x tissue interaction (Table IV.6). The concentration of total phaeophytins was significantly affected by tissue and date, and the interaction of tissue and date was significant at p= 0.07.

Table IV.6. P-values associated with the different treatment effects for the gas exchange parameters and phaeophytins.

Source of variation	Gas exchange and phaeophytin		
	A	g_s	Phaeophytin
Treatment (T)	0.0001	0.35	0.25
Tissue (Ti)	0.0001	0.0002	0.0001
Date (D)	0.0001	0.0001	0.0004
T x Ti	0.0001	0.15	0.68
T x D	0.0001	0.11	0.39
Ti x D	0.0001	0.0001	0.07
T x Ti x D	0.0001	0.05	0.45

Net photosynthesis of 1-year-old needles was significantly ($p<0.01$) higher than current needles on all dates (Fig. IV.20). Treatment differences in net photosynthesis were significant ($p<0.001$). Controls show a slightly higher rate of net photosynthesis than the treatments during the first 25 days, after which the stressed seedlings recover to rates similar to the controls. For the current needles, net photosynthesis increased with time after transplanting to day 60, and then gradually decreased. For 1-year-old needles, net photosynthesis increased till day 34 and then gradually decreased. The lower net photosynthesis on day 109 for all treatments was a result of low light levels due to electrical problems in the growth room. The abrupt but uniform drop in net photosynthesis on day 46 for all the treatments was most probably a result of very high temperatures coupled with a high VPD in the growth rooms. It must be noted that the stressed treatments had recovered fully such that the artificially generated, environmentally stressful conditions did not cause any changes in their response.

Stomatal conductance was unusually high the first 18 days after transplanting for the stress treatments (Fig. IV.20), but dropped rapidly to levels that were similar to the controls on day 25. Mean stomatal conductance of 1-year-old needles were generally higher than current needles on all dates other than day 18. Surprisingly, stomatal conductance did not change with time after day 18.

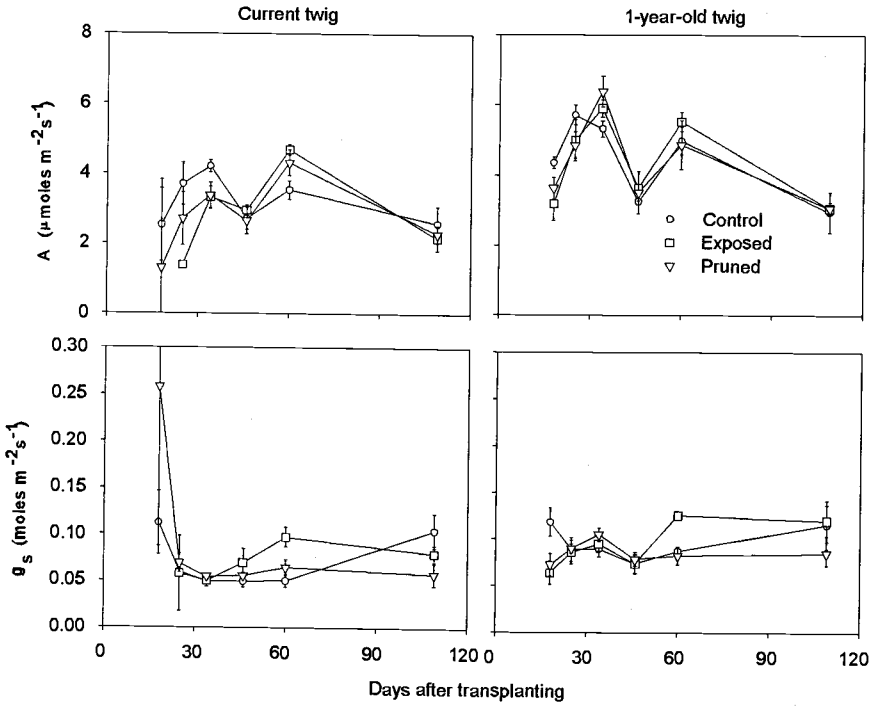

Figure IV.20. Effect of exposure and root pruning on the rate of net photosynthesis and stomatal conductance of current and 1-year-old needles of 2-year-old Douglas-fir. See table IV.6 for the level of significance of treatment effects.

Phaeophytins

The concentration of phaeophytins was significantly higher (2x) in the 1-year-old tissue than the current needles (Fig IV.21). Concentration of phaeophytins in both tissue types increased from 18 to 25 days, after which they remained fairly stable during the growing season, but decreased towards the end of the measurement period (day 109). Treatments had no effect on phaeophytin concentrations.

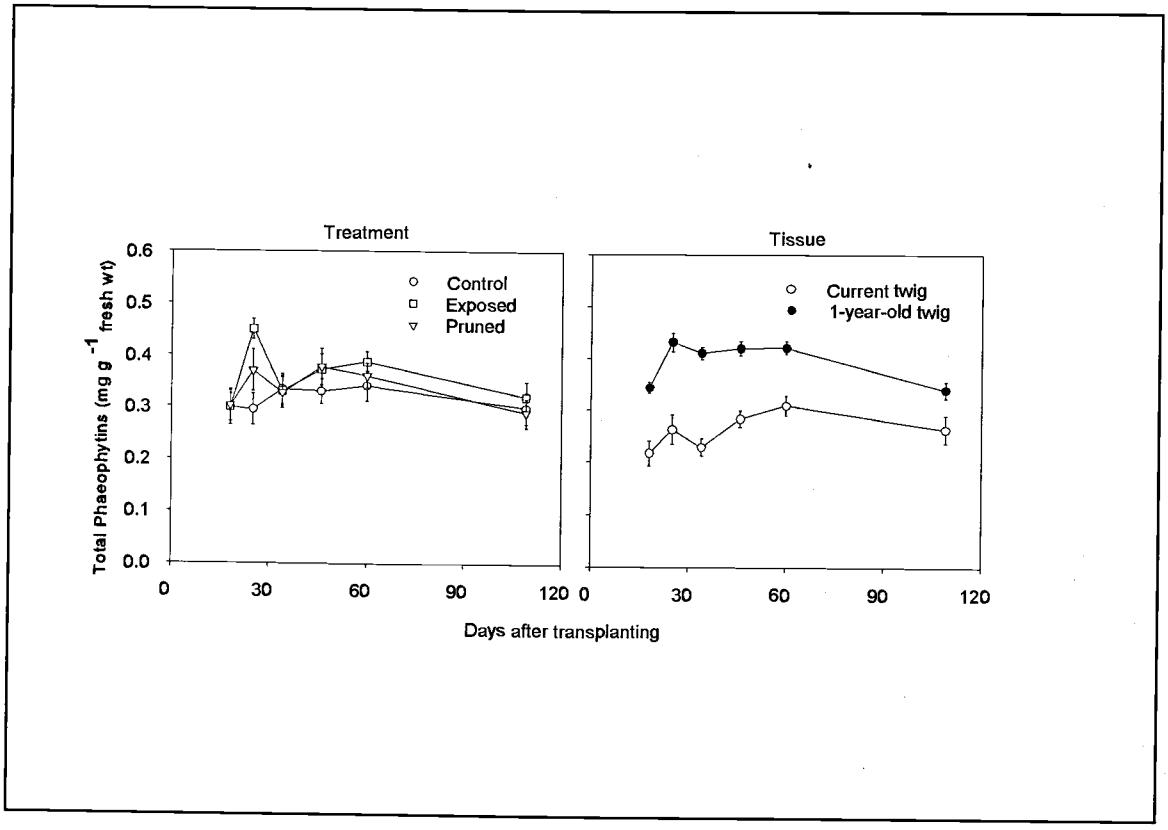

Figure IV.21. Changes in total phaeophytin concentrations of current and 1-year-old needles of Douglas-fir that were either exposed or root pruned prior to transplanting (n=4-6). Bars are one standard error of the mean. See table IV.6 for the level of significance of treatment effects.

Discussion

Exposure and root pruning prior to transplanting reduced terminal leader elongation. Simultaneous measurements of the ontogenetic and seasonal changes in tissue-water relations indicated that a reduced shoot turgor during elongation of the leader may have caused the observed growth inhibition. However, the effect of reduced turgor on shoot expansion was not associated with a decrease in the rate of cell wall hardening as measured by the modulus of elasticity. The stressed seedlings showed no indication of osmotic regulation during the period of tissue water deficit. Most of the tissue-water parameters varied largely between the two tissue types than between treatments. Stress reduced net photosynthetic rate of the developing leader and 1-year-old needles. Within 60 days after transplanting, turgor and net photosynthesis of the stressed seedlings recovered to the level of untreated controls. This suggests that the physiological damage to these seedlings as a result of exposure or root pruning was temporary, lasting only part of the first growing season. This transient transplanting stress as a result of prior exposure and root pruning is primarily associated with seedling water deficit, but compounded by a decreased rate of carbon acquisition.

Shoot expansion is primarily a function of cell turgor pressure (Green et al. 1971; Taiz 1984; Hsiao and Jing 1987); however, cell expansion is a complex physical and chemical process (Ray 1987). Turgor must be above a threshold level before extension can occur (Cleland 1959; Hsiao and Jing 1987). The rate at which cells expand above the yield threshold is a function of the growth-active turgor and

wall extensibility (Lokhart 1965; Boyer 1987). Wall extensibility may limit rates of extension growth even when turgor is adequate (Van Volkenburgh 1987). For instance, in the case of bean (Phaseolus vulgaris L.) leaf expansion, where growth-active turgor is higher in maturing cells than in rapidly growing younger cells (Van Volkenburgh et al. 1985), the growth rate of maturing cells decreases because cell wall extensibility is reduced as cells enlarge (Van Volkenburgh and Cleland 1986). In sunflower (Helianthus annuus L.), water deficits inhibit leaf expansion by reducing wall extensibility and yield threshold, while turgor is maintained (Matthews et al. 1984). Therefore, under certain conditions, the inhibition of cell expansion may occur even when turgor is adequate.

Exposed and root pruned seedlings may incur substantial damage to their root systems, thus impairing water absorption and consequently increasing the water deficit, leading to a drop in turgor. In the current experiment, the physiology and shoot growth response of the exposed and root pruned seedlings was similar, although damage to the root system may have differed. Exposure of seedlings not only decreased root hydraulic conductance, but may also decrease the functional conducting area of the stems as a result of embolized trachieds (Chapter II). This increased resistance to water transport within a newly transplanted seedling would increase seedling water deficit, therefore reducing turgor. Root pruning, on the other hand, removed 40% of the secondary roots, therefore significantly reducing the absorptive surface area and the water absorbed (Chapter III). The lack of an

adequate root system could also lead to increased water stress, which may be compounded by embolisms in the stem at lower water potentials (Sperry and Tyree 1990).

The lack of any difference in either π_{100} or π_0 among treatments suggests that osmotic adjustment does not play a role in turgor regulation and consequently growth. This supports the conclusions of Joly and Zaerr (1987), who found that Douglas-fir seedlings did not adapt to short- or medium-term water deficits by osmotic adjustment. Similarly, Blake et al. (1991) working with black spruce seedlings found that π_{100} was not altered by repeated short-term conditioning water stress, suggesting that osmotic adjustment was not important to turgor regulation. However, shoots of Jack pine and white spruce did show active osmotic adjustment during water stress, by a measured increase in organic solutes during water stress (Koppenaal et al. 1991). Therefore, it appears that both mechanisms for turgor regulation can be found among the conifers. In contrast to conifers, osmotic adjustment is well documented in several agronomic species (Turner and Jones 1980; Morgan 1984; Fekade and Krieg 1992).

Contrary to expectations, the rate of change in the elasticity of the cell wall during shoot elongation did not differ among treatments (Fig IV.18), although the turgor pressure was lower for the stressed treatments. Higher tissue elasticity may be one adaptation that allows plants to maintain turgor under moderate water stress (Tyree and Jarvis 1982; Joly and Zaerr 1987). The ability of the stressed Douglas-fir seedlings to maintain control-level tissue elasticity, and the lack of osmotic

regulation in these seedlings, supports the contention that cell wall characteristics are more important in turgor regulation of Douglas-fir (Joly and Zaerr 1987) and black spruce (Blake et al. 1991) than is osmotic adjustment.

The lack of a measurable effect of stress on cell wall elasticity of these seedlings suggests that the inhibition of elongation may be due to turgor induced changes in cell wall plasticity. Nonami and Boyer (1990b) have characterized the extensibility of the cell walls as having two physical properties: an viscoelastic component, and a plastic component. The elastic component of elongation is reversible, while the plastic component is irreversible. Soybean (Glycine max L.) seedlings subjected to low water potentials decreased the plastic properties of the cell wall and the conductance of cells to water (Nonami and Boyer 1990b). However, the elastic compliance (reciprocal of bulk elastic modulus) did not change in the elongating or mature tissue of soybean. Recently, Chazen and Neumann (1994) showed that the irreversible plastic extension capacity in expanding leaves of maize (Zea mays L.) was reduced within minutes to hours after onset of a moderate water stress. In the same study, found that the reversible elastic extension capacity was not reduced, confirming that the elastic and plastic components represent independent processes with the latter being more closely related to growth inhibition. In my study, the elasticity of cell walls in Douglas-fir seedlings decreased as the shoot developed, but was unaffected by the stress treatments, indicating that plastic properties of the cell wall were primarily affected.

The biochemical mechanism by which the cell wall properties are affected is not entirely clear. Endogenous auxin is involved in cell wall loosening, particularly the plastic component (Kutschera 1987; Moore 1989; Hohl and Schopfer 1992). On the other hand, endogenous abscisic acid (ABA) increases in the xylem sap of droughted maize and sunflower, with a concomitant decrease in leaf growth (Zhang and Davies 1990). ABA reduces cell wall extensibility (Van Volkenburgh and Davies 1983; Kutschera and Shopfer 1986b) by inhibiting proton secretion through the plasmalemma into the apoplast (Chen and Kao 1988). So, in the case of shoot elongation in post-dormant buds of Douglas-fir subjected to water stress, it is possible that the ratio of ABA to auxins increases, and that this affects the plastic component of cell wall extensibility, reducing stem elongation. Kutschera and Schopfer (1986a; 1986b) found that a higher ratio of ABA:IAA (an auxin) inhibited growth, while a lower ratio promoted growth of the maize coleoptile. They attributed this effect to a hormone-induced change in the plastic component of wall extensibility.

A possible increase in the yield threshold (Y) would also contribute to growth inhibition (Hsiao and Jing 1987). They show that the yield threshold of expanding leaves of drought conditioned maize seedlings shifted to cause slower growth, in spite of full osmotic adjustment. Any increase in Y, would increase the level of growth-active turgor needed for elongation at the same level of available bulk turgor potential. Therefore, increasing the turgor required to initiate elongation (Hsiao and Jing 1987). In contrast to maize shoots, Frensch and Hsiao (1995) have shown that

maize roots can readily reduce the yield threshold during moderate water stress, thereby reducing the level of turgor required for elongation to proceed. Similarly, in immature Douglas-fir shoots, Y could potentially vary in response to moderate stress, which would also affect shoot expansion.

Shoot elongation in post dormant seedlings is the result of both cell elongation and cell division (Fielder and Owens 1988). According to Owens et al. (1985), shoot elongation in Douglas-fir before flushing results primarily from cell divisions, while shoot elongation after flushing results primarily from cell expansion. However, it is not known to what extent cell division and cell elongation contribute independently to the final shoot lengths. It is also not known to what degree these two processes vary in their relative sensitivity to water stress in Douglas-fir. In general, cell elongation is more sensitive to water deficits than cell division (Bradford and Hsiao 1982; Boyer 1970).

In Douglas-fir, a large number of cells are present in the embryonic shoot prior to growth activity in spring (Fielder and Owens 1988). It is possible that the elongation of these cells in spring primarily determines the magnitude of shoot length, rather than current cell division. Current meristematic activity may contribute to tissue differentiation in the stem and may also be a source of hormones that are necessary for elongation, viz., auxins (Moore 1989). Therefore, if low water potentials are "sensed" by the embryonic shoot prior to or during initial elongation, all the preformed cells may be collectively affected by the stress induced hormone complex.

Consequently, cell elongation is uniformly reduced throughout the length of the spring shoot.

Unlike the observed physiological recovery of stressed seedlings during shoot elongation of Douglas-fir seedlings (Chapter II), the seedlings in this experiment recovered physiologically only after shoot elongation had ceased. There are several possible reasons for this difference. Seedlings in the first experiment (Chapter II) were transplanted 3 months earlier than those in this experiment. I and others (Hermann 1967; Coutt 1981; Ritchie 1984) have demonstrated that the longer that transplanting is delayed after deep dormancy, the poorer the performance of seedlings. This reduced performance is due to the combination of a decreasing stress tolerance of seedlings, and increasingly warmer and drier weather. Seedlings in the present experiment may have sustained more damage and been subsequently under physiological stress for a longer duration. In addition, the level and type of exposure stress to which the seedlings were subjected differed between the two experiments. Finally, the difference in ambient conditions during the growing period may have also contributed to the difference in stress responses between the two experiments.

The age of the Douglas-fir tissue affected tissue-water dynamics more than did the different stress treatments. During the early stage of expansion growth, the current and 1-year-old shoot were able to maintain similar RWC_0 (Fig IV.15), despite differences in π_0, by using different water-relation strategies. Early in development, the current shoot had higher (less negative) π_{100} than the 1-year-old shoot. This

suggests that newer needles with higher π than older needles are inherently less able to osmotically adjust turgor than older needles (Eamus and Narayan 1990). This inability of the immature shoot to osmotically adjust is reflected in a lower predawn turgor (Fig IV.13) and a lower % saturation turgor (Fig IV.14) than that of the 1-year-old shoot. However, ϵ^{max} of the current shoot was much lower (higher tissue elasticity) than that of the old needles (Fig IV.18). The higher elasticity of cell walls suggests that at a particular value of ψ, the tissue will have a lower π and a higher p (Joly and Zaerr 1987; Eamus and Narayan 1990; Kim and Lee-Stadelmann 1984). Therefore, the higher elasticity of cell walls of new needles enables the shoot to retain a similar RWC_0 to that of the old needles despite a larger loss in symplastic water. Symplastic water was relatively higher in the growing shoot as demonstrated by relatively higher TWDWT ratios (aprox. 50% of turgid weight - dry weight, Fig IV.16). As the current shoot matured, ϵ^{max} increased, symplastic volume, π_{100} and π_0 decreased to the values for 1-year-old needles. It seems plausible that the ontogenetic changes in π_{100} may in part be due to a changing dynamic balance between the rates of photosynthesis, respiration, translocation, and shoot elongation. Osmotic potentials have been reported to vary with tissue age (Teskey 1982; Abrams 1988), thus indicating a progressive adjustment with tissue development.

The ontogenetic trends in π_{100}, π_0 and ϵ^{max} are comparable to those observed by Ritchie and Shula (1984) for Douglas-fir seedlings during a similar time in the year. However, these values are not closely comparable to mine because these authors generated PV curves on whole seedlings, while I used twigs of differing age from the

same seedlings. They were unaware of the substantial error introduced by over saturation at RWC_{100} (the plateau effect) (Kubiske and Abrams 1991), and did not make appropriate corrections to their PV analysis.

Both root exposure and pruning decreased net photosynthesis (A) of the current and 1-year-old needles during the first 34 days after transplanting (Fig IV.20). Net photosynthesis had recovered to the level of the control seedlings within 46 days of transplanting. Net photosynthetic rate is primarily limited by stomatal conductance (gas-phase limitation) when other environmental factors are held nonlimiting (Nobel 1991). Several studies have reported very a high correlation between g_s and A (Chapter I; Delucia 1986; Meinzer 1982; Teskey et al. 1986; Mitchell and Hinckley 1993). However, non-stomatal factors such as mesophyll conductance and carboxylating enzyme activity can also limit photosynthetic rate, independent of stomatal conductance (Delucia 1986; Teskey et al. 1986). In the present study, stomatal conductance of both tissues did not correlate with A, particularly during the first 34 days when A of stressed seedlings decreased. For example, on day 18 the root pruned seedlings showed a disproportionately high g_s while A was low. This suggests that A was primarily limited by nonstomatal factors. Larger C_i / C_a ratios generally indicate a higher degree of mesophyll resistance (liquid-phase limitation) to CO_2 diffusion (Delucia 1986; Osunabi and Davies 1980), but contrary to expectation this ratio showed no difference between the treatments (data not shown). However, this estimated ratio may not be accurate, because gas exchange measurements made with the IRGA do not provide reliable estimates of the average internal CO_2

concentrations in stressed leaves, because the stomata do not close uniformily over the surface of the leaves (Downton et al. 1988; Terashima et al. 1988; Mansfield and Atkinson 1990). Calculations of the C_i / C_a ratio assumes that the conductance is uniform across the leaf (Long and Hällgren 1993).

Net photosynthesis of stressed seedlings was probably reduced as a result of the effect of decreased tissue turgor on mesophyll conductance to CO_2 (Kozlowski et al. 1991). Mean turgor pressure and turgor as a % of maximum were both lower for the stressed seedlings than the controls during the period when A was lower. Decreased turgor is generally associated with lower g_s, which subsequently limits A as discussed above, but this did not occur in the present study. On the other hand, differences in the osmotic adjustment of stomatal guard cell turgor and leaf mesophyll cells may account for the lack of a decrease in g_s, and a reduction in A (Brown et al. 1976; Beadle et al. 1978; Ludlow 1980). For instance, Kaiser (1982) found that photosynthesis in several species was sensitive to changes in cell volume. Decrease in turgor of the stressed seedlings in the present study is indicative of a decrease in cell volume, which may have increased mesophyll resistance to CO_2 fixation (Dietz and Heber 1983; Schulze 1986). Nonstomatal limitation of A during leaf water deficit has been reported for a number of species (von Caemmerer and Farquhar 1981; Briggs et al 1986; Guehl et al. 1991). A concomitant decrease in protein synthesis with a drop in turgor, primarily the carboxylating enzymes may also limit A (Hsiao et al. 1976). Turgor reduction had apparently no effect on the ontogenetic changes in total phaeophytins (which are 1:1 acid derivatives of chlorophyll, Moran

1982) between the treatments. This suggests that the reduction in net photosynthesis in stressed seedlings was not related to tissue chlorophyll levels.

The 1-year-old needles had higher photosynthetic rates than the current needles (Fig IV.20) probably due to several reasons. Tissue turgor was substantially higher in the 1-year-old needles than the current needles (Fig IV.13) at least for the first 34 days after transplanting. Increased turgor not only increases stomatal conductance to CO_2 but also increases the mesophyll conductance to CO_2 (Schulze 1986). In the present study, the measured stomatal conductance of 1-year-old needles was also significantly higher than current needles. Secondly, total phaeophytin concentrations of 1-year-old needles was almost 2x the concentration found in the current needles throughout the measurement period (Fig IV.21). Camm (1993) reports a similar relative difference in chlorophyll concentrations and net photosynthesis between 1-year-old and current needles of Douglas-fir branches in the shade. This suggests that the 1-year-old needles were able to absorb more light than the current needles, resulting in higher photosynthetic rates.

Summary

Exposure to air or root pruning prior to transplanting reduced terminal leader elongation. Simultaneous measurements of the ontogenetic and seasonal changes in tissue-water relations indicated that a reduced shoot turgor during elongation of the leader may have caused the observed growth inhibition. However, the effect of reduced turgor on shoot expansion was not associated with a decrease in the rate of

cell wall hardening as measured by the modulus of elasticity. The stressed seedlings showed no indication of osmotic regulation during the period of tissue water deficit.

Most of the tissue-water parameters varied largely between the two tissue types rather than between treatments. New needles and year-old needles maintained the same level of RWC_0. This is most probably a result of high initial tissue elasticity (small ϵ^{max}). The relatively higher π_0 and π_{100} in newly expanding needles suggests that they are inherently less able to adjust osmotically at this stage than the 1-year-old needles. Therefore, the different tissues appear to have different mechanisms to regulate turgor. These differences disappear as the new shoot matures, on completion of expansion growth. Changes in wall elasticity almost parallel the ontogeny of the leader.

The net photosynthetic rate of the developing leader and 1-year-old needles was reduced for the stressed seedlings. This reduction in photosynthesis is apparently limited by non-stomatal factors rather than by decreased stomatal conductance. The 1-year-old needles had higher photosynthetic rates than the current needles for all the treatments, throughout the season. A higher turgor pressure and total phaeophytin concentration of 1-year-old needles than the current needles may have contributed to the higher photosynthetic rates of 1-year-old needles.

In conclusion, within 60 days of transplanting, turgor and net photosynthesis of the stressed seedlings recovered to the level of untreated controls. This suggests that the physiological damage to these seedlings as result of exposure or root pruning was relatively temporary, lasting only part of the first growing season. This transient

transplanting stress is primarily associated with seedling water deficit, but compounded by a decreased rate of carbon acquisition. Although internal mechanisms regulating shoot elongation may be the same under field conditions; competition for resources and inhospitable field conditions could potentially increase the intensity of stress and prolong the duration of recovery of seedlings.

CHAPTER V. SUMMARY AND CONCLUSIONS

The process of transplanting Douglas-fir seedlings inevitably causes the stunting of new growth. Such stunting is usually characterized by shorter stem unit lengths and needles that may be chlorotic. This phenomenon is often referred to as transplant shock or transplanting stress (Cleary et al. 1978; Rietveld 1989). However, the specific factors and mechanisms that cause this are not well understood. Although both pre-planting and post-planting factors may contribute to the observed symptoms, research reported in this dissertation focusses on the effect of preplanting factors such as seedling exposure and root pruning on transplanting stress. This research entailed subjecting 2-year-old Douglas-fir seedlings to differing levels of exposure and root pruning. Subsequent physio-morphological responses were characterized during the first year of establishment.

This chapter summarizes the most important findings of 3 experiments described and discussed in detail in earlier chapters. Experiment 1 (chapter II) was designed to understand the effect of seedling exposure on the physiology and growth of Douglas-fir seedlings. Experiment 2 (chapter III) determined the effects of root pruning on the physiology and growth of Douglas-fir seedlings. Experiment 3 (chapter IV) focused on the water relations and gas exchange characteristics of developing leaders and 1-year-old laterals of Douglas-fir seedlings that had been either exposed or root pruned prior to planting. The purpose of experiments 1 and 2 was to determine the magnitude and nature of the effect of preplanting stresses on

first-year growth and physiology of newly planted Douglas-fir seedlings. The goal of experiment 3 was to determine the physiological mechanisms that may explain the observed stress-induced growth symptoms.

In chapter II, the first hypothesis stated that exposing seedlings to air affects growth primarily by damaging the root system rather than the shoots. Regardless of whether only roots were exposed or both shoots and roots were exposed prior to planting, the subsequent effect on physiology and growth was the same. Although root and shoot exposure (RS3) resulted in a larger loss of water (lower water potential) than the root exposed (R3) seedlings immediately after exposure, this had no effect on subsequent growth, indicating that roots are the locus of damage. Similarly, roots of Sitka spruce were more sensitive to exposure than were shoots (Coutts 1981). In his study, Coutts found that either root or shoot exposure alone showed a similar reduction in moisture content, but only seedlings that had roots exposed showed a reduction in survival and a decrease in growth of both roots and shoots after planting. In the same study, fine roots with a higher surface:volume ratio, lost considerably more water than coarse roots when exposed.

The second hypothesis stated that exposure damage to the roots causes a decrease in water potential and reductions in new root initiation, stem and root hydraulic conductance, stomatal conductance and net photosynthetic rates. During the first 2 months after transplanting, water potentials were always lower for exposed seedlings than untreated controls. Seedlings exposed and transplanted in January recovered from this early water deficit sometime during bud break (May 21), whereas

April transplanted seedlings did not recover until midway through leader elongation (June 7). This water deficit may primarily arise from poor root-soil contact (Sands 1984), or a reduction in root hydraulic conductance as a result of embolized root trachieds. Recently, Alder et al. (1996) have shown the roots of Acer grandidentatum Nutt. are more susceptible to cavitation than stems possibly due to larger pore size in the intervessel pit membrane. They also found that root embolism was partially reversed with increased soil moisture. It is possible that roots of Douglas-fir are likewise more susceptible to cavitation than the stems, and that such embolism may be partially reversed under high soil moisture. More research is needed to understand the vulnerability of roots of Douglas-fir and other conifers to cavitation, and how this may contribute to the development of transplanting stress.

Exposure reduced new root growth by 50% of untreated controls 2 months after transplanting. In related studies, new root growth of Douglas-fir and Sitka spruce was found to be very sensitive to any form of disturbance to the root system prior to planting (Tabbush 1986; Deans et al. 1990). Exposure may have reduced new root initiation by directly desiccating the meristematic tissue (Coutts 1980). Coutts also found that fine roots of Sitka spruce lost 2x the water as woody roots. Secondly, a corresponding decrease in the seedling ψ may have had a direct turgor-mediated negative effect on new root elongation (Kaushal and Aussenac 1989). Thirdly, root growth may be inhibited by a lack of photosynthates, because reduced water uptake can decrease the ψ and reduce net photosynthesis. This mechanism may

be more critical in Douglas-fir, which is dependent on current photosynthates for new root growth in spring (Philipson 1988).

Root hydraulic conductance may decrease immediately on exposing roots to dry air, most probably as a result of embolized roots. Although no one has measured root cavitation as a result of exposure, embolism may occur since roots are apparently more susceptible to cavitation than the shoots (Alder et al. 1996). Root hydraulic conductance was lower in exposed seedlings after 30 days in the soil. This was confounded by a corresponding decrease in new root growth in these seedlings. For instance, Carlson (1986) showed that the root conductance of loblolly pine seedlings increased substantially when new roots were present.

Stem conductance indicated by the % of stem cross-section dyed (conducting area) decreased for both unexposed and exposed (RS3) January seedlings by ~20%, but decreased by ~35% when seedlings were exposed in April. Also, a decrease in stem conductance correlated with a decrease in terminal elongation across all treatments and transplanting dates. Poor root-soil contact (Sands 1984), combined with an increasing evaporative demand may be sufficient to decrease ψ <-2.0 MPa to induce cavitation in Douglas-fir (Cochard 1992). Exposure of roots not only reduces new root growth, but may also reduce root conductance, which would impair water uptake and further decrease ψ, making seedlings more vulnerable to cavitation. Kavanagh (1993) found that loss in stem conductivity of newly transplanted western hemlock seedlings may be an important factor contributing to transplanting failure in

that species. It is likewise possible that in Douglas-fir, loss in stem and root conductivity may be critical factors that contribute to transplant shock.

Stomatal conductance of exposed seedlings was substantially decreased during the first 2 months after planting. A lower g_s coupled with low ψ may have also decreased net photosynthesis (Teskey et al. 1986), subsequently reducing the pool of photosynthates available for root growth. New root growth in Douglas-fir is dependent on current photosynthates (Philipson 1988), therefore factors affecting photosynthesis during establishment may be important for the recovery of transplanted seedlings from stress. Midday net photosynthesis and g_s of exposed seedlings transplanted in April were low shortly after budbreak (May 21) but recovered midway during stem elongation (June 7). In contrast, A and g_s of January exposed seedlings recovered to control levels by May 21. This decrease and recovery appear to be caused by low and high ψ respectively. Low plant ψ can directly reduce A by decreasing mesophyll conductivity (Bunce 1977; Teskey et al.1986), indirectly through stomatal closure (Teskey et al. 1995), or some combination of both. The decrease in A observed in my study is probably due to both stomatal closure and a potential increase in mesophyll resistance to carbon dioxide. Without a measure of mesophyll resistance to CO_2, it is impossible determine the relative contribution of these two processes in limiting net photosynthesis.

The third hypothesis stated that exposed seedlings regardless of the date of transplanting recover a favorable water balance and gas exchange characteristics after shoot elongation ceases in late spring. Seedling water balance and associated gas

exchange characteristics recovered prior to or during rapid elongation. For instance, January transplants recovered by May 21, whereas April transplants recovered by June 21, both prior to full terminal elongation. Recovery may be related to growing an adequate root surface area to meet the evapotranspirational demand imposed by the growing shoot and ambient VPD (Cleary et al. 1978). Faster recovery of the January transplants may be associated with 2 factors: 1) January transplants have a higher RGP (Stone et al. 1962), therefore better able to exploit the longer period in the soil (90 vs 27 days for April transplants) prior to active shoot elongation, and 2) they may be more tolerant to stress than April transplants (Hermann 1967; Ritchie 1984).

Regardless of time of recovery, leader elongation was inhibited irreversibly. However, seedlings that flushed again (lammas growth) showed no signs of physiological stress such as short internodal length. Elongation of the terminal leader may be sensitive to water deficits during early stages of development. Although very little is known about the internal factors regulating conifer stem elongation, work on crop plants suggest that cell division during early stages of new growth initiation may be more sensitive to water deficits than cell division in the expanding leaf (Clough and Milthorpe 1975; Creelman and Mullet 1991). Since all the needle primordia are present prior to the current-year's growth (Fielder and Owens 1988), any stress-induced effect prior to elongation can potentially influence the entire process of terminal development.

The fourth hypothesis stated that delayed (spring) transplanting would aggravate the negative effects of seedling exposure more than winter transplanting (January) by reducing new root growth, decreasing shoot ψ and reducing g_s within the first 2 months of establishment. New root growth was substantially higher for April transplants than January transplants irrespective of exposure treatments. Increased new root growth for April transplants is most probably an effect of warmer temperatures in spring than in winter (12°C vs 8°c). For instance, root growth in Douglas-fir increases with soil temperatures above 5-8°C (Lopushinsky and Kaufmann 1984; Tabbush 1986). However, ψ and g_s of exposed seedlings were both lower for the April than January transplants. Moreover, reduced new root growth for the April transplants decreased ψ more than for the January transplants. This suggests that new root growth may be more critical to water balance when VPD begins to increase in spring than when VPD is lower in winter. Therefore, vigorous new root growth of April transplants is apparently inadequate to meet the water demand during this period.

Exposing seedlings delayed budbreak, decreased shoot elongation, stem unit length, stem diameter, root and shoot biomass in the first season after transplanting. The common observation of "bottle brushing" associated with transplanted Douglas-fir seedlings is symptomatic of seedlings that are not only stunted, but also have reduced biomass and radial growth. Since exposure also reduced the terminal bud widths, second year growth may also be impacted although the effect may be small. Minimizing root exposure will enhance radial, vertical and

biomass growth of newly planted Douglas-fir seedlings. Winter transplanting (vs. spring transplanting) may further alleviate the effects of exposure. Winter transplanting may be more beneficial as a result of more favorable planting conditions, reduced loss of water to exposure, a longer period in the soil prior to active shoot growth in spring, and subsequently a shorter period of physiological stress.

In chaper III, the first hypothesis stated that root pruning Douglas-fir seedlings at the time of transplanting will significantly decrease new root growth, biomass and elongation growth during the first growing season. Root pruning (30-50%) decreased the number of new roots by 50% of the unpruned controls at the end of 2 months in the soil. Because pruning removed the fibrous roots, many of the active root tips that elongate after planting were lost (Stone et al. 1962). For instance, Deans et al. (1990) found most new roots of Sitka spruce growing after 14 days in the soil were due to the elongation of pre-existing root apices. In my study, when new root growth was expressed on a per unit volume basis, the pruning treatments had no effect on relative new root growth rates. The similar root initiation rates suggests that the roots of pruned seedlings were not stressed during the first 2 months after planting and the lack of plant moisture stress during this period is evident from high predawn ψ.

Root pruning >30% of the original root volume decreased both the biomass (20% of control) and elongation growth (10% of control) of Douglas-fir seedlings. On the other hand, relative growth rates were higher for the pruning treatments than

the untreated controls. This apparent discrepancy between absolute and relative growth rates is hard to explain. Smaller Douglas-fir seedlings had higher relative growth rates than larger seedlings, and the relative biomass differences decreased with time (van den Driessche 1992). It is possible that the pruned seedlings in my study may outgrow or equal the untreated controls over a longer period of time. For example, Hobbs et al. (1987) found that Douglas-fir seedlings planted after several nursery undercutting treatments showed no treatment effects after 4 years in the field. However, pruning in my study reduced final bud widths indicating that next year's growth may also be affected. Bud width has been related to spring terminal length in several conifers (Clements 1970; Graham and Hobbs 1994). A long-term field study may help confirm the predicted long-term growth patterns of root pruned Douglas-fir seedlings.

There was little difference in growth or physiology between seedlings that had 30% or 50% of their roots removed. Mortality of pruned seedlings was <1 %. Pruning reduced the number of seedlings flushing twice, but those seedlings that flushed showed no symptoms of stress, such as short stem unit lengths. These results suggest that Douglas-fir seedlings are relatively tolerant of severe root pruning. However, minimizing root loss at lifting will enhance first year height and biomass growth, and alleviate transplant shock.

The second hypothesis stated that the negative effects of root pruning will be ameliorated for seedlings transplanted in November>January>March. Transplanting seedlings in November or March, rather than in January slightly ameliorated the

negative effects of root pruning. Seedlings transplanted in November or March showed relatively little difference in net biomass between the levels of root pruning unlike seedlings planted in January. On the other hand, January and November transplants attained a larger mean biomass than March transplants. Actually in both November and January transplants, pruned seedlings had a higher biomass than the pruned March transplants. Therefore, seedlings planted in November or January will perform better than those planted in March.

However, the positive effect of the length of time that seedlings are in the soil prior to active spring growth is dependent on the degree of dormancy, cold hardiness, and the associated physiological vigor/stress resistance of seedlings. Winter (January) transplants showed better seedling performance in terms of vertical, radial and biomass growth than either November or March transplants. This supports the observation that winter transplants are more resistant to stress than spring transplants (Hermann 1967; Ritchie 1986). Winter transplants in my study had a cold hardiness of -21.5°C which is generally indicative of high stress resistance, although the mechanisms that underlie such resistance are still unknown (Ritchie 1986).

The third hypothesis stated that November and March transplants will show higher root growth due to warmer soils than January transplants, but shoot ψ will be higher for January>November>March transplants during the first 2 months of establishment due to increasing VPD. March transplants had more than 2x the new root growth of January transplants. Higher soil temperatures in March than on either of the other dates is the most probable cause for higher new root growth. New root

growth in coastal Douglas-fir does not start until soil temperatures are above 8-10°C (Lopushinsky and Kaufmann 1984; Tabbush 1986). Although, the root growth potential (RGP) for the January transplants was almost 2x that of March and November transplants, lower soil temperatures negated the expression of that potential when planted under natural conditions.

New root growth of the November transplants was substantially lower than either March or January seedlings even though soil temperatures in November were higher than January and closer to March temperatures. However, towards the end of the 2 month period in the soil, the November transplants were exposed to sub-freezing temperatures when the soil froze. These low temperatures may have killed freshly initiated root tips in the November transplants, and further delayed new root initiation. Roots are also more sensitive to low soil temperatures because roots are significantly less cold hardy than the aerial parts of trees and may not have an inherent dormant period (Kozlowski et al. 1991).

Predawn ψ during the first 2 months after transplanting remained relatively unchanged for all transplanting dates. Although the November seedlings reached the lowest ψ due to unexpected soil freezing, they recovered to pre-freezing levels. Contrary to expectations, increasing VPD in March did not cause a subsequent decrease in seedlings ψ. March transplants avoided water deficits by increasing water uptake by increasing new root growth (Fig III.3) and root conductivity (Lopushinsky and Kaufmann 1984) in response to rising soil temperatures.

The fourth hypothesis stated that root pruning will decrease ψ, reduce g_s and A. Root pruning did not affect seedling ψ during the first 2 months after planting irrespective of transplanting date. Water stress in pruned seedlings did not develop until active spring elongation. This delayed development of water stress may correspond to an increased water demand due to the combination of increasing VPD and newly expanding needles (Cleary et al. 1978). As shown in experiment 1, new root growth may be more critical when seedlings are increasingly exposed to a higher atmospheric VPD. Pruning seedlings also caused a decrease in the midday g_s and A during the same period. Therefore, shoot growth in pruned Douglas-fir seedlings may be limited by low midday ψ combined with a reduction in net photosynthesis during the period of active elongation. Any decline in current photosynthates may limit new growth which is energetically expensive and dependent on transported photoassimilates (Webb 1977).

The better performance and apparent stress resistance of seedlings transplanted in January (experiment 1; experiment 2; Hermann 1967; Ritchie 1986) rather than in November (fall) or March / April (spring) is not reflected by root growth or internal water status during the first 2 months after transplanting. Neither, is it reflected in bud break phenology. The ability of January transplants to tolerate stresses may be closely tied to their physiological status just prior to and during the period of active shoot growth in spring. It is postulated that January transplants may be more effective in conserving water and promoting rapid photosynthesis and root growth

during this period than the other transplants. Future research may help clarify the mechanisms that determine seasonal stress resistance in Douglas-fir seedlings.

Exposure and root pruning caused less than 1% mortality of seedlings regardless of when they were transplanted. Damage to seedlings as a result of exposure or root pruning was not severe enough to cause death. It is possible that seedlings may have suffered higher mortality if they had been outplanted in the field. Under field conditions, poor planting, competing vegetation, and summer drought could substantially decrease the survival of already stressed seedlings. In contrast, the high survival in these experiments may have been the result of careful planting, minimum competition, and adequate soil moisture throughout the summer. However, it is important to note that this study demonstrates that Douglas-fir seedlings can tolerate relatively severe preplanting stresses (exposure for 3 hrs under cool-dry conditions and root pruning up to 50% of original root volume) if planted under wet conditions. In the northern Coast Range, seedling mortality resulting from various causes average 20% (Mitchell et al. 1990), which suggests that under field conditions, preplanting stresses may have to be more severe, seedlings less vigorous, and / or post-planting conditions such as wind, competition, and summer drought have to be more extreme than the planting beds to have an adverse effect on Douglas-fir seedling mortality.

An often overlooked, but subtle factor contributing to reduced shoot growth associated with transplant shock may be related to the relative root:shoot biomass allocation patterns. Both exposure and root pruning caused an increase in the

allocation of biomass to the roots relative to the shoots. Reduced shoot growth would reduce the transpirational surface area therefore reducing the demand for critical water resources. On the other hand, this may also reduce the surface area for carbon assimilation. Therefore, newly transplanted seedlings may be adapted to avoid water stress by rapidly growing roots at the expense of total carbon assimilation. This adaptation inherently decreases shoot growth in favor of root growth. These observations in stressed newly planted Douglas-fir supports the contention of Lavender (1990) that newly planted seedlings use photosynthates for root growth at the expense of vigorous shoot growth, which may be an useful adaptation to moisture stress associated with the transplanting process.

In chapter IV, the first hypothesis states that the elongating terminal leader of Douglas-fir seedlings that were exposed or root pruned prior to planting lose tissue elasticity faster than unstressed controls, and that this increase in the rate of cell wall hardening is associated with a lower measurable turgor pressure. The predawn turgor of the developing leader and ψ of stressed seedlings remained lower than the untreated controls during the entire period of elongation. However, the effect of reduced turgor on shoot expansion was not associated with a decrease in the rate of cell wall hardening as measured by the modulus of elasticity. The increase in the modulus of elasticity (increase in cell wall hardening) corresponded closely with the ontogeny of the leader rather than changes in turgor. Relatively high tissue elasticity (low ϵ^{max}) during the period of shoot elongation probably results from an increase in the proportion of young shoot cells without secondary thickening (Ritchie and Shula

1984). As shoots mature, more cells produce secondary thickening with a corresponding drop in cell wall elasticity. Cell expansion is primarily a function of cell turgor (Green et al. 1971; Hsiao and Jing 1987); however, reduced turgor inhibits elongation by its effect on cell wall properties (Ray 1987). Since the stress-induced decrease in turgor was not sufficient to change the elastic component of the cell wall properties (Nonami and Boyer 1990b), it most probably decreased the plastic extensibility of the cell wall (Chazen and Neumann 1994) subsequently inhibiting elongation.

The second hypothesis states that elongating leaders of Douglas-fir seedlings that were exposed or pruned prior to planting may show some degree of osmotic adjustment to alleviate turgor deficits. Developing leaders of stressed seedlings showed no indication of active osmotic regulation of turgor during the period of tissue water deficit. This supports the conclusions of Joly and Zaerr (1987) that Douglas-fir seedlings do not adapt to short- or medium-term water deficits by osmotic adjustment. Turgor adjustment in newly elongating shoots of Douglas-fir may be more a function of cell wall properties such as the modulus of elasticity rather than osmotic adjustment. The cell wall elasticity of expanding needles of stressed seedlings were slightly higher than the untreated controls suggesting that it may play some role in turgor regulation. On the other hand, older needles inherently have lower π_{100} and π_0 than newly expanding tissue which is probably associated with moderate turgor adjustment in these needles. For instance, Ritchie and Shula (1984) found that π_{100} and π_0 of mature Douglas-fir shoots were lowest during midwinter and

midsummer and highest in spring indicating seasonal changes in osmotically influenced turgor maintenance. Nevertheless, active osmotic adjustment has been shown in other species such as Jack pine and white spruce during water stress (Koppenaal et al. 1991).

Most of the tissue-water parameters varied largely between the two tissue types rather than the stress treatments. New needles and 1-year-old needles maintained the same level of RWC_0, but had widely differing modulus of elasticity, π_{100} and π_0. One-year-old needles may compensate for lower tissue elasticity by having a lower π_{100} and π_0 (more negative). This suggests that older needles with a lower initial π than newer needles are inherently more capable of osmotically influencing turgor (Eamus and Narayan 1990). Turgor in mature needles was higher for the first 34 days than new needles, indicating that lower π does allow for some degree of turgor maintenance in mature needles. On the other hand, expanding new needles have an inherently higher elasticity than 1-year-old needles, therefore enabling the current shoot to retain a similar RWC_0 to that of older needles despite a larger loss in symplastic water (Joly and Zaerr 1987; Eamus and Narayan 1990). However, this capacity to regulate turgor by inherently higher tissue elasticity in newer needles was not sufficient to increase turgor to that of mature needles. These differences between current and 1-year-old needles disappear as the new shoot matures, on completion of expansion growth.

Finally, measurements of gas-exchange parameters of mature and expanding needles in stressed and untreated controls were made in order to characterize patterns

of carbon acquisition, and stomatal regulation of water loss during early establishment. The net photosynthetic rate of the developing leader and 1-year-old needles was reduced for stressed seedlings. This reduction in A was apparently limited by non-stomatal factors rather than by decreased stomatal conductance. Stomatal conductance of both current and 1-year-old needles did not correspond to changes in A. Non-stomatal factors such as mesophyll conductance and carboxylating enzyme activity can limit A independent of g_s (Delucia 1986; Teskey et al. 1986). Decreased turgor in stressed seedlings may have reduced A by changing the mesophyll conductance to CO_2 (Kozlowski et al. 1990). Although the specific non-stomatal mechanism limiting A in stressed Douglas-fir seedlings is not known, a similar limitation of A during leaf water deficit has been reported for several other species (von Caemmerer and Farquhar 1981; Briggs et al. 1986; Guehl et al. 1991).

In conclusion, I integrate some possible mechanisms involved in the development and expression of transplanting stress symptoms in newly transplanted Douglas-fir. Root damage as a result of exposure or pruning not only reduces the functional root surface area but also retards its expansion. Such a root system probably reduces water uptake and also delays the time to achieve an adequate absorptive area to meet the increasing water demand of a planted seedling. Impaired root systems increase the vulnerability to stem cavitation as water deficits increase in response to increasing atmospheric VPD as the temperature increases in spring. Water deficit characterized by a decrease in turgor during active cell elongation apparently inhibits the extensibility of cell walls, thereby reducing growth. This

inhibition of cell wall extensibility may depend on cell turgor or some other non-hydraulic signal. Recovery of terminal elongation in the secondary growth (lammas) and a discrete stunting of the primary growth (spring flush) suggests that the inhibition of cell wall extensibility affects all the cells in the predetermined spring growth but not lammas growth. During the period of active elongation, new shoots of stressed Douglas-fir seedlings are unable to regulate turgor by osmotic adjustment. During the same period, mature needles maintain higher turgor most probably as a result of inherently lower osmotic potentials than new needles. Net photosynthesis of both new and mature needles is reduced with or without a corresponding decrease in g_s, therefore decreasing the photosynthates available for new growth. Carbohydrate supply to the terminals may be further limited by an increased allocation of photosynthates to the roots. Such a sequence of events may partially explain the stunting of new shoot growth of Douglas-fir seedlings that is induced by exposure or root pruning prior to transplanting.

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