An Abstract of the Dissertation of

Douglass Frederick Jacobs for the degree of Doctor of Philosophy in Forest Science presented on November 30, 2001. Title: Influence of Root Architectural Development on Douglas-fir Seedling Morphology and Physiology.

Abstract Approved: Signature redacted for privacy.

Robin Rose

A series of experiments were established to gain a better understanding of the extent to which Douglas-fir seeding root architecture may be manipulated and subsequent influences on seedling morphological and physiological development.

The incorporation of amendments into nursery soils changed root architecture to some degree, but did not produce large differences in morphology at lifting or following two growing seasons under field-fertilized and non-fertilized conditions. The application of controlled-release fertilizers (CRF) to the planting hole, however, produced an interesting response in which aboveground growth was enhanced during the first field season but negatively affected thereafter. The resulting hypothesis was that drought stress was responsible for the growth reduction.

To investigate rooting response to locally-applied CRF, two greenhouse experiments were established. In the first experiment, differences in seedling morphological and physiological development over time were observed under two comparable CRF types and this was attributed to variations in nutrient release. Roots proliferated in the soil zone above the locally-applied CRF, though root penetration into lower soil zones was not restricted. With increasing CRF rates in the second greenhouse experiment, however, root penetration into soil zones below the CRF decreased with increasing CRF rate six months following transplant ($R^2 = 0.72$), likely due to the creation of a toxic osmotic gradient between rhizosphere and root. It was

hypothesized that this response might intensify seedling drought stress following field fertilization.

A field study investigated the influence of initial root volume and field fertilization at a relatively high rate on seedling drought resistance. Regardless of initial root volume, fertilized seedlings became more drought stressed during summer and had lower rates of stomatal conductance near the end of summer. An increase in shoot:root dry weight, which was greater for fertilized seedlings, was inversely correlated with xylem pressure potential ($R^2 = 0.54$). There was no distinct proliferation of roots near the CRF layer as root growth in all vertical soil zones was negatively affected for fertilized seedlings.

Influence of Root Architectural Development on Douglas-fir Seedling Morphology and Physiology

by

Douglass Frederick Jacobs

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

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Douglass F. Jacobs, Author

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Contribution of Authors

Chapter 2: Dr. Robin Rose and Diane Haase were instrumental in the implementation of this project and the editing of the manuscript. Dr. Paul Morgan also contributed to the design and provided significant resources for this project.

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Chapter 5: Dr. Robin Rose and Diane Haase contributed ideas to this project and to the editing of the manuscript. Patricio Alzugaray provided initial root volume measurements and seedling height/diameter measurements following outplanting.

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This dissertation is dedicated to my grandmother

Helen Danow Jacobs
(Born December 2, 1917)

...a pioneer in the quest for gender equality in science

Influence of Root Architectural Development on Douglas-fir Seedling Morphology and Physiology

Chapter 1. Introduction

1.1 Introduction

The ultimate objective of reforestation operations is to produce seedlings with target characteristics (Rose et al. 1990) that remain on nearly the same growth trajectory when outplanted into the field that they were on in the nursery. Reforestation managers are no longer concerned simply with survival following planting operations and attaining tree heights of two meters within two years following planting appears to be a realistic goal on many sites. To maximize reforestation productivity, a secure link between nursery and outplanting operations must be maintained. An example is the modification of nursery cultivation techniques based on the conditions of a designated outplanting site. Selection for morphological and physiological characteristics that encourage vigorous root system development in the nursery and field may also enhance reforestation success dramatically.

1.2 Importance of Roots in Reforestation Success

Roots supply the plant with water and nutrients from the soil, provide structural support, and control the flow of certain hormones, which act to modify seedling morphological and physiological development. Because small seedlings have little capacity to store water, shoot growth immediately following planting is typically most limited by water (Burdett et al. 1984). When bareroot seedlings are lifted from the nursery, a significant portion of roots, particularly fine roots may be lost (Wakeley 1954; Nambiar 1980). Thus, the survival of newly planted seedlings is largely

dependent on the rapid extension of roots, which reestablish root-soil contact and absorb water to reduce transpirational water loss (Ritchie and Dunlap 1980; Burdett et al. 1983; Sands 1984; Carlson 1986). Without efficient water extraction, seedlings continue to transpire, resulting in a condition of physiological drought, which could contribute to transplant shock (Rietveld 1989). Root fibrosity (Deans et al. 1990) and an optimal balance between the root and shoot (Atkinson and Ofori-Asamoah 1987) also improve seedling establishment.

The ability of seedlings to produce new roots following transplanting is termed root growth potential (RGP) and has been the focus of considerable research regarding root system development in recent years (Ritchie and Dunlap 1980; Tanaka et al. 1997). Though likely correlated with other important physiological aspects (e.g. bud dormancy, carbohydrate content, frost hardiness, etc.), RGP may be of questionable benefit in itself because it is typically measured after seedlings have been transplanted into controlled environments that are highly favorable to root growth. Root system response may be quite different when seedlings are transplanted into a less hospitable field environment. Thus, assessing root architectural development by destructively harvesting seedlings in a nursery or field environment may be a more valuable means of evaluating the efficacy of various treatments on subsequent seedling performance. For a variety of reasons, these types of root measurements are challenging and rarely performed.

1.3 Difficulty in Studying Roots

Due to their below-ground nature, roots are more difficult to study than the seedling top and there is little information on their early growth as compared to that related to the shoot (Mackie Dawson et al. 1995). Another impediment to studying roots is the ability to directly measure them in a natural state (Wraith and Wright 1998) and roots are generally sampled only following destructive harvest. Though measurements of root volume (Burdett 1979) and biomass are fairly rapid to perform,

more intensive measurements are often needed to adequately characterize root system development (Nambiar 1980).

For the purposes of this dissertation, seedling root architecture is defined as the quantification and spatial distribution of hierarchical branching components of the root system and active root tips throughout the soil profile. Seedling root systems are functionally divided between the tap root and successively higher-order lateral roots. First-order lateral roots branch directly from the tap root, while second and third-order laterals branch directly from roots of the previous order. Various methods have been used previously to quantify root "fibrosity" (Tanaka et al. 1976; Deans et al. 1990; Kainer and Duryea 1990; Dewald et al. 1992), and hence, characterize root system quality. Because no standard method has been established for determining root fibrosity, however, the term is ambiguous. Instead, the evaluation of active root tips offers a readily transferable methodology that may help to provide a better descriptor of root architectural development.

Assessments of root architecture may help to characterize seedling condition and be used an indicator for future productivity. For example, active root tips are important sites for ion uptake (Peterson et al. 1999) and represent points of active root elongation. Lateral root length is indicative of available root surface area for extracting water and nutrients. The presence of roots deep in the soil profile may provide access to critical subsoil water under drought conditions. These measurements are tedious and time consuming and often ignored in lieu of biomass measurements. Thus, few studies have investigated the relationship between intensive measurements of root architecture and conifer seedling performance. These measurements may be justifiable, however, if they help to explain seedling response to various nursery and outplanting treatments and are indicative of seedling performance. Furthermore, much of the literature that has intensively studied root system development is now outdated in light of dramatic stocktype and cultural advances.

1.4 Manipulation of Root Development

1.4.1 Root Wrenching

Various methods have been used in attempts to positively manipulate root development in the nursery. Root wrenching, involving the pruning of roots at a specific depth typically using mechanical means, has been the focus of considerable research. Wrenching acts to slow shoot growth, preparing seedlings for hardening off and modifies shoot:root ratios to reach a desired standard. Wrenching may also improve root fibrosity (Tanaka et al. 1976; Kainer and Duryea 1990) and has been linked to improved outplanting performance (Tanaka et al. 1976; van den Driessche 1983; Kainer and Duryea 1990). Root wrenching has thus become a standard cultural practice at most Pacific Northwest nurseries (Duryea 1984). Surprisingly little research, however, is available concerning nursery or field soil properties and conifer seedling root growth.

1.4.2 Soil Physical and Chemical Properties

A primary influence on root growth is the relationship of the seedling root system with the soil. Root growth is influenced by numerous soil physical properties such as structure, temperature, aeration, and water content (Argo 1998b; Singh and Sainju 1998). The size and distribution of pores within soil helps to determine its ability to supply roots with nutrients and water. Additionally, the mechanical impedance of the soil affects the ability of roots to penetrate and proliferate through the soil profile. Root penetration is influenced by factors such as water content, texture, aggregate size, exchangeable cations, and orientation of soil particles (Bennie 1996).

Root growth is also influenced by soil chemical properties including nutrient availability, pH, irrigation water quality, cation exchange capacity (CEC), and buffering capacity (Argo 1998a). The incorporation of organic matter and other soil amendments

into nursery soil may help to improve soil physical and chemical properties, thereby positively influencing root development (Rose et al. 1995). Though not completely understood, organic matter may also help to control common nursery pathogens and pests (Rose et al. 1995). The investigation of organic matter use in forest seedling nurseries is needed based on the paucity of organic matter literature during the past 30 years. Dramatic advances in stocktypes and nursery culture, along with the expected banning of methyl bromide as a soil fumigant further emphasizes this need.

Soil moisture content also influences plant root growth. As soils dry, roots penetrate deeper into the subsoil until dry conditions prevent root elongation (Becker et al. 1987). Dry conditions also result in the allocation of more biomass to root as compared to shoot growth, further manipulating plant development (Bongarten and Teskey 1987; Teskey et al. 1987; Livingston and Black 1988). Thus, altering nursery irrigation patterns may dramatically influence whole-plant development and the proliferation of roots within various soil depths. These changes may be further enhanced by the addition of soil amendments into nursery soils that modify soil moisture holding properties.

1.4.3 Controlled-Release Fertilizers

By slowly releasing nutrients over an extended period of time, controlled-release fertilizers (CRF) offer another means to modify plant root development. Interest in the use of CRF in the nursery and field has increased in recent years (Donald 1991; Haase and Rose 1997). CRF can supply seedlings with nutrition for as long as 2 years with a single application, providing a consistent and sustained flow of nutrients that may better coincide with plant development (Donald 1991) as compared to conventional water-soluble fertilizers. Also due to their gradual release, the potential for seedling damage associated with nutrient toxicities and nutrient loss through leaching can be reduced (Hauck 1985; Donald 1991; Goertz 1993). Benefits associated with CRF use in forest tree seedlings appear to be dependent on a complex interaction of factors including CRF type, nutrient release rate, seedling morphology/physiology, media, and environmental

growing conditions. Variable results in the nursery and field indicate that a better understanding of the mechanisms by which CRF affect seedling morphology and physiology is needed (Brockley 1988).

Polymer-coated CRF represent the culmination of many years of CRF technological ingenuity and are currently the CRF of choice for containerized nurseries (Huett and Gogel 2000). Yet, little is known about the effects of CRF on the development of seedling root architecture under nursery and field conditions. When using CRF with field plantings, researchers have advocated the application of fertilizers directly to the planting hole in field plantings to facilitate efficient nutrient uptake (Carlson 1981; Carlson and Preisig 1981; Gleason et al. 1990; Rose et al. 1991). It is possible that roots may then proliferate in these areas of localized nutrient supply as has been observed with nutrients in solution for various species, including barley (Drew 1975; Drew and Saker 1975), Sitka spruce (*Picea sitchensis* (Bong.) Carr.) (Coutts and Philipson 1976), lodgepole pine (*Pinus contorta* Dougl. ex. Loud.) (Coutts and Philipson 1977), maize (*Zea mays* L.) (Granato and Raper 1989) and Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) (Friend et al. 1990). Few studies have documented the influence of localized CRF placement on conifer seedling root architecture in the field. Additionally, the physiological mechanisms for localized root proliferation are poorly understood (Granato and Raper 1989; Robinson 1994).

Localized proliferation could have implications regarding drought resistance as reduced subsoil penetration might prevent roots from accessing deeper water when soils dry. Deep placement of fertilizer may enhance growth under drought stress when water is available in subsoil (Garwood and Williams 1967) but this technique might prove difficult when planting tree seedlings on an operational scale. As CRF release nutrients, the buildup of fertilizer salts in the soil solution may also cause the death of root apical meristems (Sherwin 1923; Skinner et al. 1945; Drew 1975; Danielson et al. 1984; Kafkafi and Bernstein 1996), restricting the ability of seedlings to access water and nutrients (Kozlowski 1987). Fertilization may also cause increased allocation of biomass to shoots as compared to roots as noted in container studies for Douglas-fir (Shaw et al. 1998), Jeffrey pine (*Pinus jeffreyi* Grev. & Balf.) (Walker and Huntt 1992; Walker and Kane 1997), singleleaf pinyon pine (*Pinus monophylla* Torr. & Frem.)

(Walker and Huntt 1992), jack pine (Tan and Hogan 1997), sycamore (*Platanus occidentalis* L.), and Sitka spruce (*Picea sitchensis* (Bong.) Carr.) (Mackie Dawson et al. 1995) and the field for Douglas-fir (Carlson and Preisig 1981). This may lead to increased water stress following transplanting, as a reduced root system must support a larger top (Gilman 1990). Thus, it is important to closely examine the effects of localized CRF supply on root development if we are to positively benefit from field fertilization with CRF.

1.5 Influence of Root Architecture on Seedling Performance

1.5.1 Morphology

Various methods may be used to assess the influence of changes in root architectural development on seedling performance. Analysis of morphological development offers a simple method to examine root architectural effects on growth in current and previous seasons. These measurements may include growth in terms of height, diameter, and shoot/root volume or biomass. Another important characteristic is shoot to root ratio, which provides an indication of how carbohydrate supplies are allocated to above and below-ground plant parts under various environmental conditions. Several different physiological measurements, however, may provide a better understanding of not only current seedling condition but also how future productivity may be affected.

1.5.2 Chlorophyll Fluorescence

First reported by Kautsky (1931), chlorophyll fluorescence is an inexpensive and non-destructive method to evaluate plant physiological status (Vidaver et al. 1989). Because chlorophyll fluorescence has been linearly correlated with net photosynthesis

(Adams et al. 1990), its potential benefit for evaluating seedling response to cultural treatments is high. The use of chlorophyll fluorescence with forest tree seedlings has been limited and its usefulness is not sufficiently understood. Chlorophyll fluorescence has been used to detect changes in the physiological status of Douglas-fir due to dormancy (Hawkins and Lister 1985; Roberts et al. 1991), freezing stress (Fisker et al. 1995), shading (Khan et al. 2000), and N-K fertilization (Birchler et al. 2001). Few studies, however, have examined changes in chlorophyll fluorescence due to CRF or other treatments that may manipulate root architectural development. Additionally, refinements in methodology for sampling of conifers are likely needed.

1.5.3 Nutrient Uptake

Roots control the efficiency of nutrient extraction from the soil. The ability of seedling roots to rapidly exploit the soil for nutrients is a major determinant of plant productivity as foliar nutrient concentrations are often correlated with rates of photosynthesis (Strand 1997) and plant biomass (van den Driessche 1980; Ingestad and Kahr 1985). The capacity of seedling roots to extract immobile soil nutrients may change with the effects of fertilization on root architectural development, the allometric relationship between the shoot and root, and rhizosphere dynamics. For instance, decreased phosphorus uptake with increasing fertilization has been observed for Douglas-fir fertilized with nitrogen in the field (Gill and Lavender 1983; Roth and Newton 1996) and nursery (van den Driessche 1980). Under conditions of high nitrogen availability, root expansion may be less vigorous. This may limit the ability of roots to intercept and extract soil-immobile phosphorus anions. Additionally, with increased shoot expansion relative to roots, foliar concentrations of immobile nutrients may become diluted. Thus, examining the relationship between root architectural development and nutrient uptake may provide further insight into the effects of treatments that manipulate root development on plant productivity and reforestation success.

1.5.4 Plant Moisture Stress

Drought stress may explain a significant portion of variation associated with successful seedling establishment. Because the ability of roots to extract soil water and supply this water to leaves is the primary method by which seedlings resist drought stress, any silvicultural treatments that modify root architectural development may substantially affect the ability of seedlings to resist drought. Simple portable instruments have been developed to assess seedling xylem water potential, providing an indication of drought stress (Waring and Cleary 1967; Cleary and Zaerr 1980) without destructively harvesting the plant. The ability of seedlings to produce new roots has been directly correlated with drought resistance in previous experiments for ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.) (Omi et al. 1991) and shortleaf pine (*Pinus echinata* Mill.) (Brissette and Chambers 1992).

1.5.5 Stomatal Conductance

The opening and closing of stomata acts to control gas exchange between the plant leaf and atmosphere. With higher rates of stomatal conductance, more CO_2 may be absorbed from the atmosphere and used for photosynthesis. However, water is also transpired from the plant while stomata are open due to the vapor pressure deficit (VPD) that exists between the leaf and atmosphere. To reduce water loss, plants often close stomata during periods of moisture stress, limiting photosynthetic capacity (Hinckley et al. 1978). Photosynthesis of Douglas-fir begins to decline at -1.0 MPa xylem water potential (Brix 1979). As plants become excessively water-stressed, respiration costs may exceed photosynthetic input, slowing growth and possibly causing mortality. The ability of seedlings to continue to photosynthesize during drought conditions is likely associated with root architectural development. Omi et al. (1991) reported a significant linear correlation between root dry weight and stomatal conductance. Thus, examining the relationship between root development and stomatal

conductance, in addition to measuring drought stress, may provide an accurate assessment of how field fertilization treatments may affect seedling growth.

1.6 Dissertation Objectives

The underlying objective of this dissertation was to enhance our knowledge of methods that may be used to modify root architecture in the nursery and field and to assess the relationship between root architecture and seedling productivity. This objective was accomplished through a series of specific experiments designed to examine the effects of various cultural treatments on root development and the subsequent influence of root architectural development on aspects of Douglas-fir seedling morphology (e.g. growth, biomass allocation) and physiology (e.g. nutrient extraction, drought resistance, photosynthetic capacities).

1.7 Research Approach

The overall dissertation hypothesis was that it is possible to manipulate Douglas-fir seedling root architecture and that changes in root development would influence seedling productivity. This overall hypothesis was tested through individual experiments designed to test smaller pieces of the larger hypothesis with the intention of forming an overarching conclusion. Results from each experiment were used to modify the hypotheses in succeeding experiment chapters.

In Chapter 2, I hypothesized that the incorporation of various soil amendments applied prior to nursery transplant into nursery soils would beneficially manipulate seedling root architectural development and subsequent growth performance under fertilized and non-fertilized conditions in the field. Though dramatic differences in root development associated with soil amendments were not realized, a distinct negative growth response to field fertilization was observed. From this experiment, it was hypothesized that drought stress, associated with restrictions in root development of

fertilized seedlings, might have been responsible for the poor growth of fertilized seedlings.

Chapters 3 and 4 were designed to examine the hypotheses that the application of CRF as a localized supply (similar to a field fertilization scenario) would manipulate root architectural development and seedling morphology/physiology under controlled conditions in the greenhouse and that potential benefits would be dependent on CRF rate. Though the method of fertilizer application was identical in these chapters, the objectives, treatments, and results were very different. The intent of Chapter 3 was to develop an understanding of how different CRF types release nutrients over time and to ascertain effects of these CRF types on plant growth, while also evaluating rooting response to localized CRF supply at rates that may be commonly used in nursery operations. The primary objective of Chapter 4, however, was to assess root penetration and whole-plant morphological and physiological response to a wide range of rates of a single CRF type that included treatments where toxicities were expected. Due to the substantial differences in objectives and expected application from these experiments, independent chapters were established for each experiment.

Based on the strong tendency of roots to proliferate relative to the localized CRF supply in Chapters 3 and 4, I hypothesized in Chapter 5 that root penetration into subsoil zones would be restricted following field fertilization and that this would limit the ability of fertilized seedlings to resist drought, helping to explain poor growth response at high fertilizer rates. In Chapter 6, I provided a synthesis for the dissertation by explaining how the chapters linked together to attain the initial objectives of the dissertation by supporting an answer to the overall hypothesis.

In each experiment, attempts were made to gain a better understanding of the biological mechanisms by which silvicultural treatments affected root growth. In cases where likely influential mechanisms were not or could not be directly measured, speculation as to the causal agent was made based on current scientific knowledge.

The exploratory nature of this dissertation should also be noted. Few previous experiments have examined the effects of various silvicultural treatments on conifer seedling root architectural development to the level of detail that was conducted in this dissertation. The intensive root morphological measurements in the multiple field and

greenhouse experiments resulted in an exceptionally large number of response variables associated with the statistical analyses.

Due to the large number of response variables in the analyses, Type I errors likely occurred. A Type I error refers to the detection of a statistical difference between treatments in the absence of a true difference. Though the chance for occurrence of a Type I error could be reduced by minimizing the number of response variables, we felt that this would detract from the novelty and fundamental objectives of this research. Thus, a 5% Type I error rate was established and all statistically significant results were reported. Type II errors (i.e. failure to recognize a treatment effect when it was in fact present) also likely occurred, again an inevitable function of the inherent nature of this work. Future research in this area will help to confirm or reject potential effects of silvicultural treatments on root architectural development that were measured in this study.

Chapter 2

Soil Amendment Use in Douglas-fir Seedling Nursery Transplant: Influence on Water Relations, Root Architectural Development, and Growth in the Nursery and Field

Douglass F. Jacobs, Robin Rose, Diane L. Haase, and Paul Morgan

2.1 Abstract

Interest in the use of organic matter in forest seedling nurseries as a source of disease control has increased with expected regulations on methyl bromide. The objectives of this experiment were to determine the influence of manure, peat, and vermiculite incorporated at two rates (0.0425 m^3 and 0.0850 m^3 per 3.3 m^2 plot) and under two soil moisture regimes on Douglas-fir (*Pseudotsuga menzieseii* (Mirb.) seedling (1+0 for 1+1) water uptake, whole-plant growth, root architectural development, and subsequent field performance under fertilized and non-fertilized conditions. Trends in soil moisture retention were observed among sampled plots (high manure > high peat > control) but no differences in pre-dawn xylem pressure potential were apparent during the experiment. After 3 months, lateral root length was significantly different among treatments in the wetter soil moisture experiment with high manure having the lowest and high vermiculite the highest mean lengths. There were no differences among treatments in either experiment at the time of lifting (after 8 months) for root length or the majority of other morphological parameters. Total height was significantly different among treatments in the wetter soil moisture experiment at the end of two field seasons, with seedlings grown in vermiculite and peat having the greatest mean height growth. Field fertilization with 35 g of Osmocote Plus® controlled-release fertilizer (CRF) in the planting hole stimulated seedling height growth during the first growing season but decreased height and diameter growth during the second growing season. Dramatic improvements in reforestation success associated with the use of nursery soil amendments were not realized in this experiment, but the failure to identify negative effects, a potential reduction in disease incidence, and improvement of nursery soil physical and chemical properties may justify their use.

2.2 Introduction

The goal of nursery operations is to produce quality seedlings with target morphological and physiological characteristics (Rose et al. 1990) known to improve

reforestation success on a given outplanting site. Survival of newly planted seedlings is largely dependent on the rapid extension of roots, which reestablish root-soil contact and absorb water to reduce transpirational water loss (Ritchie and Dunlap 1980; Burdett et al. 1983; Sands 1984; Carlson 1986). Root fibrosity (Deans et al. 1990) and an optimal balance between the root and shoot (Atkinson and Ofori-Asamoah 1987) also improve seedling establishment. Root wrenching has been used to manipulate root system development in the nursery and has been linked to improved outplanting performance (Tanaka et al. 1976; van den Driessche 1983; Kainer and Duryea 1990) and the promotion of root fibrosity (Tanaka et al. 1976). Root wrenching has thus become a standard cultural practice at most Pacific Northwest nurseries (Duryea 1984). To continue to improve seedling root system quality, identification of additional nursery cultural treatments that might improve root system development is needed.

A primary influence on root growth is the relationship of the seedling root system with the soil. Soil provides water and nutrients, allows gas exchange to and from the roots, and provides support for the plant. Root growth is influenced by soil physical properties such as structure, temperature, aeration, and water content (Singh and Sainju 1998). The size and distribution of pores within the soil helps to determine its ability to supply roots with nutrients and water. The incorporation of organic matter and other soil amendments into nursery soil may help to improve soil physical properties, thereby positively influencing root development (Rose et al. 1995).

High soil bulk densities act to restrict root growth. The mechanical impedance of the soil depends on factors such as water content, texture, aggregate size, exchangeable cations, and orientation of soil particles (Bennie 1996). Roots must apply a force greater than the mechanical strength of the soil matrix to elongate. Mechanically impeded roots are shorter, thicker, and more irregularly shaped than roots under less impeded conditions (Dexter 1986). High bulk densities also act to decrease soil pore space, limiting root aeration (Warneke and Richards 1974). Soil should contain more than 10% air filled pores to maintain proper root aeration (Box 1996). When soil moisture is high, repeated passes of heavy machinery generally increases bulk density and is an undesired outcome of common nursery operational procedures. In addition to nursery practices such as wrenching and ripping, the incorporation of soil

amendments into the soil may help to improve soil penetrability, promoting optimal root development.

The moisture content of the soil also has a dramatic influence on plant root growth. Dry soil conditions can result in a deeper penetration of the soil and a greater root distribution in the subsoil than in the surface soil. Because seedlings are typically root-wrenched before lifting, soil drying may cause a significant loss of root biomass. The capacity to store water in soil is improved by the incorporation of amendments such as organic matter and vermiculite (Warneke and Richards 1974; Brady and Weil 1996). Thus, soil amendments may act to enhance root development at upper soil depths and improve harvestable root mass. Additionally, the increased water holding capacity associated with soil amendments may decrease the needed irrigation frequency, reducing operational costs.

Root growth is also affected by changes in soil temperature. At low temperatures, root orientation can become horizontal (Box 1996), reducing water and nutrient uptake (Nielsen 1974). As temperatures increase, root angle from the vertical decreases (Sheppard and Miller 1977) and elongation rates may be reduced. Soil amendments act to moderate soil temperatures (Rose et al. 1995) and thus, may improve root architectural development and harvestable root mass.

The incorporation of organic matter into nursery soils also alters soil chemical properties, which may further improve seedling development. Organic matter acts as a slow-release fertilizer, gradually making essential nutrients available to the plant. A small fraction of nitrogen in manure may be in soluble forms and immediately available but most must be released by microbial mineralization of organic compounds (Brady and Weil 1996). Also, organic matter generally has a much greater cation exchange capacity (CEC) than that of various types of clay minerals (Brady and Weil 1996). These materials hold cations in easily exchangeable forms, improving nutrient availability over time (Rose et al. 1995). The buffering capacity of soils also increases with the incorporation of organic matter. This allows soils to resist rapid changes in pH, which may be important when considering the long-term effects of fertilizers. Higher soil buffering capacity may also decrease the incidence of pesticide-induced phytotoxicity (Mader 1956; Davey and Krause 1980).

Organic matter may also serve as a food source for heterotrophic soil organisms (Brady and Weil 1996), which could combat seedling pathogens, nematodes, and insects. The incorporation of organic matter amendments in nursery soils significantly reduced incidence of mortality associated with *Phytophthora spp.* in *Lupinus albus* L. seedlings (Aryantha et al. 2000), *Fusarium spp.* in tomato (Padmodaya and Reddy 1999), and *Rhizoctonia spp.* and *Fusarium spp.* in citrus seedlings (Dhrub et al. 1997). With future regulations on the use of methyl bromide as a fumigant in nursery soils, organic matter may become an important mechanism for disease control, particularly if other benefits associated with seedling growth, root architecture, and irrigation frequency are realized.

The incorporation of soil amendments into nursery soils may manipulate root architecture such that seedling response to outplanting fertilization is enhanced. With improvements in controlled-release fertilizer (CRF) technology, interest in field fertilization has increased in recent years (Haase and Rose 1997). Many researchers have advocated applying CRF directly to the planting hole to facilitate efficient nutrient uptake (Austin and Strand 1960; Carlson 1981; Carlson and Preisig 1981; Gleason et al. 1990; Rose et al. 1991). Seedling response to fertilization in the planting hole may then depend on the ability of the root system to efficiently extract soil nutrients while also providing water to allow seedlings to resist transplant shock. Thus, beneficial modifications to root system development associated with nursery soil amendments may improve the response of seedlings to field fertilization.

This study was designed to gain a better understanding of how the incorporation of various soil amendments into nursery soils affected transplanted Douglas-fir (*Pseudotsuga menzieseii* (Mirb.) seedling quality. We hypothesized that the incorporation of manure, peat, and vermiculite into nursery soils prior to transplant would (i) increase both soil moisture retention and plant water uptake, (ii) beneficially modify root architectural development and whole-plant growth prior to lifting, and (iii) enhance subsequent seedling outplanting performance, particularly when field-fertilized.

2.3 Materials and Methods

2.3.1 Plant Material

Coastal Douglas-fir seedlings (Seed Zones 151/152, Western Forest Tree Seed Council, State of Oregon Tree Seed Zones) were sown at Oregon Department of Forestry's D.L. Phipps Nursery near Elkton, OR (43° 36' lat., 123° 37' long.) in May 1998. The seedlings were grown using standard bareroot nursery procedures until lifting in January 1999. They were then stored at approximately 2°C until being transplanted into study plots in May 1999. Morphological measurements on a sample of 30 randomly selected seedlings indicated that seedlings averaged (±SE) 8.0 (0.3) cm height, 1.75 (0.06) mm caliper, 0.32 (0.02) g shoot dry weight, and 0.19 (0.02) g root dry weight.

2.3.2 Nursery Treatments

Three different types soil amendments were incorporated into study plots. These included a composted poultry manure (ground and sterilized, Pictsweet mushroom farm, Salem, OR), peat mix (5:1:1:1:2 (v:v:v:v:v) peat:pumice:perlite:vermiculite: coco-fiber, Growers Gold®, Pacific Soil), and vermiculite (TRUM#3 – medium grade, Teufel Nursery, Inc.). Each amendment type was applied at low (0.0425 m^3) and high (0.0850 m^3) rates. In addition, there was a control treatment where no material was added. Prior to seedling transplant, nursery beds were divided into seven 3.3 m^2 experimental plots. The seven different soil amendment treatments (three amendments types x two rates + control) were then applied. Soil amendments were distributed evenly across each plot and rototilled into the soil to a depth of 23 cm using a Rotera® tractor.

To investigate the influence of soil moisture on the response of seedlings to the soil amendment treatments, two separate soil moisture experiments were established in

different areas of the nursery. One experiment was irrigated in accordance with operational watering frequency (approximately 0 to -20 kPa) while the second experiment was maintained at a drier soil moisture range (approximately –20 to –40 kPa). Soil moisture could not be replicated due to nursery space considerations but allowed observational assessments to be made. Within each block of each experiment, two soil moisture sensors (Watermark®, Irrometer Co.) were installed into control plots and one sensor was installed into both the high manure and high peat plots. Sensors were randomly positioned within the plot and located at a soil depth of approximately 15-23 cm. Soil moisture was recorded on these plots twice daily during the summer irrigation period (June-September). Irrigation was applied using standard operational nursery equipment when the soil water potential of half of the control plots within an experiment reached the specified minimum for irrigation. Seedlings were root-wrenched in mid-September 1999 at a depth of approximately 35.5 cm.

2.3.3 Nursery Measurements

To investigate how the various soil amendment treatments affected xylem pressure potential (XPP) pre-dawn XPP measurements were recorded prior to irrigation application. Measurements were taken 6 times for both the drier (Aug 10, Aug 25, Sep 9, Sep 16, Sep 23, and Sep 28) and wetter (Aug 10, Aug 27, Sep 9, Sep 16, Sep 21, and Sep 28) experiments. At each sampling, 3 seedlings were randomly selected from each treatment replication and XPP was determined using a pressure bomb (PMS Instruments, Corvallis, OR) (Cleary and Zaerr 1980).

Seedlings were excavated at two times (August 1999 and January 2000) during the nursery portion of the experiment. Twelve randomly selected seedlings from each treatment replication (excluding border trees) were removed using shovels (August) or at lifting (January). Seedlings were measured for height from cotyledon scar to base of terminal bud, diameter at root collar, tap root length, shoot/root volume using the water displacement method (Burdett 1979), and shoot/root dry weight.

A subsample of 3 seedlings per treatment replication were randomly selected for intensive measurements of root morphology. Seedlings were first clipped at the cotyledon scar and then clipped at 6 cm segments along the tap root (Figure 2.1). This created an upper (S1), middle (S2), and lower (S3) root portion, providing a means to determine how the various soil amendment treatments affected root architectural development. Lateral roots were separated from the tap root and the number of active roots (white tips > 0.2 mm), number and length of first-order lateral roots (i.e. lateral roots connected directly to the tap root), proportion of coarse (>0.75 mm) vs. fine (<0.75 mm) roots, and fresh/dry weights of tap and lateral roots were then assessed for each root segment.

To verify that seedlings were physiologically suited for outplanting, 10 randomly selected seedlings in each block were sampled for cold hardiness (Glerum 1984) at two sampling points. Regardless of soil amendment or soil moisture, the LT_{50} was -8°C on September 18, 1999 and –21°C on February 11, 2000.

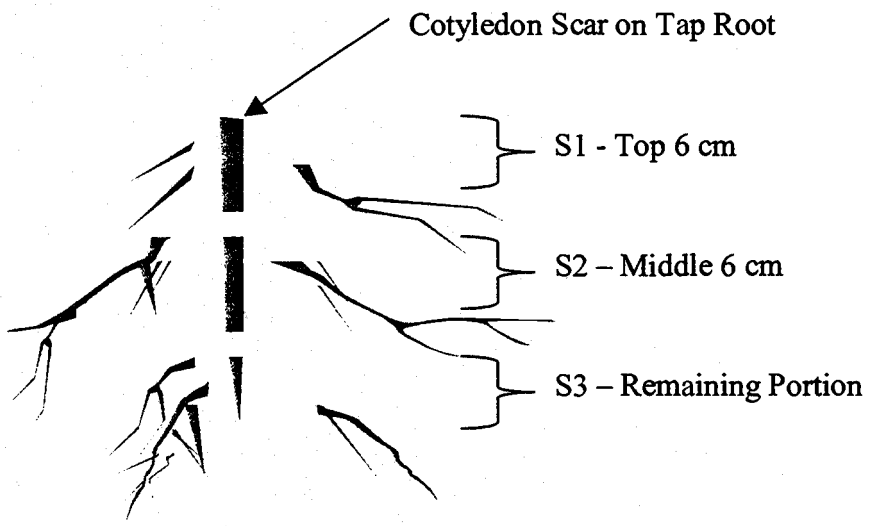

Figure 2.1. Division of root system into three segments for experimental measurements based on the position of lateral roots along the tap root.

2.3.4 Field Trial

Seedlings were lifted in early-January 1999 and 40 randomly selected seedlings (excluding border trees) in each treatment replication were held in 2°C cold storage until outplanted onto a clearcut site in Oregon State University's Paul Dunn Experimental Research Forest (44° 43' lat., 123° 20' long.) on January 19, 1999. This site was located on the eastern edge of the Oregon Coast Range, an area characterized by mild winters with heavy precipitation and hot, dry summers. Pre-plant (August 30, 1999) vegetation was controlled on the site using metsulfuron (Escort®, 0.05 kg/ha), sulfometuron (Oust®, 0.21 kg/ha), and imazapyr (Chopper®, 2.20 l/ha). Subsequent control included hexazinone (Velpar®, March 28, 2000, 7.00 l/ha), clopyralid (Transline®, June 9, 2000, 0.73 l/ha), atrazine (March 30, 2001, 4.93 kg/ha), and triclopyr (Garlon 4®, March 30, 2001, 2.91 l/ha). The objective was to attain maximum vegetation control to minimize sources of variability.

The same experimental design from the nursery portion of the study was maintained in the field. The 40 seedlings in each treatment replication were planted in two rows of 20 seedlings. Each row was randomly assigned to a field-fertilization treatment consisting of either non-fertilized or fertilized with approximately 35 g of Osmocote Plus® (Scotts Co.) 15-9-12 plus micronutrients controlled release fertilizer (CRF) in the planting hole. This CRF was coated with a polymeric-resin and designed to release approximately 80% of nutrients 12-14 months following application under a soil temperature of 21°C.

In early-February 2000 following field planting, all seedlings were measured for height (groundline to base of terminal bud) and caliper. These measurements were again recorded in September 2000 and September 2001.

2.3.5 Fertilizer Release

To determine approximate rates of fertilizer nutrient release over time, a separate experiment was established adjacent to the main outplanting experiments. Twenty-four PVC rings with a 10-cm diameter were covered with a permeable nylon material. Thirty-five g of the same CRF used in the outplanting experiment was weighed and added to the rings. The rings were then buried at the same approximate depth of planting hole fertilization (28 cm). One of the eight rings in each block was excavated from each of three blocks at approximate 6-week intervals during a period of 14 months. Fertilizer from each ring was cleaned of soil debris, dried at 70°C for 72 hr, and weighed.

2.3.6 Experimental Design

Each soil moisture experiment in the nursery portion of the study was arranged as a randomized complete block design with seven treatments and four blocks. Each soil moisture experiment was in a separate nursery area and had a unique pattern of treatment randomization. Each nursery area contained six beds. To limit variation along borders of the nursery area, the first 45 m of only the middle four beds were designated as individual blocks. Treatments were randomly assigned to the seven treatment plots (3 m in length) within each block with buffer zones (also 3 m in length) between each plot. Approximately 400 seedlings were transplanted into each treatment replication plot. At each sampling, the experimental unit was the treatment plot and the sampling unit was the individual seedling that was measured.

The two nursery soil moisture experiments remained separated during the outplanting portion of the study. Each nursery soil moisture experiment maintained the same experimental randomization of treatment replications from the nursery design. For each soil moisture experiment, four uniform areas on the outplanting site were designated as blocks. Two rows of 20 seedlings per row were planted for each treatment replication. The two rows were randomly designated as fertilized or

unfertilized, creating a split-plot randomized complete block design. In an area adjacent to the two main experiments, the separate fertilizer release experiment was established as a randomized complete block design with three blocks and eight experimental units within each block (each representing an excavation date).

2.3.7 Statistical Analysis

Data from each soil moisture experiment and each sampling were subjected to analysis of variance (ANOVA) for a randomized complete block design separately. Tests for normality, linearity, and constant variance were performed, and transformations were made when necessary to ensure the validity of these assumptions. When significant differences were detected among means for any parameter ($p < 0.05$ in F test), Fisher's Protected Least Significant Difference procedure was used to determine significant differences ($\alpha \leq 0.05$) among the soil amendment treatments. In the outplanting portion of the study, the split-plot randomized complete block design was subjected to ANOVA and the main effects of soil amendment or fertilization were assessed only in the absence of a significant ($p < 0.05$ in F test) soil amendment x fertilization interaction. SAS® software (SAS Institute, Inc., Cary, NC, USA) was used for the analysis of all data.

2.4 Results

2.4.1 Soil Moisture and Plant Moisture Stress

Daily assessments of nursery soil water potential for the control, high rate of manure, and high rate of peat plots provided curves during the summer irrigation period for each experiment (Figure 2.2 a,b). In general, the control had a lower mean soil water potential (i.e. drier) than the high peat treatment, which had a lower mean soil

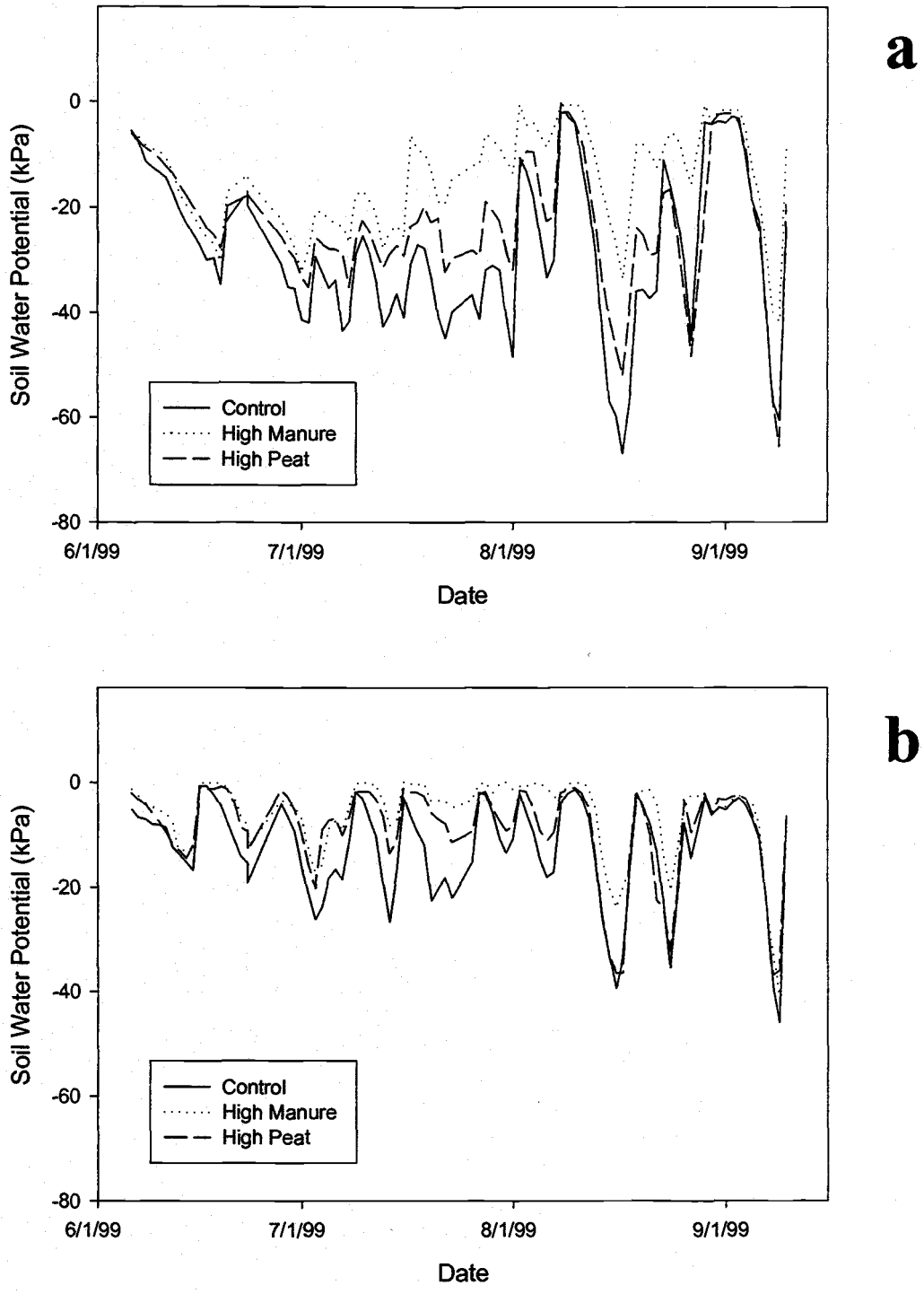

Figure 2.2. Soil moisture patterns for the control, high peat, and high manure treatments over time for the (a) drier and (b) wetter soil moisture experiments.

water potential than the high manure plots. Statistically significant differences were detected on August 17 ($p=0.0136$) and August 27 ($p=0.0013$) for the drier experiment. On August 17, mean (± 5.4 SE) soil water potential for the control (kPa) was –66.9, significantly lower than the –33.5 mean for high manure, while the –52.0 mean for high peat did not differ from either treatment. Means for the control (-45.3) and high peat (-48.4) did not differ among each other but were significantly lower than the mean for high manure (-15.6) on August 27 (± 3.7 SE). Despite the differences in soil moisture content, no significant differences or meaningful trends were detected for pre-dawn XPP at any sampling point for either experiment (Tables 2.1 and 2.2).

2.4.2 Whole Plant Morphology

All whole plant morphological parameters did not differ statistically for all treatments for both experiments at each sampling point (Table 2.3). At the August 1999 sampling in the wetter soil moisture experiment, mean root volume ($p=0.0564$) and diameter ($p=0.0690$) of control seedlings tended to be greater than that of any other soil amendment treatments, but differences were negligible in January 2000 (root volume $p=0.8545$, diameter $p=0.6132$). For the wetter soil moisture experiment, means for height (August $p=0.8087$, January $p=0.9801$), tap root length ($p=0.8640$, 0.9420), and shoot volume ($p=0.4476$, 0.9553) are shown in Table 2.3. Means for height (August $p=0.7097$, January $p=0.4400$), diameter ($p=0.6772$, 0.4742), tap root length ($p=0.3740$, 0.1602), shoot volume ($p=0.8212$, 0.3662), and root volume ($p=0.4509$, 0.7207) are also shown for the drier experiment in Table 2.3.

Mean shoot:root volume of seedlings did not differ statistically for the wetter soil moisture experiment in both August ($p=0.3735$) and January ($p=0.4647$). Similarly, no pattern in mean shoot:root volumes was observed for the drier soil moisture experiment in August ($p=0.9732$) or January ($p=0.5100$) (Figure 2.3). For each experiment and all treatments, mean shoot:root volume decreased from August to January (Figure 2.3).

Table 2.1. Mean values and standard error (SE) for pre-dawn xylem pressure potential (MPa) sampled at six points during the 1999 irrigation period for the drier soil moisture experiment. No differences were detected among treatments for any sampling at $\alpha \leq 0.05$.

Soil Amendment	Sampling Date					
Treatment	8-10	8-25	9-9	9-16	9-23	9-28
Control	-0.263	-0.325	-0.617	-0.392	-0.529	-0.546
Low Manure	-0.288	-0.354	-0.821	-0.400	-0.563	-0.525
High Manure	-0.300	-0.308	-0.567	-0.379	-0.517	-0.529
Low Peat	-0.279	-0.317	-0.608	-0.358	-0.521	-0.617
High Peat	-0.250	-0.313	-0.658	-0.379	-0.533	-0.654
Low Vermiculite	-0.246	-0.375	-0.592	-0.383	-0.538	-0.600
High Vermiculite	-0.279	-0.333	-0.571	-0.354	-0.529	-0.671
SE	-0.016	-0.025	-0.077	-0.018	-0.027	-0.056

Table 2.2. Mean values and standard error (SE) for pre-dawn xylem pressure potential (MPa) sampled at six points during the 1999 irrigation period for the wetter soil moisture experiment. No differences were detected among treatments for any sampling at $\alpha \leq 0.05$.

Soil Amendment	Sampling Date					
Treatment	8-10	8-27	9-9	9-16	9-21	9-28
Control	-0.275	-0.571	-0.663	-0.396	-0.338	-0.600
Low Manure	-0.254	-0.571	-0.733	-0.396	-0.300	-0.558
High Manure	-0.267	-0.588	-0.7000	-0.383	-0.313	-0.533
Low Peat	-0.283	-0.496	-0.696	-0.418	-0.329	-0.567
High Peat	-0.263	-0.500	-0.725	-0.44	-0.346	-0.533
Low Vermiculite	-0.263	-0.454	-0.742	-0.418	-0.325	-0.571
High Vermiculite	-0.250	-0.475	-0.650	-0.396	-0.333	-0.504
SE	-0.017	-0.039	-0.039	-0.017	-0.022	-0.032

Table 2.3. Morphology of seedlings in different nursery soil amendment treatments for each soil moisture experiment following excavation in August 1999 and January 2000. All parameters were nonsignificant for both experiments at $\alpha \leq 0.05$.

Soil Amendment Treatment	*Drier Soil Moisture Experiment*									
	Height (cm)		Diameter (mm)		Tap Root (cm)		Shoot Volume (cm^3)		Root Volume (cm^3)	
	Aug	Jan	Aug	Jan	Aug	Jan	Aug	Jan	Aug	Jan
Control	20.4	23.7	3.48	5.15	13.9	13.9	9.2	13.6	5.6	14.3
Low Manure	22.5	26.0	3.76	5.45	14.6	13.8	10.0	11.6	6.0	15.3
High Manure	20.6	25.8	3.57	5.47	14.5	14.1	9.2	11.7	5.2	16.1
Low Peat	20.8	24.5	3.59	5.35	14.1	14.6	8.4	13.4	5.4	14.4
High Peat	20.9	25.7	3.29	5.13	14.3	14.2	9.1	13.0	6.0	15.8
Low Vermiculite	21.4	27.3	3.45	5.29	14.3	14.8	9.8	13.3	6.4	16.3
High Vermiculite	20.7	25.2	3.27	5.36	14.4	14.4	8.9	14.1	5.8	15.4
SE	0.89	1.30	0.21	0.14	0.22	0.28	0.78	1.47	0.42	0.96

Soil Amendment Treatment	*Wetter Soil Moisture Experiment*									
	Height (cm)		Diameter (mm)		Tap Root (cm)		Shoot Volume (cm^3)		Root Volume (cm^3)	
	Aug	Jan	Aug	Jan	Aug	Jan	Aug	Jan	Aug	Jan
Control	23.0	25.7	4.32	5.16	14.2	13.4	13.6	16.7	8.4	14.1
Low Manure	21.3	24.9	3.61	5.24	14.5	13.9	11.6	18.2	5.9	14.5
High Manure	21.9	26.0	3.82	5.40	13.8	13.6	11.7	17.6	5.9	13.6
Low Peat	23.1	26.9	4.04	5.35	14.1	14.0	13.4	19.6	7.2	13.8
High Peat	22.3	25.8	3.95	4.82	14.4	13.9	13.0	17.5	6.4	12.4
Low Vermiculite	22.5	25.9	3.96	5.07	14.6	13.9	13.3	18.5	6.7	13.8
High Vermiculite	23.3	25.4	4.05	5.21	14.1	13.7	14.1	18.0	6.4	13.6
SE	1.04	1.42	0.14	0.22	0.42	0.40	0.95	1.87	0.55	0.99

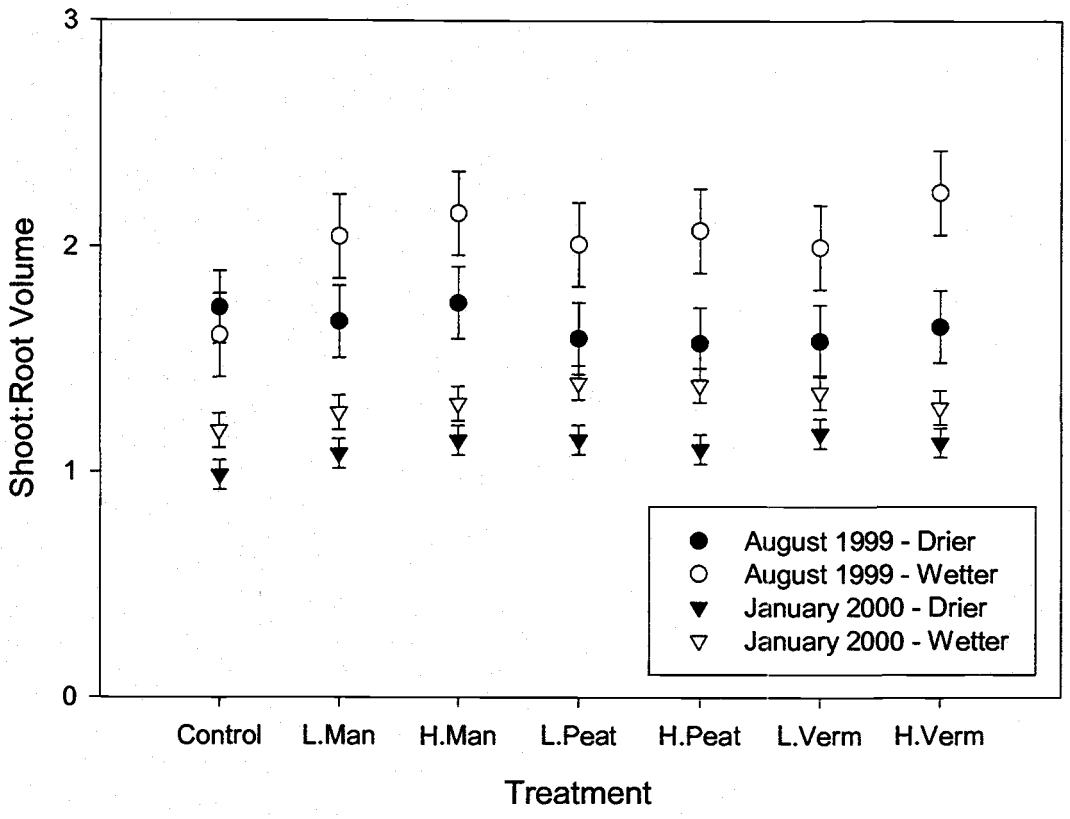

Figure 2.3. Mean shoot:root volume (±SE) for seedlings in different soil amendment treatments in each soil moisture experiment following the August 1999 and January 2000 samplings.

2.4.3 Root Architectural Development

Significant differences in mean total first-order lateral root length were detected in August 1999 for the wetter soil moisture experiment (p=0.0304) (Table 2.4). Mean total root length of seedlings in the high manure treatment was less than half that for seedlings in the high vermiculite treatment. Mean total lateral root length in the low manure treatment was also noticeably reduced compared to that of other treatments. Significant differences were also detected in August 1999 for mean first-order lateral root length along the lowest portion (S3) of the taproot (p=0.0119) in the wetter soil

Table 2.4. Mean root architecture parameters and standard error for the different soil amendment treatments at two sampling points and for each soil moisture experiment. Significant differences were only found for total first-order lateral root length in August 1999 for the wetter soil moisture experiment. Means with similar letters in this column did not differ significantly at $\alpha \leq 0.05$.

Soil Amendment Treatment	*Drier Soil Moisture Experiment*					
	Total Number of First-Order Lateral Roots		Total First-Order Lateral Root Length (cm)		Total Number of Active Root Tips	
	Aug 99	Jan 00	Aug 99	Jan 00	Aug 99	Jan 00
Control	18.1	19.1	190.6	309.7	26.9	80.7
Low Manure	15.8	18.2	160.6	291.0	27.9	83.4
High Manure	15.6	20.3	169.3	333.0	21.3	109.0
Low Peat	17.5	18.6	189.3	304.3	22.25	90.5
High Peat	19.3	22.9	233.8	388.8	32.8	97.3
Low Vermiculite	18.5	19.7	222.3	333.5	28.7	95.0
High Vermiculite	18.0	20.4	207.7	314.5	22.5	91.7
SE	1.62	1.67	21.02	35.06	5.33	10.12

Soil Amendment Treatment	*Wetter Soil Moisture Experiment*					
	Total Number of First-Order Lateral Roots		Total First-Order Lateral Root Length (cm)		Total Number of Active Root Tips	
	Aug 99	Jan 00	Aug 99	Jan 00	Aug 99	Jan 00
Control	18.1	19.4	220.5 ab	286.3	41.9	91.4
Low Manure	16.3	19.8	183.3 bc	275.5	27.3	87.8
High Manure	14.8	19.3	134.9 c	259.1	25.0	96.7
Low Peat	21.9	21.8	256.3 a	345.8	29.3	97.7
High Peat	19.3	21.7	211.3 abc	297.8	21.3	87.6
Low Vermiculite	19.0	19.0	239.5 ab	320.1	34.8	89.3
High Vermiculite	25.4	19.5	270.6 a	303.8	31.4	92.6
SE	2.85	1.90	27.67	31.54	5.42	8.33

moisture experiment (Figure 2.4). The control and high manure treatments had the lowest mean lateral root length in S3. In January 2000, mean total first-order lateral root length was not statistically different (p=0.5609). Also in the wetter experiment, the mean total number of first-order laterals did not differ statistically in August (p=0.1839) or January (p=0.8887) (Table 2.4). Mean total numbers of active root tips did not differ in August (p=0.1929) or January (p=0.9582) for the wetter experiment.

For the drier soil moisture experiment, mean total first-order lateral root length did not differ statistically among treatments in August (p=0.1990) or January (p=0.5119). The mean total number of first-order lateral roots also did not differ statistically among treatments in August (p=0.6380) or January (p=0.5205). Total numbers of active root tips did not differ in August (p=0.7070) or January (p=0.5429) among treatments. Lateral root fresh weight in S3 in January 2000 differed among treatments (p=0.0227) (Figure 2.5). The low and high vermiculite treatments had the greatest mean lateral root fresh weight. The majority of other intensive measurements of root morphology in relation to the position of the tap root were non-significant among treatments for each soil moisture experiment (Appendix 1, Tables 3-6).

2.4.4 Field Performance

In the outplanting portion of the study, no significant soil amendment x fertilizer interactions were detected for any parameter in either experiment. Fertilization at the time of outplanting affected seedling height and stem diameter growth over time for both the drier (Table 2.5) and wetter (Table 2.6) soil moisture experiments. Following the first growing season, height growth was significantly greater for fertilized than unfertilized seedlings in both the drier (p=0.0336) and wetter (p=0.0368) soil moisture experiments. Diameter growth was not significantly different for the drier (p=0.8893) or wetter soil moisture experiments (p=0.5506) following the first growing season.

During the second growing season, however, fertilized seedlings had significantly less height (p<0.0001) and stem diameter (p=0.0015) growth than that of

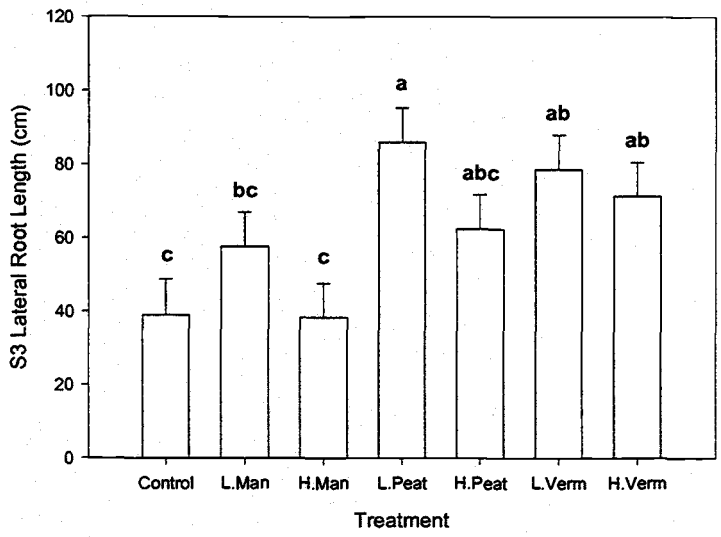

Figure 2.4. Mean first-order lateral root on the lower tap root portion (S3) and standard error for seedlings in different soil amendment treatments in the wetter soil moisture experiment following excavation in August 1999. Treatments with the same letter did not differ significantly at $\alpha \leq 0.05$.

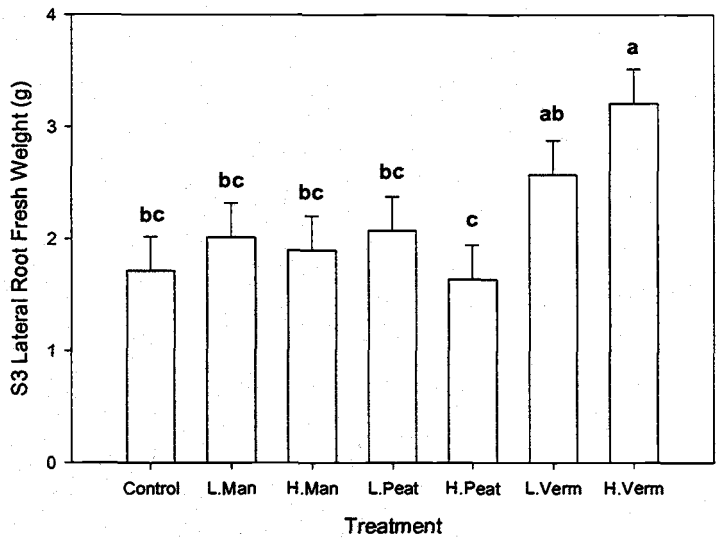

Figure 2.5. Mean lateral root fresh weight on the lower tap root portion (S3) and standard error for seedlings in different soil amendment treatments in the drier soil moisture experiment following excavation in January 2000. Treatments with the same letter did not differ significantly at $\alpha \leq 0.05$.

Table 2.5. Field morphology of seedlings for the drier nursery irrigation experiment in different nursery soil amendment treatments (averaged over fertilizer treatments) and field fertilization treatments (averaged over soil amendment treatments) following planting. For field fertilization treatments, means for each parameter within a column followed by the same letter did not differ significantly at $\alpha \leq 0.05$. All parameters for were nonsignificant for soil amendment treatments.

		Initial Height (cm)	2000 Height Growth (cm)	2001 Height Growth (cm)	Total Height in 2001 (cm)	Initial Diameter (mm)	2000 Diameter Growth (mm)	2001 Diameter Growth (mm)	Total Diameter Growth (mm)
Soil Amendment Treatment	Control	25.8	14.9	33.0	72.3	5.47	3.49	7.51	16.35
	Low Manure	27.0	13.9	38.5	79.5	5.63	2.78	7.73	16.12
	High Manure	24.3	18.7	39.0	80.4	5.46	4.40	8.46	18.14
	Low Peat	26.9	16.2	36.5	78.8	5.42	3.89	9.01	18.35
	High Peat	27.5	16.2	38.7	81.0	5.92	3.93	7.99	17.66
	Low Vermiculite	27.4	13.2	33.3	73.6	5.48	3.39	8.07	16.81
	High Vermiculite	25.4	15.8	32.5	73.2	5.34	3.79	7.61	16.73
	SE	0.85	1.99	4.40	5.47	0.14	0.42	0.73	1.03
Fertilization Treatment	Fertilized	26.3 a	16.3 a	32.6 b	74.3 b	5.58 a	3.68 a	7.55 b	16.70 b
	Non-Fertilized	26.4 a	14.8 b	39.2 a	79.6 a	5.48 a	3.66 a	8.56 a	17.64 a
	SE	0.38	1.74	3.46	4.58	0.39	0.27	0.54	0.81

Table 2.6. Field morphology of seedlings for the wetter nursery irrigation experiment in different nursery soil amendment treatments (averaged over fertilizer treatments) and field fertilization treatments (averaged over soil amendment treatments) following planting. For field fertilization treatments and total height in 2001 for soil amendment treatments, means for each parameter within a column followed by the same letter did not differ significantly at $\alpha \leq 0.05$. All parameters expect total height in 2001 were nonsignificant for soil amendment treatments.

		Initial Height (cm)	2000 Height Growth (cm)	2001 Height Growth (cm)	Total Height in 2001 (cm)	Initial Diameter (mm)	2000 Diameter Growth (mm)	2001 Diameter Growth (mm)	Total Diameter in 2001 (mm)
Soil Amendment Treatment	Control	27.1	15.5	37.8	80.5 b	5.44	4.09	9.56	19.18
	Low Manure	27.3	15.4	43.0	84.4 ab	5.53	3.69	9.98	18.94
	High Manure	26.7	16.1	41.4	84.4 ab	5.55	4.08	9.92	19.49
	Low Peat	28.6	18.7	44.0	91.2 a	5.53	4.97	10.40	20.80
	High Peat	28.5	18.5	42.9	90.5 a	5.50	5.26	10.20	21.05
	Low Vermiculite	26.8	20.1	42.9	90.4 a	5.20	5.45	10.75	21.42
	High Vermiculite	29.0	15.5	46.1	90.3 a	5.56	4.45	10.23	20.28
	SE	0.77	1.87	3.14	4.24	0.15	0.67	0.73	1.35
Fertilization Treatment	Fertilized	27.6 a	18.0 a	39.6 b	85.5 a	5.44 a	4.63 a	9.80 a	19.85 a
	Non-Fertilized	27.8 a	16.2 b	45.5 a	89.3 a	5.50 a	4.51 a	10.49 a	20.48 a
	SE	0.36	1.44	2.48	3.68	0.08	0.54	0.61	1.17

unfertilized seedlings in the drier experiment (Table 2.5). This effect was similar in the wetter soil moisture experiment for height growth (p=0.0024), though marginal for diameter growth (p=0.0510) (Table 2.6). Reduced growth during 2001 for fertilized seedlings resulted in less total height (p=0.0016) and diameter (p=0.0260) for fertilized seedlings following the two growing seasons in the drier soil moisture experiment (Table 2.5). In the wetter experiment, means of fertilized seedlings also tended to be less for total height (p=0.0549) and total diameter (p=0.1821) following the two growing seasons (Table 2.6).

In the drier soil moisture experiment, soil amendment treatments did not differ in height (p=0.1177) or stem diameter (p=0.0884) at the time of planting (Table 2.5). Though height (p=0.0555) and diameter (p=0.1260) growth during 2000 did not differ significantly in the drier experiment, means tended to be highest for the high manure treatment (Table 2.5). Height (p=0.5020) and diameter (p=0.5122) growth were similar in 2001 for the drier soil moisture experiment as were total height (p=0.3182) and total diameter (p=0.2270) at the end of 2001.

Soil amendment treatments also did not differ in the wetter soil moisture experiment for initial height (p=0.6363) or diameter (p=0.4638) (Table 2.6). Height (p=0.1152) and diameter (p=0.0804) growth during 2000 and 2001 height (p=0.3239) and diameter (p=0.6743) growth also did not differ significantly (Table 2.6). However, total height (p=0.0381) was significantly different among soil amendment treatments for the wetter experiment with the control treatment having the lowest mean height and peat and vermiculite treatments having the greatest mean total heights (Table 2.6). Total diameter in 2001 did not differ among treatments (p=0.2052).

2.4.5 Fertilizer Release

Estimates of fertilizer release based on residual fertilizer material indicated that the majority of fertilizer release occurred during the first 6 months following application (Figure 2.6). The initial 35 g appeared to stabilize at approximately 12 g of residual fertilizer material.

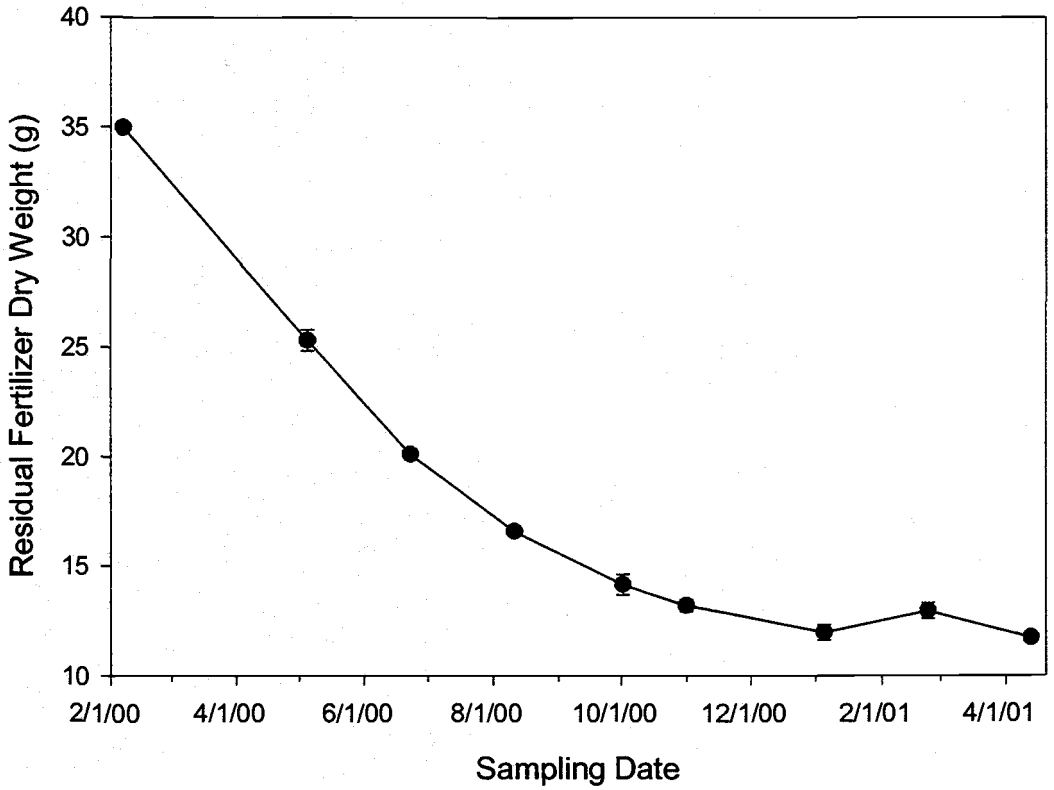

Figure 2.6. Mean fertilizer release (± standard error) for different sampling points during a 14-month period following application based on dry weight of residual fertilizer material.

2.5 Discussion

2.5.1 Soil Moisture and Plant Moisture Stress

Both experiments showed trends for an increase in soil moisture content with the addition of peat or manure at the high rate as compared to the control. The high moisture holding capacities of these materials prevented water from draining. Though it is widely documented that organic material increases soil water retention (Rose et al. 1995; Brady and Weil 1996; Havlin et al. 1999), effects on plant water uptake are less

well known. In this experiment, the increased water content associated with the addition of organic matter did not translate to increased plant water uptake.

The water in these materials may have been held tightly in a form that was unavailable for plant uptake. It was particularly surprising that no difference in XPP was found between the control and high manure treatments, considering the variation in soil water potential. This may have been associated with the stage of decomposition of the manure. When organic matter has fully decomposed to humus, more water is generally available to plants, but when not fully decomposed, slightly less water is available particularly in coarse-textured soils (Rose et al. 1995). Though consisting of highly-decomposed plant material, the peat also did not show differences in plant water uptake. This may have been due to the less pronounced differences in soil water potential between the control and peat. It should be noted that seedlings in either experiment were never truly drought stressed and there may have been differences in XPP at soil water potentials lower than those used in this experiment. However, this irrigation level would probably be considered excessively dry for operational nursery use.

2.5.2 Whole Plant Morphology

The substantial reductions in shoot:root ratios for each soil moisture experiment from August 1999 to January 2001 was related to the seasonal patterns of shoot vs. root growth. Root growth slows during the summer period of shoot growth, but accelerates in fall following the cessation of shoot growth (Cleary et al. 1978).

Mean root volume tended to be highest for the control treatment in August for the wetter soil moisture experiment. This may have been due to a higher CEC in soil amendment treatments increasing nutrient availability and limiting the need for root expansion under well watered-conditions. It may also have been associated with greater soil penetrability in soil amendment treatments, as seedlings in the control may have needed more root mass to penetrate and exploit the soil. Several possible explanations may help to explain the failure to find numerous significant differences in whole-plant

growth. The soil amendment application rates may have been too low to elucidate a response, sample sizes may have been insufficient, or the nursery soils of the control treatment may have provided conditions for optimal plant growth.

2.5.3 Root Architectural Development

Mean first-order lateral root length differed significantly among soil amendment treatments in the wetter soil moisture experiment at the August 1999 sampling. Soil toxicities may have acted to reduce the lateral root length of seedlings in the high manure treatment to the lowest mean value of any treatment. Manure tends to rapidly release NH_4^+ during the first stages of decomposition, with release gradually tapering off (Davey 1984; Rose et al. 1995). During this short period of rapid decomposition, toxicities and the depletion of soil O_2 may negatively affect plant root growth (Davey 1984). The manure was applied during spring and the warm period under frequent irrigation leading up to the August sampling created conductions conducive to rapid decay. The application of poultry manure resulted in less root mass than controls for rice seedlings (Eneji et al. 2001). The similar root length at the January 2000 sampling indicated that soil conditions no longer restricted root growth. Kaplan and Noe (1993) found that manure initially suppressed but later enhanced root weight of tomatoes. Peat consists of materials that have already undergone initial stages of humification, thus its breakdown is slow and non-toxic to roots. Lateral root length was greatest for the high vermiculite treatment likely due to the increased soil penetrability that this material provides.

Lateral root length on the distal portion (S3) of the tap root was greatest in the vermiculite and peat treatments and lowest in the control and manure treatments at the August 1999 sampling in the wetter soil moisture experiment. Explanations for this response are likely similar to those for the differences in lateral root length. Inhibitions associated with the initial breakdown of manure may have limited root penetrability deeper into the soil profile whereas vermiculite and peat materials facilitated penetration of roots into the subsurface. The significantly greater root biomass on S3 of

the vermiculite treatment in January 2000 for the drier soil moisture experiment was also likely due to the greater ability of roots grown in this material to penetrate into the subsoil.

2.5.4 Field Performance

The significantly greater height growth during the first growing season of fertilized as compared to non-fertilized seedlings (averaged over soil amendment treatments) was likely due to stimulation of shoot growth by the fertilizer. Numerous studies have shown increased aboveground growth immediately following planting for Douglas-fir with field fertilization in the planting hole (Austin and Strand 1960; Brix and Ebell 1969; Carlson and Preisig 1981; McClarnon 1999). The reduction in mean height and diameter growth during the second growing season may have been associated with drought stress.

Field fertilization of Douglas-fir may result in increased shoot growth relative to root growth (Carlson and Preisig 1981). During the summer dry period, seedlings must then supply water to a greater transpirational leaf area with a proportionally smaller root system, causing reductions in xylem pressure potential (increased drought stress). This may force stomata to close to conserve water (Meinzer 1982), limiting photosynthetic capacities and reducing growth. Release of polymer-coated CRF is largely temperature-dependent, with moisture content having little influence on release (Kochba et al. 1990). Thus, fertilizer nutrients were released into the soil solution during the summer when seedlings were not actively growing, as evidenced by the continued reduction in residual fertilizer dry weight. A decrease in soil osmotic potential within the root zone may have intensified drought stress during this period.

The significantly greater total height at the end of 2001 for seedlings grown under peat and vermiculite as compared to the control in the wetter experiment may have been due to enhanced seedling nutrition associated with the high CEC of these materials. The failure to detect additional significant differences in field growth for seedlings in the various soil amendment treatments was probably due to the lack of

differences in whole-plant morphology and minimal differences in root architecture at the time of lifting. Even substantial modifications in root development of Douglas-fir seedlings at the time of lifting have not always translated to significant differences in seedling growth in the field (Stein 1984; Hobbs et al. 1987).

A potential limitation to the field portion of this experiment should be noted. The chemical preparation of the site with metsulfuron, sulfometuron, and imazapyr may negatively affect Douglas-fir seedling growth due to the persistence of herbicide residues in the soil (Cole and Newton 1989; Vizantinopoulos and Lolos 1994). However, significant time passed (5 months) between the point of application and planting. Seedlings remained dormant for at least 2.5 additional months (7.5 months since application). Herbicide persistence is dependant upon soil type (Vizantinopoulos and Lolos 1994) and during this time, the high clay and organic matter content of the site acted to bind the herbicide and initiate breakdown, reducing potential for seedling injury. Douglas-fir was not damaged following site preparation with imazapyr at rates similar to that used in this experiment (Belz and Nishimura 1989). Few experiments have investigated effects of metsulfuron and sulfometuron applied during site preparation on subsequent performance of Douglas-fir seedlings, though Coate (2000) reported no damage with either herbicide applied at operational rates. Additionally, seedling survival on both experiments exceeded 94% following two growing seasons. Finally, these herbicides were applied as evenly as possible throughout the entire site, minimizing any chance for treatment confounding. This type of potential "confounding" is an inherent risk in the establishment of any field experiment. Many factors across a site (e.g. soil type, mechanical site preparation, microsite variations, presence of mycorrhizal mats, emergence of competing vegetation, etc.) cannot be completely controlled. In this instance, it appears likely that the relative responses to treatments will be proportional to those observed where such residues are absent, hence the findings are likely to be valid.

2.6 Conclusions

The application of high rates of peat and manure increased soil water potential as compared to the control but did not appear to enhance rates of plant water uptake or shoot growth. Thus, the incorporation of organic matter into forest nursery soils should not dismiss the need for consistent irrigation. Regardless of soil amendment treatment, seedlings in each soil moisture experiment had similar shoot:root ratios at the time of lifting.

Under well-watered conditions, the addition of peat and vermiculite into nursery soils may improve subsequent seedling growth in the field. The application of vermiculite appeared to increase root proliferation into the lower soil profile to some degree but this effect is of questionable benefit as these roots may be lost during subsequent wrenching and lifting operations. The incorporation of manure under frequent irrigation acted to decrease root length initially, but differences were no longer evident at the time of lifting. Thus, the addition of manure in nursery soils should not impact harvestable root mass and may likely improve the ability of seedlings to resist soil-borne diseases.

Field fertilization initially enhanced and then reduced seedling growth in the field. This was likely due to the greater susceptibility of fertilized seedlings to moisture stress on this drought-prone site. To attain a positive response from field fertilization with polymer-coated CRF, the anticipated drought level of the site must be considered. Conservative fertilizer rates and the use of fertilizers with moisture-dependent release characteristics (e.g. isobutylidene diurea, ureaformaldehyde) may improve seedling response to fertilization at planting on droughty sites.

The benefits to outplanting performance associated with adding soil amendments to nursery soils were not strongly conclusive in this experiment. However, no negative effects were detected and the potential advantages of soil amendments in nursery operations may justify their use.

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Chapter 3

Development of Douglas-fir Seedling Morphology, Physiology, and Root Architecture in Response to Different Polymer-Coated Controlled-Release Fertilizers

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3.1 Abstract

The objective of this experiment was to determine how different types and rates of two locally applied controlled-release fertilizers (CRF) affected seedling growth, root architectural development, and photosynthetic capacities over time. Three months following sowing, coastal Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) seedlings were transplanted into pots with Osmocote Plus® 15-9-12 and Simplot® 16-5-9 CRF applied at four rates (including a control) as a single uniform layer beneath the root system. Regardless of CRF type, seedlings allocated a greater number of active roots to the soil profile above the fertilizer layer and less below the fertilizer layer with increasing CRF rate. Fertilizer rate did not significantly affect seedling growth over time but seedlings fertilized with Osmocote grew faster during the first 4 months following transplant than those fertilized with Simplot. Growth differences between CRF types were negligible at 9 months, indicating that seedlings fertilized with Simplot grew more rapidly during the second half of the experiment. Nutrient concentrations and contents were relatively similar among fertilizer rates. After 4 months, N concentration was higher in seedlings fertilized with Simplot but content was the same indicating that N was diluted in faster-growing Osmocote seedlings. Maximal chlorophyll fluorescence (Fms) was initially higher in seedlings fertilized with Osmocote but by the end of the experiment, Fms was higher in seedlings fertilized with Simplot. This corresponded well with the growth pattern of seedlings over time. These results indicated that localized CRF application acts to modify Douglas-fir root architectural development and that different CRF types may have variable temporal patterns of nutrient release, which may affect seedling growth and physiological development over time.

3.2 Introduction

The goal of reforestation projects is to produce quality seedlings with target characteristics (Rose et al. 1990) that attain maximum biological potential after

outplanting into the field. Interest in controlled released fertilizers (CRF) has increased for tree seedlings as a method to improve seedling performance in the nursery and the field (Donald 1991; Haase and Rose 1997). CRF offer the advantage that a single application can supply seedlings with adequate nutrition throughout a growing season with less chance for seedling damage or nutrient loss through leaching as compared to conventional water-soluble fertilizers (Hauck 1985; Donald 1991). Controlled-release fertilizer use with forest tree seedlings is far from a precise science (van den Driessche 1997), however, and inconsistencies in seedling response to fertilizers in the nursery and field illustrates the need for more process-oriented research to improve our understanding of the mechanisms by which fertilizers affect seedling development (Brockley 1988).

Many different CRF are currently marketed for use with forest tree seedlings. Controlled-release fertilizers primarily differ in terms of their nutrient formulations, coatings, and estimated times for nutrient release. The majority of CRF used in containerized nursery plant production are polymer-coated (Huett and Gogel 2000) and release rates are controlled by changing the physical characteristics of the coating, either the thickness or the nature of the polymer itself (Benson 1997). CRF coating materials may include polymeric resins (Osmocote®, Scotts Co.), polyurethane (Polyon®, Simplot), and polyolefin (Nutricote®, Plantco). Nutrient release of polymer-coated CRF is determined by the diffusion of water through the membrane, which is temperature dependent (Kochba et al. 1990). Moisture content of media between 50 and 100% field capacity has little effect on nutrient release (Kochba et al. 1990). Release rates are based on manufacturer's estimates and different CRF types may in fact release at very different rates despite equivalent estimated times for release (Huett and Gogel 2000). In a study where 3-4 month polymeric-resin Osmocote was used, the most rapid N and K release occurred within the first 2 wk after potting when plant demand was low, thus resulting in significant nutrient leaching (Huett 1997a; Huett 1997b). Differences in release rates of comparable CRF types and their effects on seedling development are poorly documented. The effective use of CRF in seedling production requires a better understanding of the influence of different CRF types on seedling growth and physiology over time.

One potential method for examining the effects that the use of different CRF types might have on seedling physiological development is chlorophyll fluorescence. Chlorophyll fluorescence provides a non-invasive and non-destructive method to evaluate plant physiological status (Vidaver et al. 1989). Changes in the physiological status of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) due to dormancy (Hawkins and Lister 1985; Roberts et al. 1991), freezing stress (Fisker et al. 1995), and shading (Khan et al. 2000) have been assessed using chlorophyll fluorescence. Chlorophyll fluorescence parameters were more closely correlated to stem volume increment than photosynthetic rate or root growth potential for jack pine (*Pinus banksiana* Lamb.) and black spruce (*Picea mariana* (Mill.) B.S.P.), (Mohammed et al. 1997). The use of chlorophyll fluorescence for assessing physiological changes in forest tree seedlings associated with fertilization has been limited. Chlorophyll fluorescence was used to detect Cu and P deficiencies in Douglas-fir (Vidaver et al. 1988) and Monterey pine (*Pinus radiata* D. Don) (Lopez Gorge et al. 1985; Conroy et al. 1986). Birchler et al. (2001) found that nursery seedlings fertilized with N and K in fall had higher rates of chlorophyll fluorescence (Fv/Fm) than unfertilized seedlings. Chlorophyll fluorescence may provide a means to detect changes in photosynthetic capacities over time associated with different CRF treatments.

In addition to affecting seedling growth and physiological development, CRF may also modify seedling root architectural development, particularly when placed in localized portions of soil. Roots tend to proliferate in areas of high nutrient concentration and this effect is most pronounced when the remainder of the plant is provided with an inadequate supply (Friend et al. 1990). The proliferation of roots in zones of localized supplies of nutrients in solution has been shown for barley (Drew 1975; Drew and Saker 1975), Sitka spruce (*Picea sitchensis* (Bong.) Carr.) (Coutts and Philipson 1976), lodgepole pine (*Pinus contorta* Dougl. ex. Loud.) (Coutts and Philipson 1977), maize (*Zea mays* L.) (Granato and Raper 1989) and Douglas-fir (Friend et al. 1990). Few experiments have examined the effects on root proliferation of localized placement of CRF, particularly for tree seedlings. Roots of Douglas-fir (Carlson and Preisig 1981) and western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) (Carlson 1981) did not preferentially grow in areas of localized CRF placement during

outplanting. Under controlled conditions, proliferation of white spruce (*Picea glauca* (Moench) Voss) roots in localized zones of CRF supply was dependent on soil substrate (Krasowski et al. 1999).

Several researchers have recommended applying fertilizers directly to the planting hole in field plantings to facilitate efficient nutrient uptake (Austin and Strand 1960; Carlson 1981; Carlson and Preisig 1981; Gleason et al. 1990; Rose et al. 1991). Roots then grow down through the fertilizer layer during the critical establishment period. Changes in root architectural development due to the presence of localized fertilizer supply could have significant effects on seedling morphological and physiological development. For instance, root proliferation in upper soil zones of high nutrient concentration may affect the ability of roots to access subsoil water as soils dry. Modifications to root architecture may also affect the ability of seedlings to extract soil-immobile nutrients. Thus, a better understanding of how localized sources of CRF modify root architecture and subsequent effects on seedling development is needed.

The primary objective of this study was to assess the influence of two polymer-coated CRF types with different nutrient release technologies and a range of CRF rates on Douglas-fir seedling morphological and physiological development. We hypothesized that the application of different CRF types and rates applied as a single layer beneath the transplanted root system would affect (i) seedling whole-plant morphological development over time, (ii) chlorophyll fluorescence, (iii) nutrient uptake, and (iv) root architectural development in relation to the placement of the locally-applied CRF.

3.3 Materials and Methods

3.3.1 Plant Material

Douglas-fir seeds (seed zone 274, Western Forest Tree Seed Council, State of Oregon Tree Seed Zones) were sown into containers with 39 cm^3 cavities in March

1999 at The Timber Company's nursery near Cottage Grove, OR. Seedlings were grown under standard nursery cultural practices until being lifted and transplanted into pots in mid-June 1999. Initial measurements of height and diameter taken at the time of lifting indicated that seedlings had a mean height (±SE) of 5.1 (0.2) cm and a mean stem diameter of 1.05 (0.02) mm.

3.3.2 Treatments

Following measurement, seedlings were transplanted into cylindrical pots 30.5 cm in length and 10.2 cm in diameter (Figure 3.1). A metal screen for drainage was installed 1 cm from the bottom of the pots. Pots were filled to a depth of 13 cm with a 4:4:1:1 (v:v:v:v) peat:composted plant material:pumice:perlite (Organic mix, Pacific Soil). Two different CRF types (Scott's Osmocote Plus® 15-9-12 and Simplot® 16-5-9) (Table 3.1) were then applied at low, medium, and high rates as a single uniform layer (Figure 3.2). The coating material to control the release of soluble nutrients within the fertilizer prills of Osmocote Plus was a polymeric resin while that of Simplot was polyurethane. Each fertilizer was designed to release over 80% of its nutrients within 5-6 months based on a media temperature of 21°C. The purpose of layering the CRF beneath the transplanted root system was to examine seedling vertical root architectural development over time in relation to the proximity of the CRF layer. Fertilizer rates were adjusted to provide equivalent amounts of bulk N for the different CRF types, resulting in rates of 4, 8, and 12 g (Osmocote) and 3.75, 7.5, and 11.25 g (Simplot) for the low, medium, and high rates respectively. For each fertilizer type, a control was also included in which no fertilizer was added. An additional 2.5 cm of potting mix was applied above the fertilizer layer and seedlings were then transplanted and soil was filled to 2.5 cm below the top of the pots. The total volume of soil in each pot was approximately 2206 cm^3.

All pots were thoroughly watered following planting and placed in a controlled-environment greenhouse at Oregon State University's Oak Creek Plant Facility (44° 38'

lat., 123° 30' long.). Fans and coolers were used to cool the greenhouse below 32°C. Pots were watered to field capacity when a representative sample (2 pots/block) dried to a water content of 39% (using formula in Haase and Rose (1994)). This provided a water content range known to promote optimum Douglas-fir morphological and physiological development (Khan et al. 1996). All seedlings received 50 ppm Peters® (20-20-20 plus micronutrients) water-soluble fertilizer during 1 in 3 watering cycles to prevent mortality due to nutrient stress. Watering occurred approximately every 11 d during the first 4 months of the experiment and every 17 d during the final 4 months. Temperature data recorders (R-2100, Telog Instruments, Inc.) positioned at the approximate depth of the fertilizer layer in three pots recorded a mean soil temperature of 18°C during the period of active nutrient release, which varied over time (Figure 3.3).

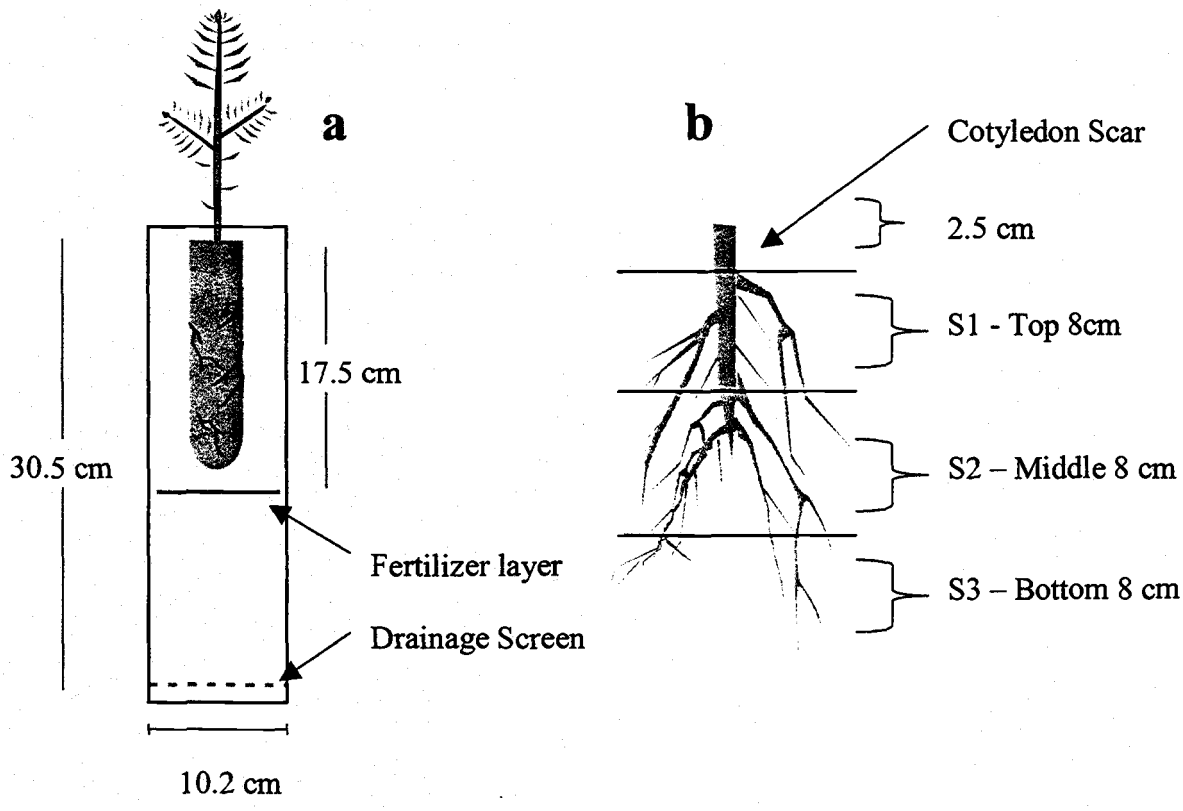

Figure 3.1. Placement of fertilizer (a) within pots and division of root system based on proximity of the fertilizer layer (b)

Table 3.1. Nutrient composition (%) of each fertilizer type.

	Osmocote Plus	Simplot
	---------- (%) ----------	
Nitrogen	15.0	16.0
NH_4	7.0	8.8
NO_3	8.0	7.2
Phosphorus (P_2O_5)	9.0	5.0
Potassium (K_2O)	12.0	9.0
Mg	1.0	1.0
S	2.3	5.0
B	0.02	0.0
Cu	0.05	0.05
Fe	0.45	1.25
Mn	0.06	0.5
Mo	0.02	0.0003
Zn	0.05	0.05

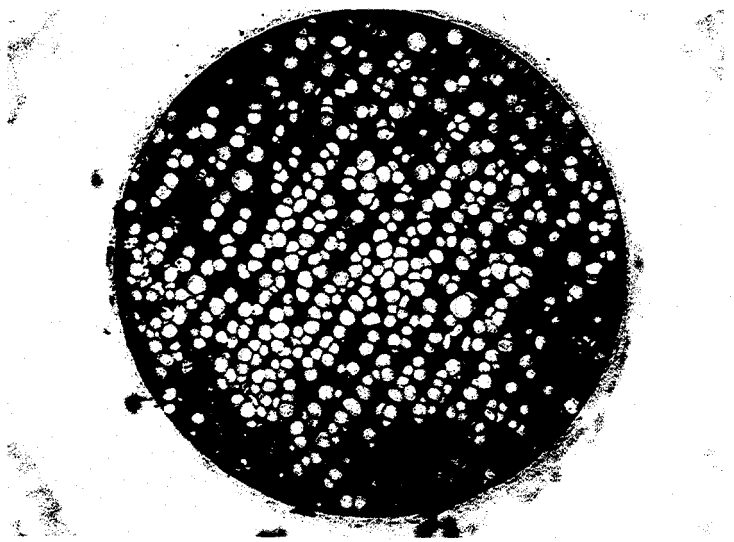

Figure 3.2. Osmocote Plus® CRF at the high (12 g) treatment showing the distribution of fertilizer as a single layer beneath the transplanted seedling.

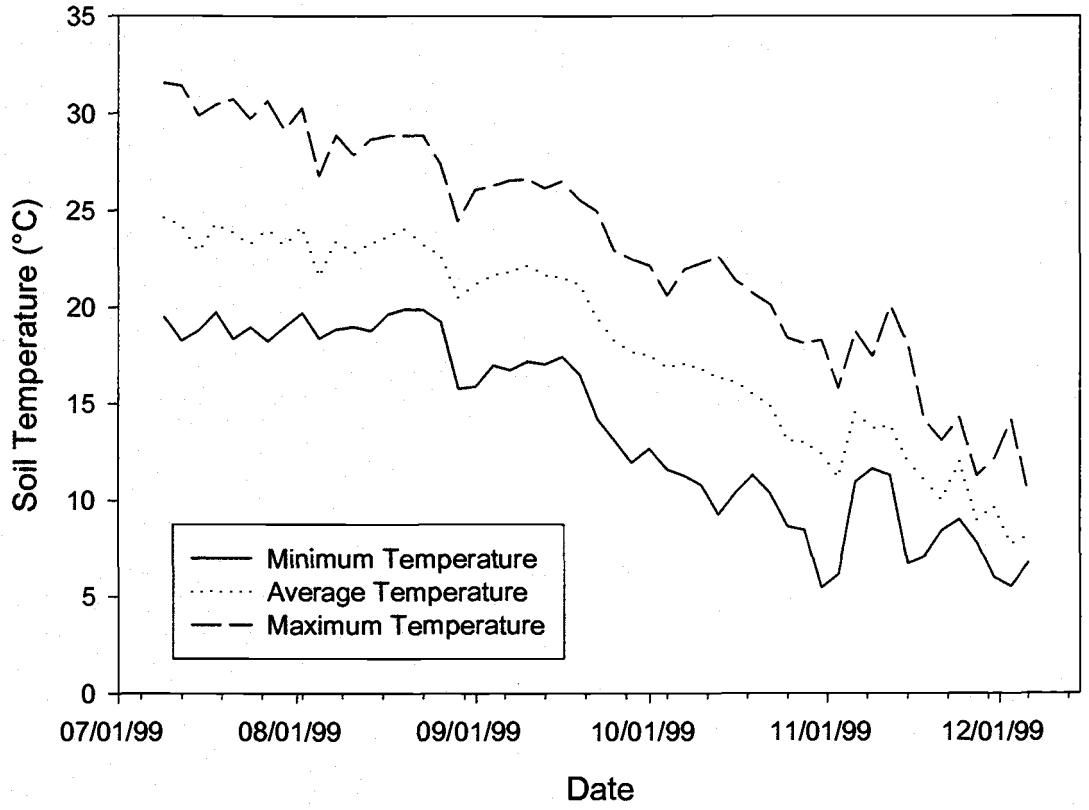

Figure 3.3. Soil temperature at the position of the fertilizer layer during the expected period of active nutrient release.

3.3.3 Measurements

In mid-October (first sampling) and again in early-March (second sampling), half of the seedlings in each treatment within each block (192 total at each sampling) were randomly selected for harvest and measured for height, stem diameter, shoot/root volume using water displacement (Burdett 1979), and shoot/root dry weight.

A subsample of 2 seedlings in each treatment/block combination were randomly selected for measurements of root morphology within soil zones relative to the placement of the fertilizer layer. Seedlings were first clipped at cotyledon scar and

again 2.5 cm below this point, which represents the position at which lateral roots tend to initiate. The remaining roots were then sliced into three 8 cm sections with the middle (S2) section representing the portion of roots where the fertilizer layer was present and the top (S1) and bottom (S3) being above and below the fertilizer layer (Figure 3.1). Within each root segment, the number of active root tips (white tips > 1 mm in length), the number of first-order lateral roots (>1 cm length), and dry root weights were assessed.

At each sample point, a composite of the 4 seedlings in each treatment for 4 blocks was sampled by removing needles. Each composite sample was dried at 70° C for 72 hr. Dry weight was measured on a sample of 100 needles/composite sample for use in calculating nutrient contents and vector analysis (Haase and Rose 1995) and foliage was then ground in a Wiley mill (40-mesh screen). Nutrient concentrations (USAg Analytical Services, Inc., Pasco, WA) were determined from foliage samples using methodology in Gavlak (1994).

Chlorophyll fluorescence (Opti-Sciences Pulse Modulated Chlorophyll Fluorometer OS5-FL) was sampled on September 10, 1999, October 12, 1999, and March 7, 2000 on a subsample of seedlings (3 at sample 1 and 2, 4 at sample 3) from each treatment/block. A single needle from both the top and middle portion of the terminal seedling shoot was removed from each seedling shoot. Within 3 min following removal, each needle was exposed to a pulse of light from a light emitting diode (LED) passing through a short pass filter (Opti-Sciences 1997). Measurements of Fs (steady state fluorescence), Fms (maximal fluorescence), and the quantum yield ((Fms-Fs)/Fms) of photochemical energy conversion were then recorded.

3.3.4 Experimental Design and Statistical Analysis

The experiment was arranged in a 2 x 4 factorial, randomized complete block design (2 CRF types X four CRF rates (control, low, medium, and high)). Each of six benches in the greenhouse was designated as one block due to discrepancies in light availability, temperature, and air circulation. Within a block, 64 pots (8 pots/treatment)

were randomly distributed. The experimental unit was the group of pots/treatment in each block and the sampling unit was the individual seedling.

Data were subjected to analysis of variance (ANOVA) for a randomized complete block design with factorial treatments. Tests for normality, linearity, and constant variance were performed, and transformations were made when necessary to ensure the validity of these assumptions. The main treatment effects of fertilizer type and rate were examined only in the absence of a significant ($p < 0.05$ in F test) type x rate interaction. Fisher's Protected Least Significant Difference procedure was used to detect significant differences among treatments ($\alpha \leq 0.05$) when main effects or the interaction was significant in the ANOVA. To account for the correlation between measurements made on top and middle needles of the same experimental unit, needle position was used as a source of variation in the ANOVA for chlorophyll fluorescence data. The absence of significant interactions for chlorophyll fluorescence at any sampling point allowed the main effects of fertilizer type, rate, and needle position to be assessed. Regression analyses were used to examine the relationship of nutrient concentrations to seedling morphology. Orthogonal contrasts were used to determine the statistical significance of higher order regression models for explaining variability associated with the data but only linear relationships were significant. The mean values of treatment replicates were used in the regression and an adjusted R^2 value was determined to indicate the fit of the model. SAS® software (SAS Institute, Inc., Cary, NC, USA) was used for analysis of all data.

3.4 Results

3.4.1 Summary of Results

Temporal patterns of seedling morphological and physiological development were affected by CRF type. Seedlings grew more rapidly during the first four months following transplant when fertilized with Osmocote but seedlings fertilized with

Simplot were the same size at the end of the experiment. Chlorophyll fluorescence followed a similar pattern over time to that of growth for the different CRF types. Maximal rates of chlorophyll fluorescence were higher for seedlings fertilized with Osmocote prior to the first sampling but were higher for Simplot-fertilized seedlings at the end of the experiment. The application of CRF affected the spatial root architectural development of seedlings. Seedlings allocated more active root tips above the CRF layer and less below the CRF layer with increasing fertilizer rate.

3.4.2 Morphology

No significant fertilizer type x rate interactions were detected for any whole-plant morphological parameters at the first sampling (4 months). The two CRF types affected patterns of seedling growth over time. Seedlings fertilized with Osmocote (averaged over fertilizer rate) had a significantly greater height growth ($p=0.0384$), diameter growth ($p=0.0035$), root volume ($p=0.0162$), shoot volume ($p=0.0185$), root dry weight ($p=0.0098$), and shoot dry weight ($p=0.0147$) 4 months (first sampling) following transplant than those fertilized with Simplot (Table 3.2). At 9 months (second sampling) no significant fertilizer type x rate interactions or differences between the CRF types were established for height or root dry weight although mean shoot:root dry weight ratio was significantly greater ($p=0.0332$) for trees fertilized with Osmocote. Mean values for diameter growth and shoot volume/dry weight were similar between fertilizer types at the second sampling although significant fertilizer type x rate treatment interactions (diameter growth, $p=0.0185$; shoot volume, $p=0.0271$) prevented statistical comparison of the main effects. This interaction was due to reduced values for these parameters at the highest fertilizer rate for seedlings fertilized with Simplot while seedlings fertilized with Osmocote had greatest mean values at the high rate. No significant differences between fertilizer rates were established for any morphological responses at either sampling point, although seedling fertilized at the highest CRF rate tended to have greater mean values for most responses than controls (Table 3.3).

Table 3.2. Mean values and standard error (SE) for morphology of seedlings grown with Osmocote Plus (OS) and Simplot (SM) CRF (averaged over fertilizer rate) at two sampling points. For each parameter and within each sampling point, means followed by the same letter did not differ significantly at $\alpha \leq 0.05$.

	October 1999			March 2000		
	OS	SM	SE	OS	SM	SE
Height Growth (cm)	22.3a	20.6b	0.90	22.1a	21.4a	0.59
Diameter Growth (mm)[1]	3.8a	3.5b	0.21	4.0	4.0	0.24
Root Volume (cm^3)	17.9a	15.7b	1.74	23.3b	26.1a	2.90
Shoot Volume (cm^3)[1]	17.7a	15.3b	1.34	18.0	17.8	1.94
Shoot:Root Volume	1.07a	1.05a	0.07	0.86a	0.68a	0.71
Root Dry Weight (g)	2.7a	2.3b	0.26	4.2a	5.0a	0.63
Shoot Dry Weight (g)[1]	3.6a	3.1b	0.26	4.7	4.7	0.49
Shoot:Root Dry Weight	1.43a	1.46a	0.10	1.20a	1.04b	0.11

[1]A fertilizer type/rate-treatment interaction occurred at March 2000.

3.4.3 Root Architecture

The presence of a CRF layer affected the root architectural development of transplanted seedlings and this effect was more pronounced with increasing CRF rate. No significant fertilizer type x rate interactions were observed for the total number of active root tips at 4 months (p=0.7793) or within any of the three soil zones at either sampling. Although the total number of active root tips did not differ among fertilizer rates at 4 months (p=0.3630) (Table 3.4), different patterns of active root distribution within the soil profile were established. Total numbers of active root tips were significantly different between treatments in the upper soil zone (S1) (p=0.0024). The mean number of active root tips in S1 appeared to increase with fertilizer rate (Table 3.4). The opposite effect was observed in the lower soil zone (S3), with greater fertilizer rates resulting in significantly less active root tips at 4 months (p=0.0174). A significant type x rate interaction for total number of active root tips at 9 months (p=0.0413) prevented the analysis of the fertilizer rate main effect, although means were similar (Table 3.4). At 9 months, the number of active root tips in S1 differed significantly among treatments (p=0.0016) and means numbers again increased with

Table 3.3. Mean values and standard error (SE) for morphology of seedlings grown under different CRF rates (averaged over fertilizer type) at two sampling points. There were no significant differences between any parameters at $\alpha \leq 0.05$.

	October 1999					March 2000				
	Control	Low	Med.	High	SE	Control	Low	Med.	High	SE
Height Growth (cm)	21.3	21.8	21.3	21.2	1.06	20.4	22.0	22.3	22.4	0.84
Diameter Growth (mm)	3.5	3.6	3.6	3.8	0.23	3.9	3.9	4.2	4.0	0.28
Root Volume (cm^3)	16.7	16.9	15.8	17.7	1.84	23.0	25.6	24.3	25.8	3.06
Shoot Volume (cm^3)	16.1	16.6	15.6	17.7	1.51	17.7	17.6	17.9	18.3	2.16
Shoot:Root Volume	0.97	1.07	1.08	1.13	0.86	0.74	0.70	0.79	0.86	0.10
Root Dry Weight (g)	2.5	2.5	2.4	2.7	0.28	4.4	5.3	4.3	4.5	0.80
Shoot Dry Weight (g)	3.3	3.3	3.2	3.6	0.30	4.6	4.6	4.8	4.8	0.56
Shoot:Root Dry Weight	1.35	1.49	1.46	1.50	0.11	1.08	1.09	1.19	1.13	0.09

Table 3.4. Mean number of active root tips and standard error (SE) by fertilizer rate (averaged over fertilizer type) in three root zones – S1 (upper), S2 (middle), and S3 (lower) at two sampling points. Within each sampling point, means followed by the same letter within a column did not differ significantly at $\alpha \leq 0.05$.

Fertilizer	October 1999				March 2000			
Rate	S1	S2	S3	Total	S1	S2	S3	Total[1]
Control	38 c	40 a	60 a	137 a	31c	37 a	44 a	111
Low	46 bc	40 a	39 b	126 a	39 bc	33 a	42 a	112
Medium	55 ab	44 a	35 b	134 a	48 ab	39 a	32 a	119
High	62 a	46 a	39 b	147 a	54 a	36 a	29 a	121
SE	7	5	8	14	4	4	8	13

[1]A fertilizer type/rate-treatment interaction occurred.

fertilizer rate (Table 3.4). No statistically significant difference among treatments was established at 9 months (p=0.0509) for active root tips in S3, though means again decreased with increasing fertilizer rate. No significant difference was ever present in the middle soil zone (S2) at either sampling (p=0.5855, 0.3755 respectively) for active root tips.

At the first sampling, no significant type x rate interactions were detected for total root dry weight or dry weight of roots within the soil zones. Root dry weight differed significantly among treatments at in S1 (p=0.0422), though not in S2 or S3 (p=0.1748, 0.7913 respectively) at 4 months. In S1, the same type of root allocation response noted for active roots was again observed with seedlings having greater root biomass in S1 with increasing rate (Table 3.5). As with active roots, there was no significant difference for total root dry weight in the three soil zones at four months (p=0.1306). At the second sampling, a significant fertilizer type x rate treatment interaction prevented statistical comparison of the main effect of root dry weight for S1 (p=0.0047), S2 (p=0.0413), and total root dry weight (p=0.0304), although the mean dry weights of S1 again increased with fertilizer rate (Table 3.5). Mean dry weights of S3 were not significantly different (p=0.6879). No significant differences in the distribution of active root tips or root biomass were noted at either sampling point between the two fertilizer types.

Table 3.5. Mean root dry weight (g) and standard error (SE) by fertilizer rate (averaged over fertilizer type) in three root zones – S1 (upper), S2 (middle), and S3 (lower) at two sampling points. Within each sampling point, means followed by the same letter within a column did not differ significantly at $\alpha \leq 0.05$.

Fertilizer	October 1999				March 2000			
Rate	S1	S2	S3	Total	S1[1]	S2[1]	S3	Total[1]
Control	0.95b	0.60a	0.73a	2.28a	1.38	1.07	1.15a	3.59
Low	1.05b	0.56a	0.62a	2.23a	1.61	1.05	1.24a	3.90
Medium	1.10ab	0.55a	0.55a	2.20a	1.91	1.14	1.14a	4.18
High	1.28a	0.74a	0.73a	2.76a	2.13	1.17	1.02a	4.31
SE	0.12	0.09	0.11	0.26	0.28	0.17	0.23	0.65

[1]A fertilizer type/rate-treatment interaction occurred.

3.4.4 Nutrients

No significant fertilizer type x rate interactions were observed at either sampling for any nutrient concentrations or contents with the exception of Ca content at 9 months ($p=0.0065$) and Mn concentration at 9 months ($p=0.0338$). Both P ($p=0.0409$) and K ($p=0.0029$) concentrations differed by fertilizer rate at the first sampling with the highest fertilizer rate having a lower mean concentration than the control (Table 3.6). Vector diagrams illustrated the reductions in P concentration and content at the highest fertilizer rate treatment after 4 months and for all fertilizer application rates as compared to the control at 9 months (Figure 3.4 a,b). Mean concentrations of N tended to increase with increasing fertilizer rate at both samplings, but there were no significant differences among treatments (4 months, $p=0.1254$ and 9 months, $p=0.3996$) (Table 3.6). At the first sampling, mean N content differed significantly among fertilizer rates ($p=0.0197$) with the medium rate having the highest mean content (Table 3.6). Vector diagrams (Figure 3.4. a,b) showed that the greatest increase in concentration, content, and unit dry weight occurred for the medium fertilizer rate. There were no significant differences in concentration or content at either sampling for Fe, B, Cu, and Na.

Mean concentrations of N ($p=0.0349$), P ($p=0.0415$), Ca ($p=0.0342$), Mg ($p=0.0191$), and Mn ($p<0.0001$) were significantly greater for seedlings fertilized with

Table 3.6. Mean nutrient concentration (%) and content (based on mean weight of 100 needles) and SE for fertilizer rate (averaged over fertilizer type) at two sampling points. For each nutrient and within each sampling point, means followed by the same letter within a column did not differ significantly at $\alpha \leq 0.05$.

	October 1999		March 2000	
	Concentration (%)	Content (g)	Concentration (%)	Content (g)
Nitrogen				
Control	2.95a	0.71b	3.35a	1.07a
Low	3.13a	0.81ab	3.58a	1.10a
Medium	3.29a	0.93a	3.49a	1.21a
High	3.34a	0.78b	3.66a	1.18a
SE	0.13	0.05	0.13	0.08
Phosphorus				
Control	0.90a	0.22ab	0.84a	0.28a
Low	0.88a	0.23a	0.72a	0.23a
Medium	0.89a	0.25a	0.68a	0.24a
High	0.79b	0.18b	0.68a	0.22a
SE	0.06	0.02	0.09	0.04
Potassium				
Control	2.93a	0.70a	2.41a	0.75a
Low	2.89a	0.74a	2.65a	0.79a
Medium	2.45b	0.69a	2.78a	0.95a
High	2.58b	0.61a	2.49a	0.79a
SE	0.14	0.04	0.33	0.07
Calcium[1]				
Control	0.80a	0.19b	0.83c	0.264
Low	0.86a	0.22ab	1.01b	0.3059
Medium	0.90a	0.25a	1.20a	0.4114
High	0.93a	0.22ab	1.22a	0.3892
SE	0.05	0.01	0.08	0.02
Magnesium				
Control	0.24a	0.057b	0.22a	0.070a
Low	0.25a	0.064ab	0.24a	0.073a
Medium	0.25a	0.069a	0.25a	0.087a
High	0.23a	0.054b	0.24a	0.076a
SE	0.01	0.004	0.01	0.005
Sulfur				
Control	0.25a	0.060a	0.24b	0.08b
Low	0.30a	0.077a	0.36a	0.11a
Medium	0.30a	0.085a	0.34a	0.12a
High	0.27a	0.064a	0.35a	0.12a
SE	0.03	0.009	0.04	0.016
Manganese[2,3,4]				
Control	64.0d	0.0015c	59.00	0.0018d
Low	84.6c	0.0022b	113.25	0.0035c
Medium	115.5b	0.0032a	154.50	0.0054b
High	141.4a	0.0032a	237.88	0.0077a
SE	12.45	0.0003	23.69	-

[1]A fertilizer type/rate-treatment interaction occurred for content in March 2000.
[2]Concentration is ppm.
[3]Means for content in March 2000 were back-transformed from log values used for analysis.
[4]A fertilizer type/rate-treatment interaction occurred for concentration in March 2000.

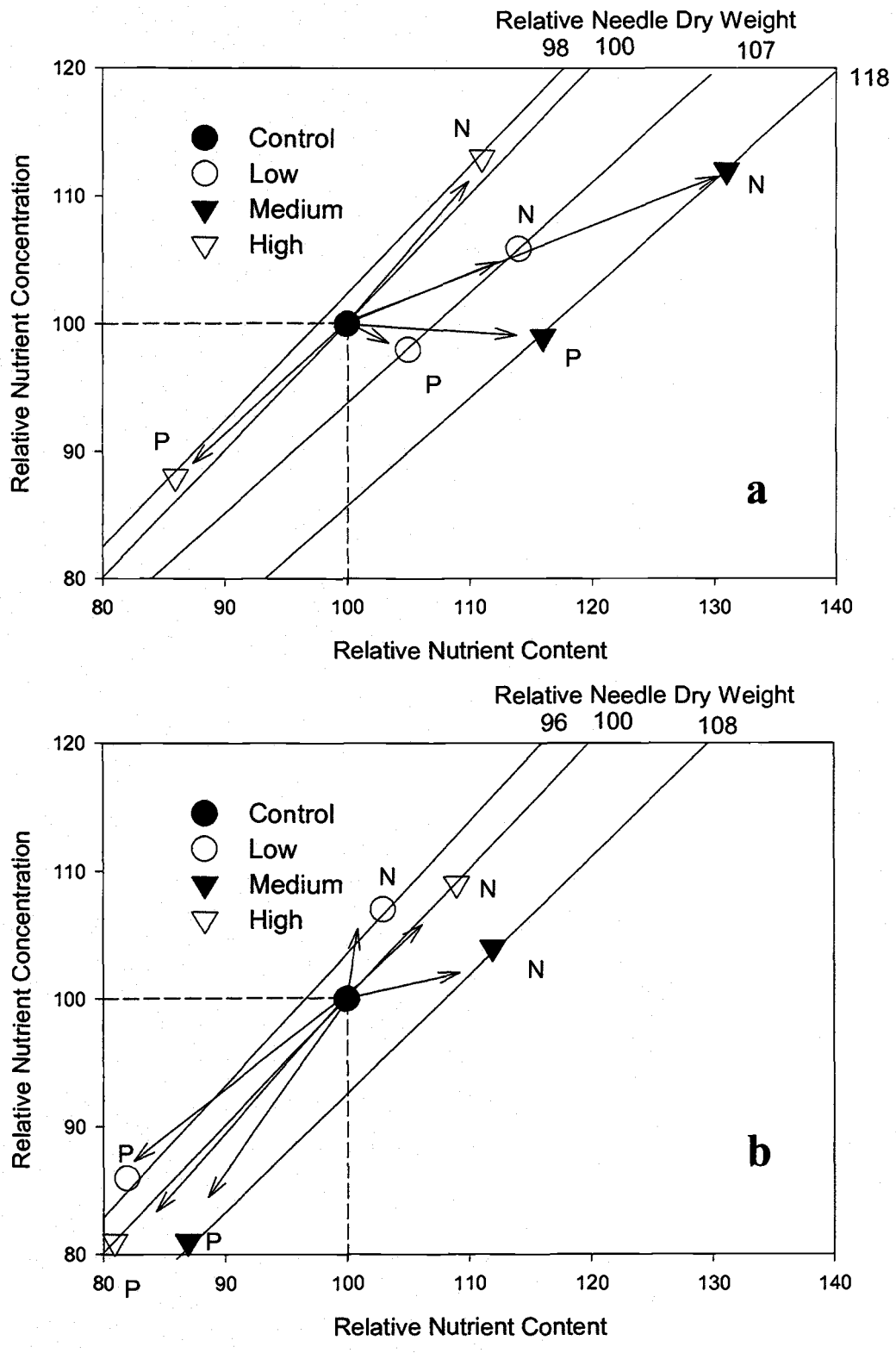

Figure 3.4. Vector diagrams for total nitrogen and phosphorus by fertilizer rate in October 1999 (a) and March 2000 (b).

Simplot as compared to Osmocote at the first sampling (Table 3.7). Mean contents, however, were similar due to the greater mean needle weight of Osmocote (Table 3.7, Figure 3.5). At the second sampling, most nutrient concentrations were not statistically different although the concentration of Ca was significantly greater for Osmocote ($p=0.0019$) and Mn was significantly greater for Simplot ($p=0.0245$) (Table 3.7). Though not statistically significant, mean N concentration ($p=0.3090$) and content ($p=0.0937$) as well as mean P concentration ($p=0.4106$) and content ($p=0.2099$) were greater for seedlings fertilized with Simplot at the second sampling (Table 3.7, Figure 3.5).

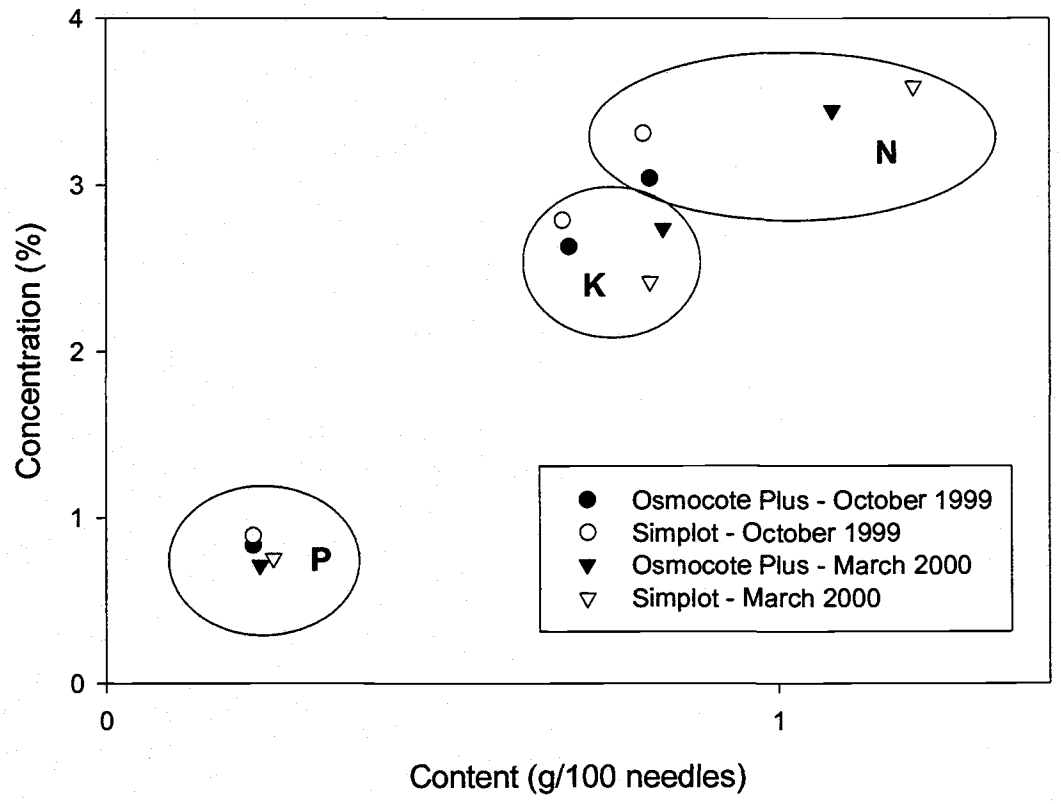

Figure 3.5. Vector diagram showing absolute concentration and content of total nitrogen, phosphorus, and potassium by fertilizer type in October 1999 and March 2000.

Table 3.7. Mean nutrient concentration (%) and content (based on mean weight of 100 needles) and SE for fertilizer type (averaged over rate) at two sampling points. For each nutrient and within each sampling point, means followed by the same letter within a column did not differ significantly at $\alpha \leq 0.05$.

	October 1999 Concentration (%)	Content (g)	March 2000 Concentration (%)	Content (g)
Nitrogen				
Osmocote	3.04b	0.81a	3.45a	1.08a
Simplot	3.31a	0.80a	3.59a	1.20a
SE	0.10	0.03	0.09	0.07
Phosphorus				
Osmocote	0.83b	0.22a	0.71a	0.23a
Simplot	0.89a	0.22a	0.75a	0.25a
SE	0.06	0.02	0.08	0.04
Potassium				
Osmocote	2.63a	0.69a	2.74a	0.83a
Simplot	2.79a	0.68a	2.42a	0.81a
SE	0.13	0.03	0.30	0.05
Calcium[1]				
Osmocote	0.83b	0.22a	1.12a	0.35
Simplot	0.91a	0.22a	1.01b	0.33
SE	0.05	0.009	0.08	0.01
Magnesium				
Osmocote	0.23b	0.06a	0.25a	0.08a
Simplot	0.25a	0.06a	0.23a	0.08a
SE	0.01	0.003	0.01	0.003
Sulfur				
Osmocote	0.26a	0.07a	0.31a	0.10a
Simplot	0.30a	0.07a	0.33a	0.11a
SE	0.02	0.007	0.03	0.016
Manganese[2,3,4]				
Osmocote	82.13b	0.22b	112.88	0.0036 b
Simplot	120.63a	0.29a	169.44	0.0056 a
SE	11.81	0.0003	17.00	-

[1]A fertilizer type/rate-treatment interaction occurred for content in March 2000.
[2]Concentration is ppm.
[3]Means for content in March 2001 were back-transformed from log values used for analysis, preventing the report of standard error.
[4]A fertilizer type/rate-treatment interaction occurred for concentration in March 2000.

A regression analysis of P concentration (%) on shoot:root dry weight showed a strong negative linear correlation ($p<0.0001$, Adjusted $R^2 = 0.51$) at the second sampling (Figure 3.6) with P concentration decreasing with increasing shoot:root dry weight.

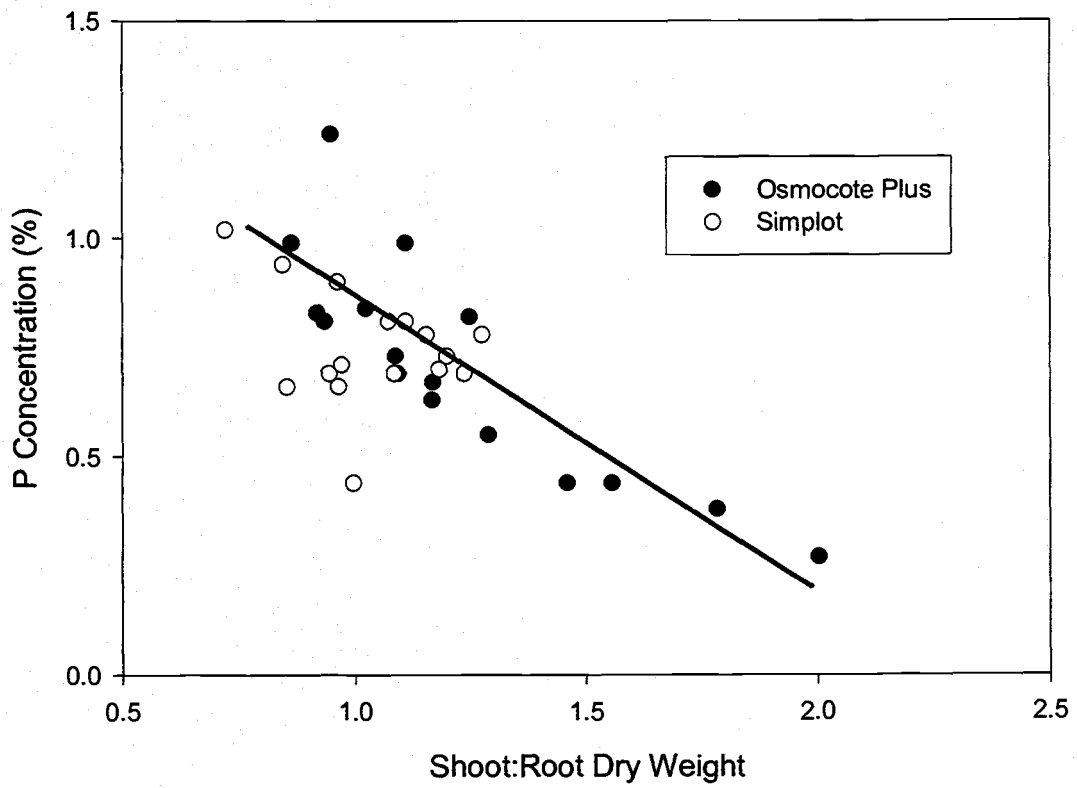

Figure 3.6. Phosphorus concentration on shoot:root dry weight in March 2000 for seedlings fertilized with Osmocote Plus and Simplot. Regression equation is P (%) = 1.35 – 0.55 (SDW/RDW), Adjusted $R^2 = 0.51$.

3.4.5 Chlorophyll Fluorescence

In September 1999, chlorophyll fluorescence parameters were consistently higher in needles sampled from the middle of the plant as compared to actively elongating needles from the top of the plant ($p<0.0001$ for all parameters) (Table 3.8).

Table 3.8. Mean values and standard error for chlorophyll fluorescence parameters at three sampling points for fertilizer type, rate, and needle position. For each parameter and within each sampling point, means followed by the same letter within a column did not differ significantly at $\alpha \leq 0.05$.

		Sampling Date								
		September 10, 1999			October 12, 1999			March 7, 2000		
		Fs	Fms	Quantum Yield	Fs	Fms	Yield	Fs	Fms	Quantum Yield
Fertilizer Type	Osmocote	156a	597a	0.72a	266a	1264a	0.78a	181a	841b	0.78a
	Simplot	146b	536b	0.71a	275a	1273a	0.78a	195a	929a	0.79a
	SE	4.6	28.7	0.008	10.1	36.9	0.005	5.1	26.2	0.007
Fertilizer Rate	0	146a	525a	0.70a	269a	1252a	0.78a	187a	881a	0.78a
	4	145a	539a	0.71a	285a	1305a	0.77a	191a	888a	0.78a
	8	155a	597a	0.73a	267a	1261a	0.79a	194a	915a	0.79a
	12	158a	606a	0.72a	263a	1255a	0.79a	179a	857a	0.79a
	SE	5.7	34.0	0.010	12.0	50.8	0.006	7.2	34.5	0.008
Needle Position	Middle	176a	690a	0.74a	266a	1311a	0.80a	191a	904a	0.79a
	Top	126b	444b	0.69b	275a	1225b	0.77b	185a	866a	0.78a
	SE	4.5	26.9	0.007	9.8	32.0	0.005	5.1	25.5	0.007

This same trend was noted for Fms ($p=0.012$) and quantum yield ($p<0.0001$) in October 1999 but this response was no longer significant in March 2001 for any parameter (Fs, $p=0.3869$; Fms, $p=0.2082$; quantum yield, $p=0.5302$) (Table 3.8).

There were distinct temporal changes in Fms associated with fertilizer type. In September, Fms was higher ($p=0.023$) for seedlings fertilized with Osmocote, though quantum yield was unaffected ($p=0.3618$). Although no differences for Fms ($p=0.8594$) or any other parameter were observed in October 1999, mean Fms ($p=0.0097$) was greater for seedlings fertilized with Simplot in March 2000. No significant differences were established for fertilizer rate at any sampling point, though means for Fms in September 1999 ($p=0.0778$) tended to increase with increasing fertilizer rate (Table 3.8).

3.5 Discussion

3.5.1 Fertilizer Effects on Roots Architectural Development

The layering of CRF below the transplanted root system had a distinct effect on seedling root architectural development over time. Root dry weight increased in the upper soil zone with increasing fertilizer rate but dry weights in the middle and lower zones and total root dry weight were unaffected (Table 3.5). Coutts and Phillipson (1976) found that the dry weight and diameter of Sitka spruce roots increased only in the half of the root system that was supplied with a NPK solution. Root dry weight of lodgepole pine increased in areas of localized nutrient supply in solution but did not change total root growth (Coutts and Philipson 1977). Friend et al. (1990) reported that Douglas-fir fine root proliferation and root dry weight increased in areas of localized nutrient solution supply when the remainder of the plant was nutrient stressed but total root growth again was unchanged. Eckhard et al. (1997) found that root dry weight and root length of Douglas-fir was unaffected in localized nutrient supply in mineral soil, whereas increases were reported for Scots pine (*Pinus sylvestris* L.) and Norway spruce

(*Picea abies* (L.) Karst.). Previous work with localized supplies of polymer-coated CRF has provided conflicting results. No uneven root development was found due to planting hole or adjacent placement of polymeric-resin-coated Osmocote for western hemlock (Carlson 1981) and Douglas-fir (Carlson and Preisig 1981) seedlings following field excavation. Increased numbers of root tips, root length, and root surface area occurred in white spruce seedlings when polyurethane-coated CRF was added to mineral soil compartments only, with no difference in proliferation between fertilized and unfertilized compartments when CRF was added to organic soil compartments (Krasowski et al. 1999).

When studying roots, it is important to use criteria other than simply root mass to quantify root architecture (Nambiar 1980). The distribution of white root tips increased in the upper soil zones and decreased in the lower soil zones with increasing fertilizer rate at each sampling in this experiment with no difference in the total number of white root tips (Table 3.4). White root tips are anatomically suited for efficient ion uptake due to the presence of a living cortex in white roots (Peterson et al. 1999) and the immaturity of the endodermis in the zone of elongation. Coutts and Phillipson (1977) found that lodgepole pine roots turned white and elongated in nutrient rich solution whereas in nutrient poor solutions, they turned brown and elongation slowed or stopped.

The biochemical and physiological bases for root proliferation in zones of localized nutrient supply is not adequately understood (Granato and Raper 1989; Robinson 1994). Roots cannot sense areas of superior nutrient content and grow into them by random chance (Stout 1956). The plant may then respond by allocating a preferential supply of assimilates and carbohydrates to the nutrient-rich area of the root system (Drew and Saker 1975; Barta 1976). This shifts growth to the portion of the roots where nutrients can be rapidly exploited without increasing growth in the entire root system (Friend et al. 1990). Granato and Raper (1989) attributed the proliferation of corn roots in localized sources of nutrient supply to in situ reduction and utilization of absorbed NO_3. Root response to localized nutrient sources may also be hormonal (Coutts and Philipson 1976) as abscisic acid (ABA), for example, acts to control root extension (Wraith and Wright 1998).

Another possibility is that root proliferation below the fertilizer layer was restricted due to the buildup of fertilizer salts. Root apical meristems are subject to death due to toxic ion concentrations and osmotic effects (Drew 1975; Danielson et al. 1984; Kafkafi and Bernstein 1996). In this experiment, the presence of the fertilizer did not affect the ability of roots to penetrate to the lower portion of the pots, as indicated by similar root biomass in the lower soil zone among treatments (Table 3.5). However, the greater numbers of white root tips for trees fertilized at higher rates was observed in the upper soil zone, despite the location of the fertilizer layer in the middle zone. Though fertilizer nutrients probably moved into the upper soil zone, the majority of nutrient salts likely leached into the lower portion of the pots with the flow of irrigation water. Proliferation of white roots above the middle zone and not actually in the zone of fertilizer suggests that osmotic effects may have restricted the ability of root tips to proliferate in the middle zone.

3.5.2 Seedling Morphological Development

There were no significant differences in overall seedling growth by fertilizer rate at either sampling point in this experiment, although means for most morphological parameters tended to be greater in the highest fertilizer rate (Table 3.3). This result was unexpected, as several studies have documented enhanced growth of seedlings fertilized with CRF. After 12 months, Jeffrey pine (*Pinus jeffreyi* Grev. & Balf.) seedlings fertilized with Sierra CRF had a greater total volume and dry weight than seedlings fertilized with Agriform and Peters water-soluble fertilizer (Walker and Huntt 1992). Jeffrey pine fertilized with polymeric resin-coated High N® and Sierra® CRF had greater height and diameter after 12 months than those fertilized with Peters (Walker and Huntt 2000). It is possible that growth differences by fertilizer rate were negligible in this experiment because all seedlings had adequate nutrition for growth due to the application of Peters water-soluble fertilizer to all seedlings. However, in an experiment involving Engelmann spruce (*Picea engelmannii* Parry ex Englelm.) where all seedlings in the experiment were fertilized with Peters water-soluble fertilizer, the

height, diameter, and total weight of seedlings given supplemental Osmocote or polyolefin-coated Nutricote was greater than controls, though root weight was reduced (Hunt 1989). It is also possible that the application of CRF as a single uniform layer compared to uniform mixing prevented a growth response. However, Robinson (1994) noted that growth usually increases or hardly changes in plants supplied with localized nutrients and Krasowski et al. (1999) reported that locally supplied CRF improved aboveground growth as compared to controls.

Despite equivalent estimated times for nutrient release and similar nutrient formulations, there were distinct differences in growth response over time for CRF type with seedlings fertilized with Osmocote having greater initial growth than those fertilized with Simplot (Table 3.2). Growth differences were negligible at the end of the experiment however, indicating that seedlings fertilized with Simplot grew at a more rapid rate between the first and second sampling. In a study investigating the release of various types of CRF, Huett and Gogel (2000) found that longevities, measured as weeks to 90% nutrient leachate recovery at 30°C, were considerably shorter than the nominated release periods for all formulations. Osmocote released faster than all other CRF types, however, with release periods up to 10 wk shorter than comparable Nutricote and polyurethane-coated Apex Gold® (Simplot) products. Osmocote seemed to release nutrients more rapidly than the Simplot CRF in this experiment as well, resulting in differences in seedling growth rates over time.

One explanation for inconsistencies in release rates between Osmocote and Simplot CRF is the difference in release technology. Though both polymer-coated, Osmocote is composed of granules of dry plant nutrients, which are encapsulated within multiple layers of polymeric resin. Nutrients in Simplot are encapsulated within multiple layers of a polyurethane coating. These materials react differently following exposure to moisture. The polymeric resin on Osmocote tends to swell soon after application, resulting in a thinner coating and exposing a greater surface area of the fertilizer prill, which may result in a more rapid release as nutrients diffuse through enlarged pores in the coating. The polyurethane coating of Simplot CRF resists swelling following application and the original thickness of the coating is maintained, allowing nutrients to be released more consistently and over a longer time period

(Personal Communication-Gary Furze, Simplot). Knowledge of differences in release rates among CRF types with equivalent estimated times for nutrient release is important for nursery managers. With this information, adjustments in application rates and time periods for nutrient release will help to maximize nutrient availability during the active growing period. This will act to minimize the potential for nutrient leaching and the buildup of toxic concentrations of fertilizer salts in the soil. Additionally, a better understanding of CRF release patterns may be used to prevent significant nutrient release late in the growing period, helping to properly condition plants for dormancy.

3.5.3 Nutrients

Though N concentrations tended to increase by fertilizer rate at both samplings (Table 3.6), there were no significant differences among rates at either sampling. N for all treatments was in the upper range of concentrations considered adequate for Douglas-fir seedling growth (Landis 1989). The same trend was noted for most other nutrients, with concentrations of all elements well within the range considered adequate by Landis (1989). The failure to detect significant difference in growth due to CRF application in this experiment may be partly explained by the lack of significant differences for most nutrient concentrations. Growth differences due to CRF treatments noted in Walker and Huntt (1992) also corresponded with substantially higher nutrient concentrations in seedlings treated with CRF. Vector diagrams as interpreted by Timmer and Stone (1978), however, (Figure 3.4. a,b) showed that N was limiting to growth of controls at both samplings, though more prominently at the first sampling based on the magnitude of the vectors. The medium rate resulted in the greatest increase in N content and needle dry weight at each sampling, suggesting that this may have been the ideal fertilizer rate (Figure 3.4. a,b).

Nitrogen concentration was significantly higher for seedlings fertilized with Simplot than Osmocote at the first sampling due to the dilution of N in faster-growing trees fertilized with Osmocote, as indicated by nutrient content (Table 3.7, Figure 3.5). At the second sampling, both the concentration and content of N were not statistically

different between the fertilizer types but Simplot seedlings had a higher mean concentration and content of N (Table 3.7, Figure 3.5). The greater mean N content for seedlings fertilized with Simplot from the first to second sampling helps to explain the improved growth during this period and further supports the conclusion that Simplot released nutrients over a longer period than Osmocote.

Mean concentrations of P tended to decrease with increasing CRF rate (Table 3.6). This effect has been observed for Douglas-fir fertilized with N in the field (Gill and Lavender 1983; Roth and Newton 1996) and nursery (van den Driessche 1980). There is currently no generally accepted physiological explanation to explain this response. Visser et al. (1993) found that with increasing N as NH_4^+, less P was taken up than as NO_3^- and attributed this to acidification of the rhizosphere when N was taken up as NH_4^+. The negative linear correlation between P concentration and shoot:root dry weight noted in this study (Figure 3.6) suggests that P became diluted as shoot volume increased. Additionally, because P is immobile in the soil, less root expansion relative to the shoot implies that roots were limited in their ability to exploit the soil and supply P to the foliage.

Despite approximately double the P composition in the Osmocote CRF (Table 3.1), mean P concentrations and content were lower than that for Simplot at the second sampling (Table 3.7, Figure 3.5). This was likely associated with the greater shoot:root ratios for Osmocote-fertilized seedlings (Table 3.2, Figure 3.6) acting to dilute P concentrations. Additionally, the significantly greater root volume of Simplot-fertilized seedlings in March 2000 (Table 3.2) allowed a greater soil volume to be exploited for P extraction. This implies that efficient P uptake may be more dependent on seedling morphological development than quantity of P in fertilizer applications.

3.5.4 Chlorophyll Fluorescence

Initially, chlorophyll fluorescence parameters were higher in needles sampled from the middle of the plant as compared to actively elongating needles from the top of the plant, but this response was no longer significant at the end of the experiment (Table

3.8). Many researchers have reported higher photosynthetic rates in current year foliage as compared to foliage from previous seasons (Hom and Oechel 1983; Teskey et al. 1984; Kajimoto 1990; Oleksyn et al. 1997). Needles sampled from seedlings in this experiment, however, were all from the same growth flush. Active foliar growth is generally associated with a low photosynthetic capacity and high respiration rates (Radoglou and Teskey 1997). Radoglou and Teskey (1997) found that the highest photosynthetic rates in loblolly pine (*Pinus taeda* L.), occurred when the foliage was 90%+ expanded. This helps to explain the reduced chlorophyll fluorescence of needles that were still actively elongating. In March 2001 when seedlings were dormant and not actively growing, needles probably moved from being a net carbon sink to a net carbon source as indicated by similar chlorophyll fluorescence values for top and middle needles. This illustrates the importance of developing a methodology to sample needles for chlorophyll fluorescence from a specific part of the plant, particularly during the active growing period.

Chlorophyll fluorescence values were never significantly different by fertilizer rate (Table 3.8). Birchler et al. (2001) found that chlorophyll fluorescence (Fv/Fm) of seedlings fertilized with N and K in the nursery was consistently higher than that for unfertilized seedlings. In this study, the absence of a chlorophyll fluorescence response corresponded with the nominal differences in seedling nutrient uptake by fertilizer rate and the failure to detect significant differences in seedling growth.

The significantly greater maximal fluorescence (Fms) in September 1999 (Table 3.8) of seedlings grown with Osmocote corresponded to the higher growth rates observed for these seedlings at the first morphological sampling (Table 3.2). In October 1999, Fms was no longer significantly different between the fertilizer types and in March 2000 Fms of seedlings grown with Simplot was significantly greater than that for Osmocote seedlings. This corresponded with the improved growth of Simplot trees during the period between the first and second morphological sampling. Thus, the higher Fms may have been a reflection of enhanced photosynthetic capacity due to differential patterns in CRF nutrient release, resulting in more rapid growth rates during periods of higher Fms. Reduced growth and lower maximal rates of fluorescence have

been recorded previously and attributed to P deficiency in sunflower (Guidi et al. 1994) and to high temperatures in wheat (Ferguson et al. 1993).

3.6 Conclusions

With increasing CRF rate, seedling roots tended to proliferate in the upper soil zones, indicating that localized CRF placement affected root architectural development. Though the use of pots and the fact that water was never limiting limits conclusions regarding root development when outplanted with CRF in the field, the same type of localized root proliferation in the upper soil zones could occur when seedling roots grow through a layer of CRF applied to the planting hole. In areas characterized by hot, dry summers, root proliferation in upper soil zones may limit the ability of roots to extract water from deeper in the soil profile, thereby increasing the potential for drought stress.

Despite equivalent manufacturer's estimated release rates, Osmocote released nutrients at a more rapid initial rate than Simplot, resulting in a difference in seedling growth response over time. This has important implications to nursery managers regarding issues such as maintaining acceptable soil electrical conductivity (EC) levels, minimizing nutrient leaching, and conditioning plants for dormancy. A more compatible system for assessing release rates among CRF types is needed such that confident recommendations regarding CRF use can be implemented based on fertilizer formula and coating material/thickness.

Chlorophyll fluorescence was able to detect subtle differences in seedling development between the two CRF types, which seemed to correspond to changes in seedling growth over time. It is essential that foliage for chlorophyll fluorescence be sampled in a systematic manner to avoid discrepancies based on the developmental stage of foliage. Chlorophyll fluorescence may provide a cost-effective, non-destructive means to detect changes in seedling physiological development associated with mineral nutrition.

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Chapter 4

Controlled-Release Fertilizer Effects on Growth, Root Architecture, and Chlorophyll Fluorescence of *Pseudotsuga menziesii* Seedlings

Douglass F. Jacobs, Robin Rose, and Diane L. Haase

4.1 Abstract

The objective of this experiment was to determine how different application rates of locally applied controlled-release fertilizers (CRF) affect seedling growth, lateral root penetration, and photosynthetic capacities over time. Three months following sowing, coastal Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) seedlings were transplanted into pots with Osmocote Plus® 15-9-12 poymeric-resin coated CRF applied at rates of 0, 8, 16, and 24 g per 2200 cm^3 soil as a single uniform layer beneath the root system. Root penetration into the soil layers below the fertilizer layer was severely restricted at the 16 and 24 g CRF rate. Vector analysis indicated that N improved seedling growth at the 8 and 16 g CRF rates as compared to controls but was inhibitory at 24 g. Concentration and content of P were reduced in all CRF treatments at 4 months following transplant but P content at the 8 g rate was greater than that of all other treatments after 8 months, likely due to larger root systems in this treatment improving uptake of this soil-immobile nutrient. Quantum yield of photosynthesis was significantly higher in all CRF treatments than for the unfertilized control 3 months following transplant, which corresponded with the rapid initial release of fertilizer nutrients. These results indicate that despite improvements in CRF technology, high application rates appear to release toxic concentrations of fertilizer nutrients, which restrict lateral root penetration and negatively affect seedling growth. Conservative CRF application rates and techniques such as exponential fertilization will help to reduce the potential for adverse effects on seedling development.

4.2 Introduction

Reforestation efforts are most successful when target seedling morphological and physiological characteristics (Rose et al. 1990) are promoted in the nursery and maintained during the establishment period following field planting. Interest in using controlled-release fertilizers (CRF) to enhance forest seedling productivity in the nursery and field has increased (Donald 1991; Haase and Rose 1997). As compared to

conventional water-soluble fertilizers, CRF can supply seedlings with nutrition throughout a growth cycle with a single application. Due to their gradual release, the potential for seedling damage associated with nutrient toxicities and nutrient loss through leaching can be reduced (Hauck 1985; Donald 1991). Benefits associated with using CRF depend on a complex interaction of factors including CRF type/rate, seedling morphology/physiology, media, and environmental growing conditions. Variable results in the nursery and field indicate that a better understanding of the mechanisms by which CRF affect seedling morphology and physiology is needed.

Many types of CRF are available for use with forest tree seedlings. The primary differences among types are the nutrient formulations and prill coatings, which determine the rate at which nutrients are released. Many of the early CRF were sulfur-coated urea (SCU) mixtures, which produced a rapid initial flush of nutrients due to imperfections in the coating. The use of SCU has decreased with the advent of longer-lasting polymer-coated CRF, which are currently the CRF used in the majority of nursery plant production (Huett and Gogel 2000). Nutrient release of polymer-coated CRF is determined by the temperature-driven diffusion of water through the semi-permeable membrane, with little effect associated with media water content (Kochba et al. 1990).

Several types of coating materials are used with polymer-coated CRF, including polymeric resins, polyurethane, and polyolefin. The degree with which the fertilizer prill swells following application differs among material types with polymeric resin coatings tending to swell more than polyurethane and polyolefin coatings due to the nature of the material. This swelling may result in a thinner coating that causes a more rapid initial release of nutrients. Osmocote® (Scotts Company), a polymeric resin coated CRF, released nutrients more rapidly than comparable polyurethane- and polyolefin-coated CRF (Huett and Gogel 2000). Rapid nutrient release from CRF can restrict plant growth due to a reduction in nutrient availability associated with excessive leaching (Huett and Morris 1999) or toxic buildup of fertilizer salts. Thus, it is important to examine the relationship between polymeric resin coated CRF rates and seedling development to prevent negative results.

The architectural development of roots offers a means to investigate potential negative effects associated with high CRF rates. Researchers have advocated the application of fertilizers directly in the planting hole in field plantings to facilitate efficient nutrient uptake (Austin and Strand 1960; Carlson 1981; Carlson and Preisig 1981; Gleason et al. 1990; Rose et al. 1991). Roots then grow through the fertilizer layer while extending into the soil profile. Experiments have documented the proliferation of roots in areas of high nutrient concentrations in solution for barley (Drew 1975; Drew and Saker 1975), Sitka spruce (*Picea sitchensis* (Bong.) Carr.) (Coutts and Philipson 1976), lodgepole pine (*Pinus contorta* Dougl. ex. Loud.) (Coutts and Philipson 1977), maize (*Zea mays* L.) (Granato and Raper 1989) and Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) (Friend et al. 1990). Results from the few studies that have investigated root response to localized supply of CRF have provided variable results. No localized proliferation of roots due to CRF placement was reported for outplanted Douglas-fir (Carlson and Preisig 1981) and western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) (Carlson 1981). When roots of white spruce (*Picea glauca* (Moench) Voss) were divided into mineral and organic soil compartments, roots grew preferentially in mineral compartments when CRF was added to this compartment only (Krasowski et al. 1999).

Most experiments have reported that seedling growth usually increases or hardly changes with the application of localized nutrient supplies (Robinson 1994). However, toxicities associated with high fertilization in the root zone would be apt to impair root architectural development, which may then have a negative impact on plant growth. As fertilizer is released, salts diffuse outward into the soil media, which acts to lower soil osmotic potential and causes changes in soil pH. Buildup of fertilizer salts can then cause the death of root apical meristems (Drew 1975; Danielson et al. 1984; Kafkafi and Bernstein 1996), preventing seedlings from accessing water and nutrients (Kozlowski 1987). While there are numerous studies in agricultural and horticultural literature (Karim and Touchton 1983; Lamont et al. 1992; Moody et al. 1995a; Moody et al. 1995b), few studies have documented the effects of high fertilization rates, particularly with CRF, on the root development of coniferous species. Thus, it is

important to determine how different rates of CRF affect seedling root growth and the subsequent effect on whole plant development.

Chlorophyll fluorescence offers another method for examining changes in seedling development due to fertilization. First reported by Kautsky (1931), chlorophyll fluorescence is an inexpensive and non-destructive method to evaluate plant physiological status (Vidaver et al. 1989). Chlorophyll fluorescence has been used to detect changes in the physiological status of Douglas-fir due to dormancy (Hawkins and Lister 1985; Roberts et al. 1991), freezing stress (Fisker et al. 1995), and shading (Khan et al. 2000). Few studies have reported the use of chlorophyll fluorescence to detect physiological changes associated with fertilization in forest tree seedlings, although chlorophyll fluorescence was used to detect Cu and P deficiencies in Douglas-fir (Vidaver et al. 1988) and Monterey pine (*Pinus radiata* D. Don) (Lopez Gorge et al. 1985; Conroy et al. 1986). Birchler et al. (2001) found that nursery-fertilized Douglas-fir had higher rates of chlorophyll fluorescence (Fv/Fm) than unfertilized seedlings. More research is needed to determine how chlorophyll fluorescence can be used to evaluate the physiological status of seedlings in response to fertilization.

The primary objective of this study was to quantitatively assess the effects of a wide range of CRF rates applied as a single layer beneath the transplanted root system on the morphological and physiological development of Douglas-fir seedlings. We hypothesized that with increasing fertilizer rates (i) whole-plant morphological development might be negatively affected, (ii) roots would proliferate in the upper soil zones, limiting penetration into subsoil zones, (iii) excessive nutrient uptake would result in toxicities, and (iv) chlorophyll fluorescence would vary among CRF rates over time.

4.3 Materials and Methods

4.3.1 Plant Material

Douglas-fir seeds (seed zone 274, Western Forest Tree Seed Council, State of Oregon Tree Seed Zones) were sown into containers with 39 cm^3 cavities in March 2000 at the Timber Company's nursery near Cottage Grove, OR. Seedlings were grown under standard nursery cultural practices until being lifted and transplanted into pots in mid-June 2000. Measurements of height and diameter taken at the time of transplanting indicated that seedlings had a mean height (±SE) of 5.6 (0.1) cm and a mean stem diameter of 1.07 (0.02) mm.

4.3.2 Treatments

Following measurement, seedlings were transplanted into cylindrical pots 30.5 cm in length and 10.2 cm in diameter (Figure 4.1). A metal screen for drainage was installed 1 cm from the bottom of the pots. Pots were filled to a depth of 13 cm with a 4:4:1:1 (v:v:v:v) peat:composted plant material:pumice:perlite (Organic mix, Pacific Soil). Osmocote Plus® 15-9-12 CRF (Table 4.1) was then applied at three rates (8, 16, and 24 g) as a single uniform layer. A control treatment was also included in which no CRF was added. An additional 2.5 cm of potting mix was applied above the fertilizer layer and seedlings were then transplanted and soil was filled to 2.5 cm below the top of the pots. The total volume of soil in each pot was approximately 2200 cm^3. The purpose of layering the CRF beneath the transplanted root system was to examine seedling vertical root architectural development over time in relation to the proximity of the CRF layer.

Osmocote Plus® CRF is composed of granules of dry plant nutrients, which are encapsulated within multiple layers of polymeric resin. By adjusting the thickness of the polymer resin coating, the release of rate of nutrients within the fertilizer prill can be

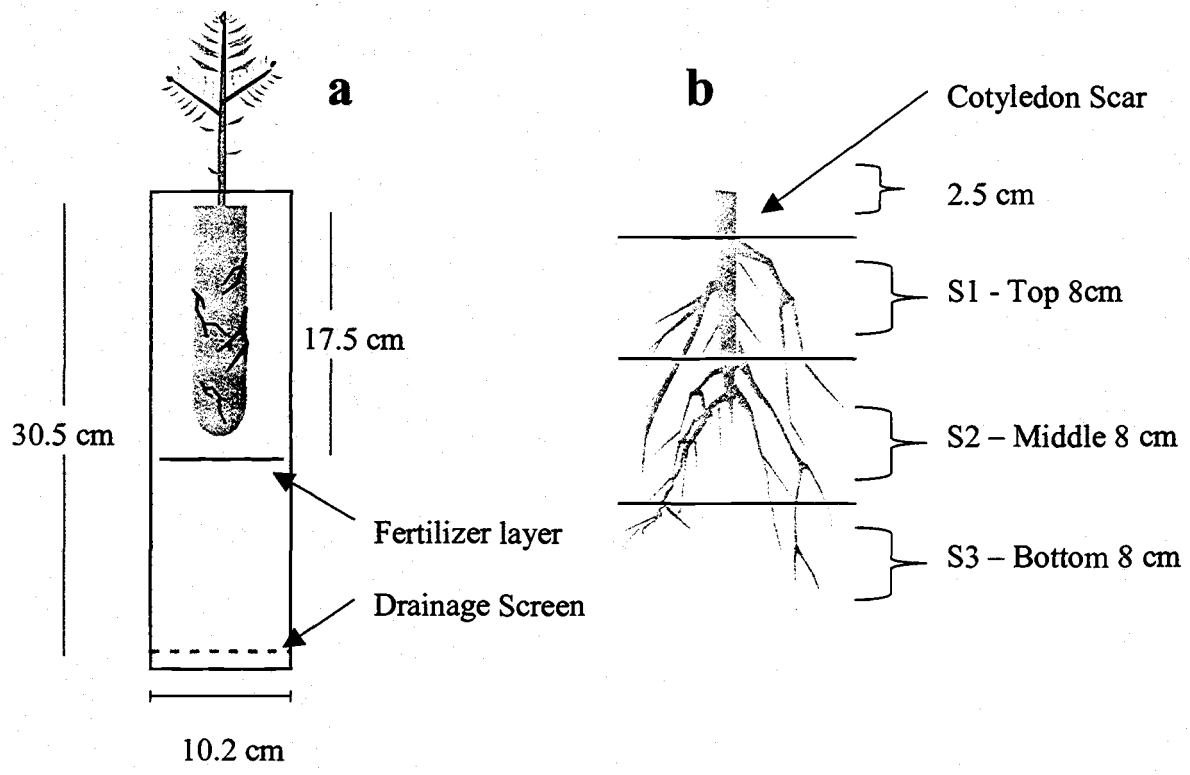


Figure 4.1. Placement of fertilizer (a) within pots and division of root system based on proximity of the fertilizer layer (b).

controlled. This CRF was designed to release approximately 80% of its nutrients within 5-6 months based on a potting media temperature of 21°C.

All pots were thoroughly watered following transplanting and placed in a controlled-environment greenhouse at Oregon State University's Oak Creek Plant Facility (44° 38' lat., 123° 30' long.). Fans and coolers were used to keep the greenhouse below 32°C. Pots were watered to field capacity when a representative sample (2 pots/block) dried to a water content of 39% (using formula in Haase and Rose (1994)). This provided a water content range known to promote optimum Douglas-fir morphological and physiological development (Khan et al. 1996). Watering occurred approximately every 10 d during the first 4 months of the experiment and every 15 d during the final 4 months. All seedlings received Peters® 20-20-20 plus micronutrients

Table 4.1. Nutrient composition (%) of Osmocote Plus® (15-9-12) CRF.

Nutrient	---------- (%) ----------
Nitrogen	15.0
NH_4	7.0
NO_3	8.0
Phosphorus (P_2O_5)	9.0
Potassium (K_2O)	12.0
Mg	1.0
S	2.3
B	0.02
Cu	0.05
Fe	0.45
Mn	0.06
Mo	0.02
Zn	0.05

water-soluble fertilizer at a rate of 50 ppm in irrigation water every other watering cycle to prevent mortality of controls due to nutrient stress.

4.3.3 Measurements

Seedlings were transplanted in mid-June and then 25% of the seedlings in each treatment within each block (96 total at each sampling) were randomly selected for harvest at approximately 2-month intervals (i.e. August 16, 2000, October 17, 2000, December 15, 2000, and February 17, 2001) during the 8-month experiment. Seedlings were measured for height, stem diameter, shoot/root volume using water displacement (Burdett 1979), and shoot/root dry weight. At the final harvest (February 2001), two blocks were not sampled because of poor seedling health associated with heat stress due to insufficient air circulation at the position of these blocks.

All seedlings at each harvest were then examined for root architectural development within soil zones relative to the placement of the fertilizer layer. Seedlings were first clipped at cotyledon scar and again 2.5 cm below this point, which represents the position at which lateral roots tend to initiate. The remaining roots were then sliced into three 8 cm sections with the middle (S2) section representing the portion of roots where the fertilizer layer was present and the top (S1) and bottom (S3) being above and below the fertilizer layer (Figure 4.1). Roots in each section were then divided into tap and lateral roots, although the tap root rarely extended into S2. Within each root section, the number of active root tips (white tips > 1 mm in length), the number of first order lateral roots (>1 cm length), first order lateral root length (cm), and dry tap/lateral root weights were assessed.

At 4 and 8 months following transplanting, a composite foliar sample of the 4 seedlings sampled at these points in each treatment for 4 blocks was created by removing needles and drying at 70° C for 72 hr. Foliage was then ground in a Wiley mill (40-mesh screen). Nutrient concentrations (USAg Analytical Services, Inc., Pasco, WA) were determined from foliage samples using methodology in Gavlak (1994). Mean shoot dry weight of each treatment/block combination was used to calculate nutrient contents for vector analysis (Haase and Rose 1995).

Chlorophyll fluorescence (Opti-Sciences Pulse Modulated Chlorophyll Fluorometer OS5-FL) was sampled on September 13, 2000, October 16, 2000, December 14, 2000, and February 16, 2001 on a subsample of 4 seedlings from each treatment/block. A single needle from both the top and middle portion of the terminal seedling shoot was removed from each seedling. Within 3 min following removal, each needle was exposed to a pulse of light from a light emitting diode (LED) passing through a short pass filter (Opti-Sciences 1997). Measurements of Fs (steady state fluorescence), Fms (maximal fluorescence), and the quantum yield ((Fms-Fs)/Fms) of photochemical energy conversion were then recorded.

To determine estimated rates of nutrient release over time, 24 g of CRF (the highest treatment rate) was sealed in nylon fabric and positioned at the same soil depth as the fertilizer layer in 24 pots (4 pots/block) with no seedling. These pots were watered on the same schedule as other pots in the experiment. Every 2 months during

the 8-month study, the CRF in 6 pots was removed, dried at 70°C for 72 hr, and weighed. A temperature data recorder (R-2100, Telog Instruments, Inc.) was positioned at the approximate depth of the fertilizer layer in one randomly selected pot to monitor soil temperature.

Soil electrical conductivity (EC) was measured (Con-100, Oakton Instruments) on soil sampled from pots of seedlings harvested at 6 months following transplant. For each treatment within a block, soil from each of the three root zones from the sampled pots was combined separately to create composite soil samples. Each composite soil sample was then dried at 70°C for 72 hr and sifted with a 2 mm sieve. A 5 g sample of dry soil was then added to 50 ml of distilled water and after 24 hr, EC of the soil solution was measured.

4.3.4 Experimental Design

The experiment was arranged as a randomized complete block design with four CRF rate treatments (0, 8, 16, and 24 g), six blocks, and 384 total seedlings. Each of six benches in the greenhouse was designated as one block due to variations in light availability, temperature, and air circulation. Within a block, 64 pots (16 pots/treatment) were randomly distributed and rearranged monthly. For each sampling, the experimental unit was the group of randomly selected pots/treatment in each block and the sampling unit was the individual seedling.

4.3.5 Statistical Analysis

Data from each sampling were independently subjected to analysis of variance (ANOVA) for a randomized complete block design. Tests for normality, linearity, and constant variance were performed, and transformations were made when necessary to ensure the validity of these assumptions. When significant differences were detected among means for any parameter ($p < 0.05$ in F test), Fisher's Protected Least

Significant Difference procedure was used to detect significant differences ($\alpha \leq 0.05$) among CRF treatments. To account for the correlation between measurements made on top and middle needles of the same experimental unit, needle position was used as a source of variation in the ANOVA for chlorophyll fluorescence data. The significance of the main effects needle position and CRF rate were determined only if the needle position x fertilizer rate interaction was non-significant. Regression analyses were used to determine the relationship between CRF rate and measurements of root morphology. Orthogonal contrasts were used to determine the statistical significance of higher order regression models for explaining variability associated with the data. When more than one regression model was statistically significant, the model with the best fit for the data was selected based on adjusted R^2 values and analysis of the residuals. SAS software (SAS Institute Inc., Cary, NC, USA) was used for the analysis of all data.

4.4 Results

4.4.1 Summary of Results

Root penetration into the lower soil zone (S3), based on lateral root dry weight and active root tips, was restricted at CRF rates of 16 and 24 g. This resulted in a decreased overall root volume, root dry weight, and total lateral root length for these treatments 6 months following transplanting. Shoot volume and dry weight were also reduced at the highest CRF rates. Seedlings in the 8 g CRF treatment, however, had the greatest mean shoot and root development. Nutrient analyses indicated that N was limiting to the growth of controls but inhibitory at the 24 g CRF rate. P content increased at the 8 g rate but was highly depleted at the 24 g rate. Chlorophyll fluorescence analyses showed a significantly greater photochemical quantum yield for all fertilized seedlings 3 months following transplant but not at any of the other sampling times.

4.4.2 Fertilizer Release

Analysis of fertilizer release by weight over time indicated that the initial 24 g dropped to 15.8 g after 2 months and 11.3 g after 4 months, which coincided with the warmest soil temperature range recorded in the experiment (Figure 4.2). The initial 24 g had dropped to 8.3 g by February 2001, 8 months following application (Figure 4.2).

Electrical conductivity measured 6 months following transplant indicated that there were significant differences among treatments in the middle soil zone (S2) where the fertilizer layer was present ($p=0.0011$), though not in the upper (S1) ($p=0.1283$) or lower (S3) ($p=0.1332$) soil zones. All fertilizer treatments had higher EC levels than the control, though there were no differences among fertilizer treatments (Figure 4.3). EC levels were highest in S2 and S3 for CRF treatments, while the EC of S2 was similar to S1 for the control treatment.

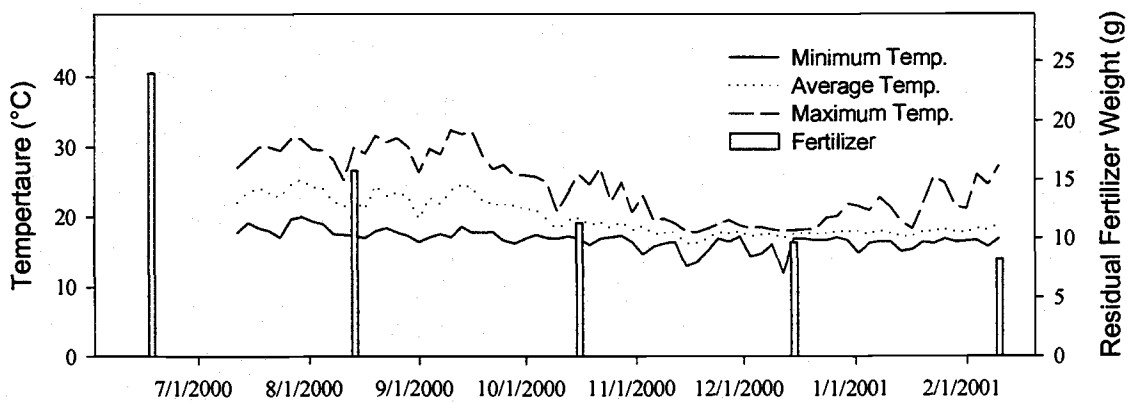


Figure 4.2. Soil temperature at depth of fertilizer layer (left y-axis) and fertilizer release as assessed by weight of residual fertilizer (right y-axis) over time. Note that temperature sensors were installed after the experiment was initiated.

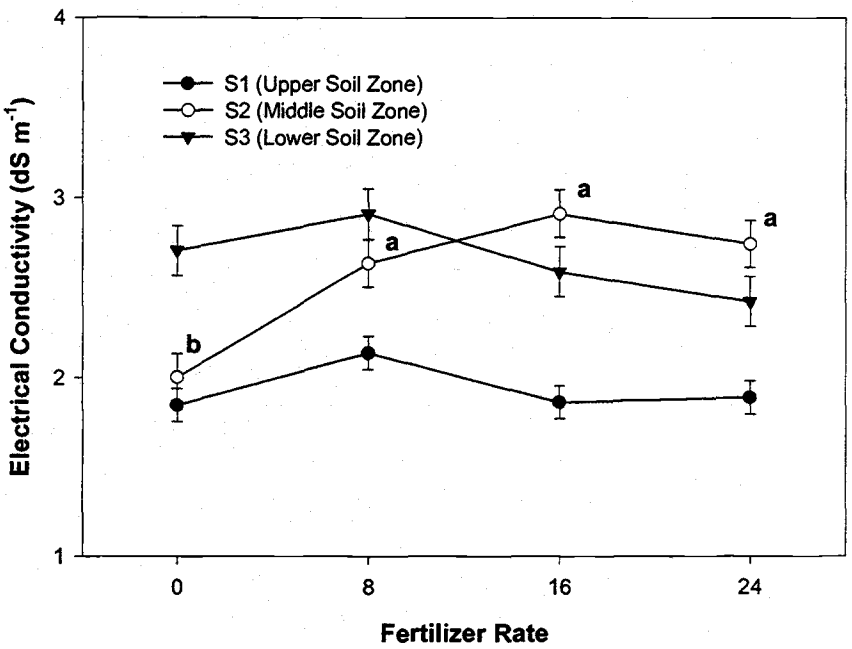


Figure 4.3. Electrical conductivity and standard error of soil from three soil zones for the four different fertilizer treatment rates six months following transplant. Statistical differences were established only in the middle soil zone where the fertilizer layer was present, with the EC of all fertilizer treatments being significantly greater than the control at $\alpha \leq 0.05$.

4.4.3 Whole Plant Morphology

Two months following transplant, seedling shoot:root volume increased linearly ($p=0.0004$) with increasing fertilizer rate (Figure 4.4). This was a function of decreased root volume with increased fertilizer rate ($p=0.0025$) since shoot volume did not differ among treatments ($p=0.8073$). Total first order lateral root length also decreased with fertilizer rate ($p<0.0001$) (Figure 4.5).

Six months following transplant, fertilizer treatments had a significant effect on seedling height growth ($p=0.0029$), diameter growth ($p=0.0046$), shoot volume ($p=0.0004$), root volume ($p=0.0026$), shoot dry weight ($p=0.0059$), and root dry weight ($p=0.0021$). Total lateral root length at this point was also significantly different among fertilizer treatments ($p=0.0002$), although the total number of active root tips was not

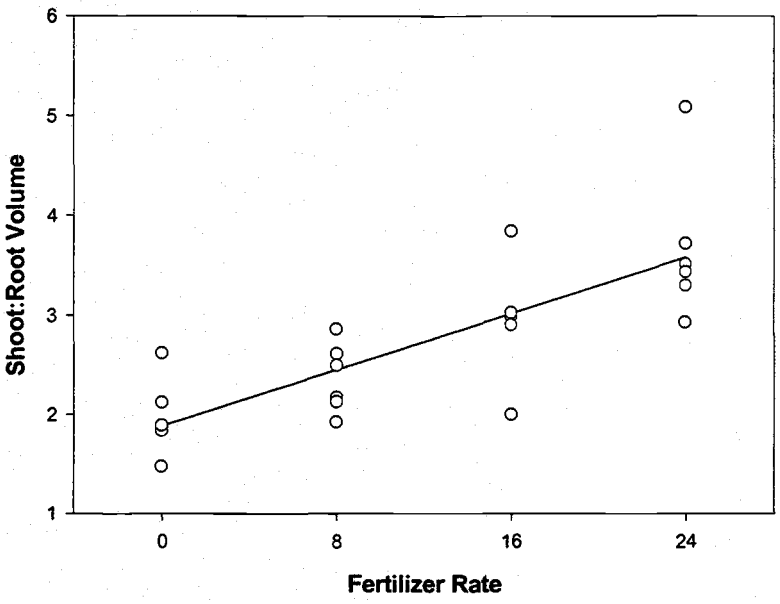


Figure 4.4. Shoot:root volume two months following transplant versus fertilizer rate. Regression equation is SV/RV = 1.88 + 0.071(Rate), $R^2 = 0.60$, $p < 0.0001$.

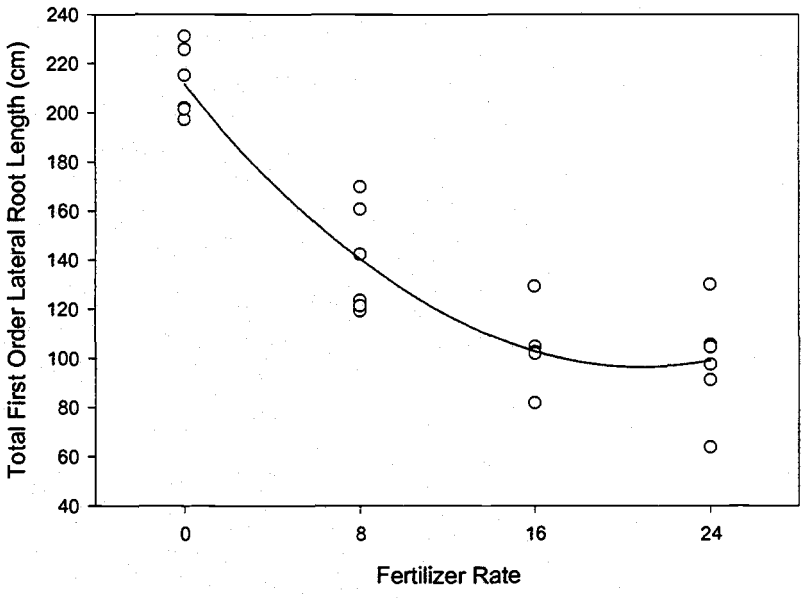


Figure 4.5. Total first order lateral root length two months following transplant versus fertilizer rate. Regression equation is Root Length = 211.81 – 11.01(Rate) + 0.263 ($Rate^2$), $R^2 = 0.87$, $p < 0.0001$.

($p=0.1605$). Seedlings grown with 24 g of CRF were significantly smaller than the other treatments for nearly all parameters, while seedlings in the 8 g fertilizer rate had the highest mean values for most parameters (Table 4.2). Due to a reduced sampling size with the exclusion of 2 blocks, parameters were generally not statistically significant at the 8-month harvest although the same trends established at the 6-month sampling were maintained.

4.4.4 Root Architecture

The presence of a CRF layer affected root architectural development among soil zones. This effect was more pronounced with increasing fertilizer rate. Two months following transplant, lateral root dry weight in S3 decreased linearly with fertilizer rate ($p<0.0001$) and root penetration was severely restricted at the highest fertilizer rate (Figure 4.6). The distribution of active root tips was also affected by fertilizer rate in S1 ($p=0.0007$), S2 ($p=0.0122$), and S3 ($p<0.0001$). Active root tips increased with increasing fertilizer rate in S1 but decreased in S2 and S3 (Figure 4.7).

Six months following transplant, distinct differences in lateral root penetration into the lower soil zones were evident (Figure 4.8). At the 16 and 24 g fertilizer rates, roots that did penetrate to the lowest soil zone tended to be large in diameter and oriented along the periphery of the pots (Figure 4.9). Fertilizer treatments affected lateral root dry weight in S1 ($p=0.0127$), S2 ($p<0.0001$), and S3 ($p=0.0010$). Regression analyses showed a quadratic trend for S1 lateral root dry weight and a decrease in root dry weight with increasing fertilizer rate for S2 and S3 (Figure 4.10).

4.4.5 Nutrients

Fertilizer treatments significantly affected N concentrations 4 months following transplant ($p=0.0198$), though not statistically significant at 8 months ($p=0.1642$). At each sampling, mean concentrations of N increased with fertilizer rate though contents

Table 4.2. Mean values and standard error in parentheses for seedling morphology by fertilizer rate in December 2000 (six months following transplant). For each parameter, means followed by the same letter in a row did not differ significantly at $\alpha \leq 0.05$.

	Fertilizer Rate (g)			
	0	8	16	24
Height Growth (cm)	16.0 (0.70) b	18.2 (0.67) a	17.0 (0.67) ab	13.7 (0.74) c
Diameter Growth (mm)	3.26 (0.16) b	3.83 (0.15) a	3.89 (0.15) a	3.06 (0.17) b
Height:Diameter	5.04 (0.17) a	4.92 (0.16) a	4.60 (0.16) a	4.82 (0.16) a
Shoot Volume (cm^3)	16.0 (0.99) ab	18.4 (0.94) a	13.8 (0.94) b	10.5 (1.04) c
Root Volume (cm^3)	8.91 (0.61) ab	10.2 (0.58) a	8.22 (0.58) b	6.14 (0.64) c
Shoot:Root Volume	1.84 (0.14) a	1.86 (0.14) a	1.81 (0.14) a	1.92 (0.15) a
Shoot Dry Weight (g)	3.04 (0.21) ab	3.61 (0.20) a	2.91 (0.20) bc	2.34 (0.22) c
Root Dry Weight (g)	1.43 (0.10) ab	1.71 (0.10) a	1.39 (0.10) b	1.01 (0.11) c
Shoot:Root Dry Weight	2.20 (0.12) a	2.17 (0.11) a	2.18 (0.11) a	2.51 (0.12) a
Lateral Root Length (cm)	188 (12) a	161 (11) a	113 (11) b	96 (12) b
Number of Active Roots	136 (14) a	135 (12) a	105 (13) a	100 (14) a

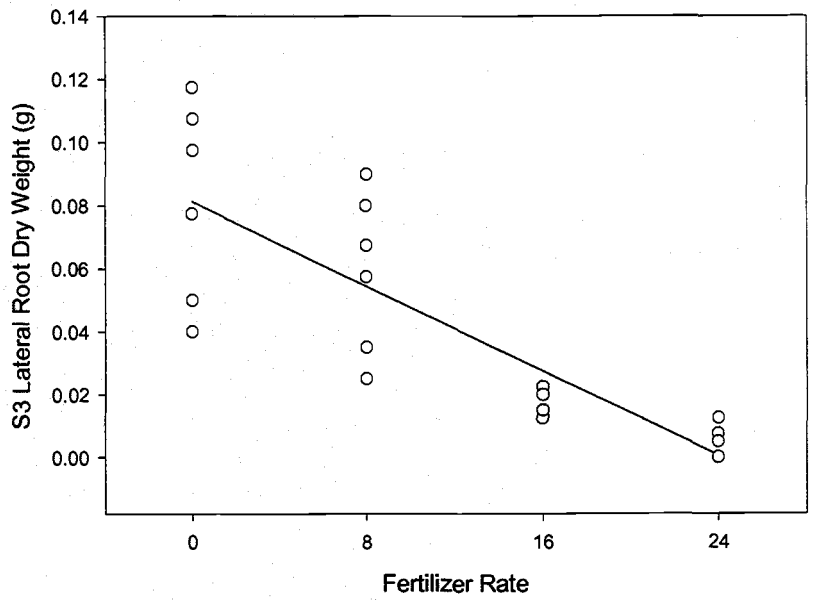
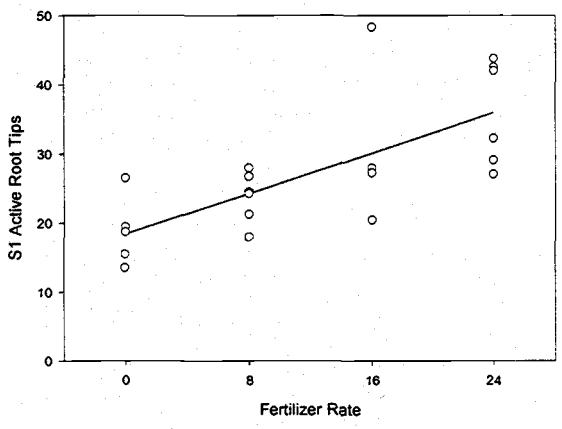


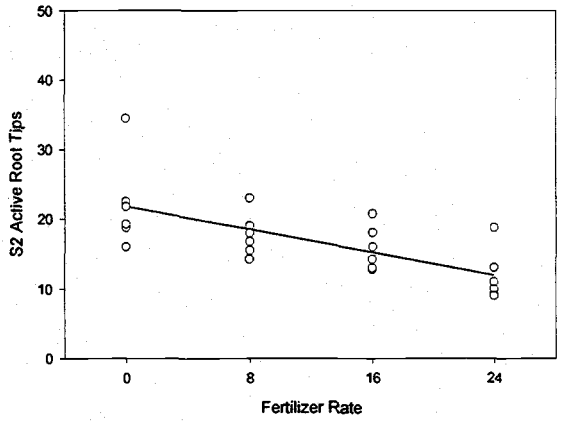
Figure 4.6. Lateral root dry weight in S3 two months following transplant versus fertilizer rate. Data presented on original scale prior to transformation. Regression equation is LOG (S3 Root Dry Wt. + 0.01) = -2.48 –0.080(Rate), $R^2 = 0.84$, $p < 0.0001$.

were reduced at the 24 g fertilizer rate (Table 4.3, Figure 4.11). Concentrations of P were affected by fertilizer treatments at 4 months (p=0.0277), though not statistically significant at 8 months (p=0.1190) (Table 4.3). Mean P concentrations generally decreased with increasing fertilizer rate at both 4 and 8 months, although concentrations of P in the 8 g treatment were equal to the control at 8 months (Table 4.3). Concentrations of Ca (p=0.0006) (Table 4.3) and Mn (p=0.0003) (Table 4.4) increased with increasing fertilizer rate after 4 months. At 8 months, concentrations of B (p=0.0175) increased, while Zn concentrations (p=0.0151) decreased with fertilizer rate (Table 4.4).

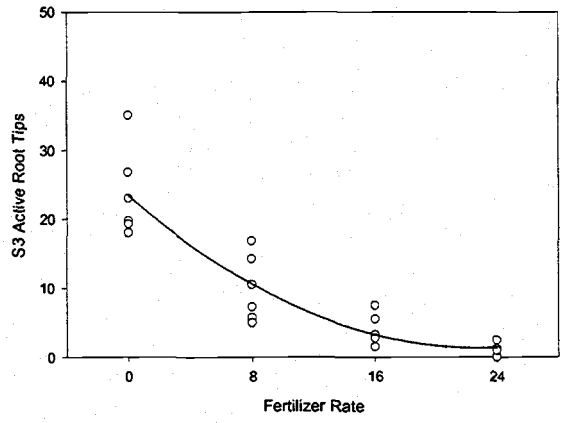
At both the 4 and 8-month sampling, vector diagrams (Figure 4.11) reflected toxicities associated with N at the 24 g rate due to increased concentration and decreased shoot dry weight (Figure 4.11). Nitrogen improved growth at the 8 and 16 g rate at each sampling based on an increase in concentration, content, and shoot dry weight (Figure 4.11). Although P concentration and content were reduced in all



a



b



c

Figure 4.7. Number of white root tips in S1 (a), S2 (b), and S3 (c) two months following transplant versus fertilizer rate. Data for (c) presented on original scale prior to transformation. Regression equations are (a) S1 Root tips = 18.425 + 0.728 (Rate), $R^2 = 0.51$, $p < 0.0001$ (b) S2 Root tips = 21.80 – 0.410 (Rate), $R^2 = 0.43$, $p = 0.0003$ (c) LOG (S3 Root tips + 1) = 3.11 – 0.173 (Rate) + 0.024 (Rate^2), $R^2 = 0.95$, $p < 0.0001$.

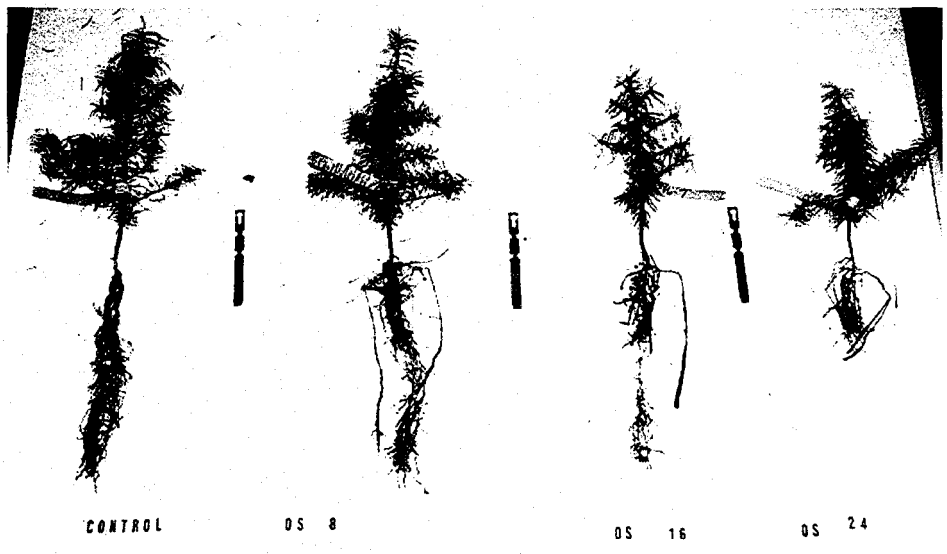


Figure 4.8. Example seedlings (0, 8, 16, 24 grams from L to R) six months following transplant showing decreased root penetration into lower soil zones with increased fertilizer rate.

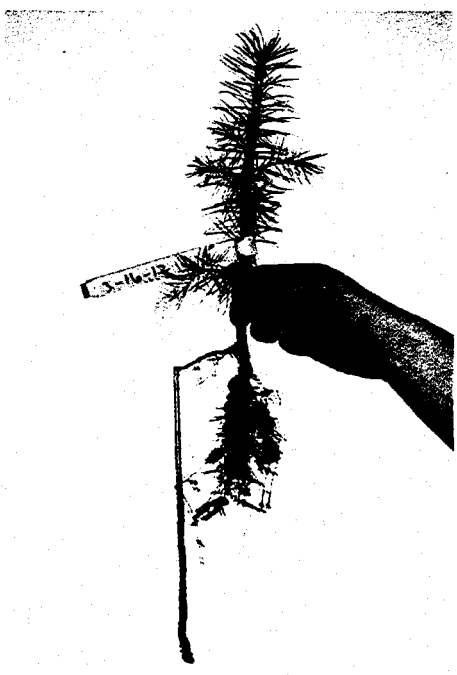
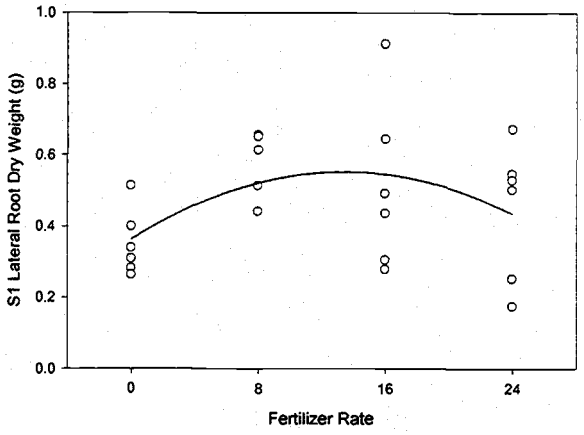
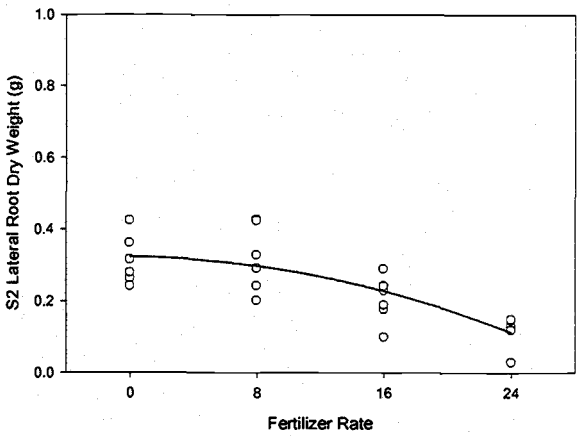


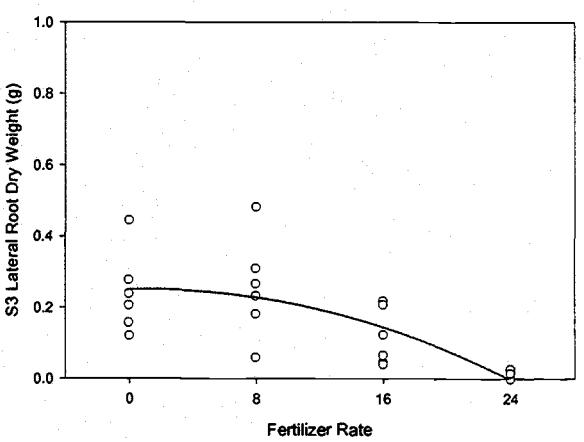
Figure 4.9. Seedling in the 16g rate six months following transplant illustrating the tendency for deep roots to be larger in diameter and oriented along the periphery of the pots.



a



b



c

Figure 4.10. Lateral root dry weight in S1 (a), S2 (b), and S3 (c) versus fertilizer rate six months following transplant. Data presented on original scale prior to transformation. Regression equations are (a) LOG (S1 RDW + 0.01) = -1.30 + 0.095(Rate) – 0.0034(Rate^2), $R^2 = 0.27$, $p < 0.0260$ (b) LOG (S2 RDW + 0.01) = -1.21 + 0.016(Rate) – 0.0026(Rate^2), $R^2 = 0.59$, $p < 0.0001$ (c) LOG (S3 RDW + 0.01) = -1.68 + 0.032(Rate) –0.0060(Rate^2) , $R^2 = 0.72$, $p < 0.0001$.

Table 4.3. Mean values for macronutrient concentration and content (based on mean shoot dry weight (g) of seedlings in experimental unit) and standard error in parentheses for fertilizer rate at 4 and 8 months following transplant. For each nutrient and within each sampling point, means followed by the same letter within a column did not differ significantly at $\alpha \leq 0.05$.

	October 2000		February 2001	
	Concentration (%)	Content (g)	Concentration (%)	Content (g)
Nitrogen				
0 g	2.79 (0.09) b	6.72 (0.50) a	3.05 (0.10) a	10.24 (1.62) a
8 g	3.01 (0.09) ab	7.87 (0.48) a	3.24 (0.09) a	12.58 (1.57) a
16 g	3.14 (0.09) a	7.75 (0.47) a	3.27 (0.09) a	12.68 (1.57) a
24 g	3.27 (0.08) a	6.69 (0.46) a	3.39 (0.09) a	10.16 (1.54) a
Phosphorus				
0 g	0.63 (0.04) a	1.52 (0.08) a	0.60 (0.06) a	2.02 (0.44) a
8 g	0.51 (0.04) ab	1.34 (0.08) a	0.60 (0.06) a	2.36 (0.42) a
16 g	0.44 (0.04) b	1.09 (0.07) b	0.52 (0.06) a	2.17 (0.42) a
24 g	0.44 (0.04) b	0.91 (0.07) b	0.42 (0.05) a	1.32 (0.42) a
Potassium				
0 g	3.47 (0.14) a	8.37 (0.52) a	4.20 (0.29) a	13.89 (1.34) a
8 g	3.36 (0.13) a	8.77 (0.50) a	3.93 (0.28) a	14.83 (1.30) a
16 g	3.42 (0.13) a	8.46 (0.49) a	4.08 (0.29) a	15.58 (1.30) a
24 g	3.46 (0.12) a	7.15 (0.48) a	4.05 (0.28) a	11.74 (1.30) a
Calcium				
0 g	0.79 (0.08) b	1.93 (0.32) b	1.45 (0.12) a	4.92 (0.66) a
8 g	0.98 (0.08) b	2.57 (0.30) b	1.66 (0.12) a	6.36 (0.64) a
16 g	1.43 (0.08) a	3.57 (0.30) a	1.77 (0.12) a	6.86 (0.64) a
24 g	1.36 (0.07) a	2.77 (0.29) ab	2.00 (0.12) a	5.82 (0.63) a
Magnesium				
0 g	0.26 (0.01) a	0.63 (0.04) a	0.31 (0.02) a	1.02 (0.10) a
8 g	0.22 (0.01) a	0.58 (0.05) a	0.28 (0.02) a	1.09 (0.10) a
16 g	0.24 (0.01) a	0.60 (0.05) a	0.25 (0.02) a	0.97 (0.10) a
24 g	0.25 (0.01) a	0.51 (0.05) a	0.26 (0.02) a	0.77 (0.09) a
Sulfur				
0 g	0.15 (0.01) a	0.35 (0.03) a	0.14 (0.01) a	0.46 (0.09) a
8 g	0.12 (0.01) a	0.32 (0.03) a	0.16 (0.01) a	0.59 (0.08) a
16 g	0.11 (0.01) a	0.27 (0.03) ab	0.13 (0.01) a	0.55 (0.08) a
24 g	0.11 (0.01) a	0.22 (0.03) b	0.12 (0.01) a	0.38 (0.08) a

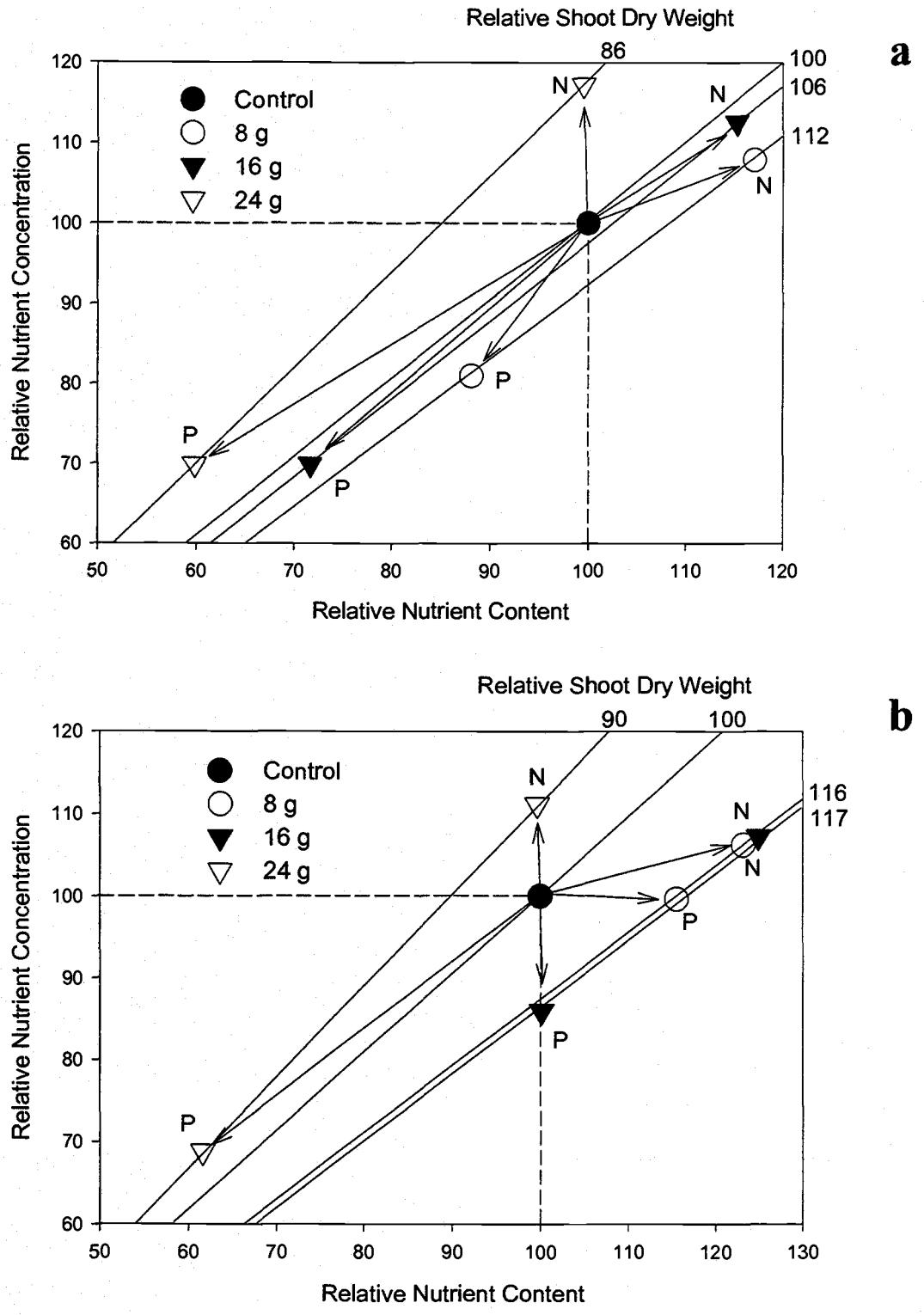


Figure 4.11. Vector diagrams for total nitrogen and phosphorus by fertilizer rate in October 2000 (a) and February 2001 (b).

Table 4.4. Mean values for micronutrient concentration and content (based on mean shoot dry weight (g) of seedlings in experimental unit) and standard error in parentheses for fertilizer rate at 4 and 8 months following transplant. For each nutrient and within each sampling point, means followed by the same letter within a column did not differ significantly at $\alpha \leq 0.05$.

	October 2000		February 2001	
	Concentration (ppm)	Content (g)	Concentration (ppm)	Content (g)
Boron				
0 g	61.0 (4.9) a	0.0148 (0.001) bc	91.4 (4.6) b	0.0308 (0.005) a
8 g	81.4 (4.8) a	0.0214 (0.001) a	110.0 (4.4) a	0.0423 (0.005) a
16 g	83.3 (4.7) a	0.0205 (0.001) ab	117.0 (4.4) a	0.0461 (0.005) a
24 g	82.8 (4.6) a	0.0170 (0.001) b	110.0 (4.3) a	0.0330 (0.005) a
Zinc				
0 g	60.1 (8.4) a	0.0144 (0.001) a	65.0 (5.3) a	0.0226 (0.004) a
8 g	53.8 (8.0) a	0.0141 (0.001) a	56.1 (5.2) ab	0.0222 (0.004) a
16 g	43.2 (7.9) a	0.0107 (0.001) a	47.7 (5.2) b	0.0190 (0.004) a
24 g	57.2 (7.7) a	0.0108 (0.001) a	35.0 (5.1) c	0.0112 (0.004) a
Manganese				
0 g	59.5 (7.0) c	0.014 (0.003) a	99.04 (19.7) a	0.035 (0.013) a
8 g	82.2 (6.7) b	0.022 (0.003) a	109.68 (19.1) a	0.044 (0.013) a
16 g	95.9 (6.6) b	0.024 (0.003) a	147.24 (19.1) a	0.061 (0.013) a
24 g	129.0 (6.4) a	0.027 (0.003) a	170.79 (18.7) a	0.055 (0.013) a

fertilizer treatments as compared to the control at 4 months, the 8 g rate showed an increase in P content at 8 months, while P uptake in the 24 g treatment remained lower (Table 4.3, Figure 4.11).

4.4.6 Chlorophyll Fluorescence

Needle position on the shoot affected chlorophyll fluorescence parameters (Table 4.5). Maximal fluorescence (Fms) of needles sampled from the middle of the shoot was significantly higher than those from the top of the shoot for all sampling points (9-13-00, $p<0.0001$; 10-16-00, $p=0.0003$; 12-14-00, $p=0.0065$; 2-16-01, $p=0.0033$). Quantum yield was significantly higher for middle needles at the February 16, 2001 sampling date only ($p=0.0011$).

Table 4.5. Mean values and standard error in parentheses for chlorophyll fluorescence parameters at four sampling points for needle position. At each sampling point for either fertilizer rate or needle position, means for each parameter followed by the same letter within a row did not differ significantly at $\alpha \leq 0.05$.

Sampling Date		Needle Position	
		Middle	Top
9/13/2000	Fs	152 (3.81) a	124 (3.81) b
	Fms	553 (16.46) a	427 (16.46) b
	Quantum Yield	0.71 (0.0096) a	0.70 (0.0096) a
10/16/2000	Fs	90 (3.16) a	83 (3.16) a
	Fms	479 (16.8) a	425 (16.8) b
	Quantum Yield	0.81 (0.0104) a	0.80 (0.0104) a
12/14/2000	Fs	189 (8.06) a	187 (8.05) a
	Fms	793 (24.0) a	721 (23.9) b
	Quantum Yield	0.74 (0.0304) a	0.71 (0.0304) a
2/16/2001	Fs	196 (7.87) a	198 (7.87) a
	Fms	873 (30.38) a	742 (30.38) b
	Quantum Yield	0.76 (0.019) a	0.71 (0.019) b

Three months following transplant (September 13, 2000), seedlings fertilized with CRF had significantly greater mean values than controls for Fms ($p=0.0037$) and quantum yield ($p=0.0389$) (Table 4.6). In subsequent samplings, this trend was no longer present and mean values for quantum yield were reduced (10-16-00, $p=0.3878$; 12-14-00, $p=0.4863$; 2-16-01, $p=0.5626$) at the highest CRF rate compared to all other treatments, though differences were not statistically significant (Table 4.6).

Table 4.6. Mean values and standard error in parentheses for chlorophyll fluorescence parameters at four sampling points for fertilizer rate. At each sampling point for either fertilizer rate or needle position, means for each parameter followed by the same letter within a row did not differ significantly at $\alpha \leq 0.05$.

Sampling Date		*Fertilizer Rate (g)*			
		0	8	16	24
9/13/2000	Fs	127 (6.3) a	142 (6.3) a	134 (6.3) a	151 (6.3) a
	Fms	411 (22.6) b	520 (22.6) a	491 (22.6) a	538 (22.6) a
	Quantum Yield	0.67 (0.013) b	0.71(0.013) a	0.72 (0.013) a	0.71 (0.013) a
10/16/2000	Fs	85 (4.5) a	84 (4.4) a	92 (4.4) a	85 (4.6) a
	Fms	468 (20.1) a	440 (19.8) a	478 (19.8) a	421 (20.3) a
	Quantum Yield	0.82 (0.013) a	0.80 (0.013) a	0.81 (0.013) a	0.80 (0.013) a
12/14/2000	Fs	186 (12.4) a	194 (12.4) a	185 (12.4) a	188 (12.5) a
	Fms	774 (41.7) a	800 (41.2) a	776 (41.2) a	676 (42.2) a
	Quantum Yield	0.75 (0.051) a	0.75 (0.051) a	0.75 (0.051) a	0.66 (0.051) a
2/16/2001	Fs	176 (13.5) a	194 (13.4) a	206 (13.8) a	211 (14.1) a
	Fms	747 (48.5) a	853 (47.6) a	866 (49.8) a	764 (50.8) a
	Quantum Yield	0.72 (0.036) a	0.77 (0.036) a	0.75 (0.036) a	0.70 (0.037) a

4.5 Discussion

4.5.1 *Root Architectural Development*

The layering of CRF as a single layer beneath the transplanted root system had a significant influence on root architectural development. Compared to the control treatment, roots tended to proliferate in the upper soil zone (S1) with increasing fertilizer rate (Figures 4.7 and 4.10). Root penetration into the middle (S2) and lower (S3) soil zones was restricted at the highest CRF rates (Figures 4.6, 4.7, and 4.10). This was based on both the numbers of active roots tips, which are anatomically suited for efficient ion uptake (Peterson et al. 1999), and lateral root dry weight. Roots tend to proliferate in areas of high nutrient supply without increasing overall root growth, particularly when the remainder of the plant is nutrient stressed (Friend et al. 1990).

No satisfactory mechanism has been provided to explain the proliferation of roots in localized sources of nutrient supply (Granato and Raper 1989; Robinson 1994). The plant may allocate a preferential supply of assimilates and carbohydrates to the nutrient-rich area of the root system (Drew and Saker 1975; Barta 1976), thus shifting growth to a portion of the roots rather than increasing growth in the entire root system (Friend et al. 1990). Granato and Raper (1989) attributed the proliferation of corn roots in localized sources of nutrient supply to *in situ* reduction and utilization of absorbed NO_3^-. Root response to localized nutrient sources may also be hormonal (Coutts and Philipson 1976) as abscisic acid (ABA), for example, is a key regulator of root extension (Wraith and Wright 1998).

In this experiment, the restriction of root penetration into S2 and S3 at the highest CRF rates was best explained by the localized increase in fertilizer salt concentrations. Trees, conifers in particular, are highly sensitive to salt injury (Tinus and McDonald 1979). Root apical meristems are subject to death due to the buildup of toxic ion concentrations and resulting decreases in soil osmotic potential (Sherwin 1923; Skinner et al. 1945; Drew 1975). Lack of adequate water in the root zone tends to accelerate the compounding of salt concentrations (Landis 1989; Huett 1997a).

Although salt tolerance in roots varies with stage of seedling development (Zekri 1993), the recommended standard for salinity (EC) in water when growing conifers is 1.5 dS m^{-1} (Landis 1989). Growth of Colorado blue spruce (*Picea pungens* Engelm.) and Douglas-fir, conifers highly sensitive to excessive EC levels, was reduced by 10% when EC increased from 1.0 to 1.4 dS m^{-1} and 50% at 2.5 dS m^{-1} (Landis 1989). Phillion and Bunting (1983) found that black spruce (Picea mariana (Mill) B.S.P.) and white spruce (Picea glauca (Moench) Voss) were healthy at an EC up to 2.5 dS m^{-1} but levels above 4.0 dS m^{-1} were lethal.

Six months following transplant, EC levels in S2 were significantly greater for all treatments receiving CRF (range of means 2.6-2.9 dS m^{-1}) compared to the control (mean 2.0 dS m^{-1}), while EC levels in S1 (range of mean 1.85 to 2.14 dS m^{-1}) were similar among treatments (Figure 4.3). Because this sampling was taken after the majority of the CRF had released (Figure 4.2), it is likely that S2 EC levels for the CRF treatments reached levels higher than that recorded despite frequent watering. Additionally, EC levels in S2 probably increased with CRF rate and this may have prevented roots from penetrating into the lower soil zones at the highest CRF rate treatments. The proliferation of roots in high CRF treatment rates in S1, and a reduction in root growth in S2 where the fertilizer layer was actually present (Figures 4.7 and 4.10) helps to support this conclusion. Although capillary action may cause some upward movement of fertilizer nutrients, it does not occur to the degree of downward leaching particularly under adequate irrigation. This is supported by the similar EC among all treatments in S1 (Figure 4.3). Additionally, roots in the 16 and 24 g CRF treatments that did penetrate into S3 tended to be oriented along the sides of the pots, where salt toxicity levels were likely reduced (Figure 4.9) as compared to the center of the pots.

4.5.2 Seedling Morphology

Two months following transplant, seedling shoot:root volume increased with fertilizer rate (Figure 4.4) due to less root volume growth with increasing rate.

Increases in seedling shoot:root volume with fertilization have been previously reported for Douglas-fir (Carlson and Preisig 1981; Shaw et al. 1998), Jeffrey pine (*Pinus jeffreyi* Grev. & Balf.) (Walker and Huntt 1992; Walker and Kane 1997), and singleleaf pinyon pine (*Pinus monophylla* Torr. & Frem.) (Walker and Huntt 1992), sycamore (*Platanus occidentalis* L.), and Sitka spruce (Mackie Dawson et al. 1995). Under high nutrient availability and no water stress, seedlings tend to allocate more resources to shoot development and less to roots, presumably because belowground resources are sufficient for growth (Marschner 1995).

Interestingly, shoot:root ratios were not significantly different among treatments at any other sampling. Osmocote® tends to release a more rapid initial flush of nutrients than many comparable CRF with different coating technologies (Huett and Gogel 2000) and most of the N and K may be released soon after application (Huett 1997a; Huett 1997b). Eight months following application, the original 24 g of fertilizer had dropped to 8.3 g (Figure 4.2). Thus, over 50% of nutrients that released during the experiment had released within 2 months following application (based on a fertilizer weight of 15.8 g) and over 80% had released 4 months after application (based on a fertilizer weight of 11.3 g). The rapid release of fertilizer nutrients during this period may have stimulated shoot growth without the need for compensatory root growth, particularly under well-watered conditions. However, based on the restrictions in root penetration at high CRF rates 2 months following transplant (Figures 4.6 and 4.7), it is more likely that high concentrations of fertilizer salts prevented adequate root growth which resulted in the greater shoot:root volume at high CRF rates. In subsequent samplings, toxicities associated with excess fertilizer salts in the soil media at high CRF rates began to negatively affect shoot growth, acting to reduce shoot:root ratios to levels proportional to that of the other treatments.

Six months following transplant, seedlings in the 8 g rate had higher mean values for most morphological parameters than any other treatment, while seedlings in the 16 and 24 g rates tended to have lower values (Table 4.2). Other studies have shown improved growth rates for forest tree seedlings fertilized with CRF compared to water-soluble fertilizer or unfertilized treatments (Walker and Huntt 1992; Walker and Kane 1997; Krasowski et al. 1999; Walker and Huntt 2000). It is difficult, however, to

find experiments that have documented growth reductions when using CRF with forest tree seedlings despite the many reports of toxicities associated with high rates of fertilization with nutrients in solution. Ingestad (1979) distinguished critical N levels of 50mg/l and toxicity at 400 mg/l for Scotch pine (*Pinus sylvestris* L.). Concentrations of 40-60 mg/L of N in solution increased height growth of slash pine (*Pinus elliottii* Engelm.), but concentrations above 180 mg/l reduced growth (Dewald et al. 1992). Lu (1998) found that root and shoot dry weight of Douglas-fir decreased when N concentrations were increased from 50 to 200 ppm and attributed this to possible toxicities to roots associated with NH_4^+ ions. The lack of literature citing negative responses associated with CRF may be because CRF gradually release nutrients, minimizing the chance for the buildup of excessive nutrient levels, particularly with adequate irrigation. Despite improved release technologies, however, high CRF rates clearly acted to reduce plant growth in this experiment.

4.5.3 Nutrient Uptake

Mean concentrations of foliar N increased with fertilizer rate at both nutrient samplings (4 and 8 months following transplant), though N content tended to decrease at the 24 g CRF rate reflecting the reduced shoot growth in this treatment (Table 4.3). Vector analysis, which allows for simultaneous comparisons of plant growth, nutrient concentration, and nutrient content (Haase and Rose 1995) showed that N was limiting to the growth of control seedlings at both samplings and that N enhanced growth at the 8 and 16 g rate (Figure 4.11). Fertilization at the 8 and 16 g rates enhanced N uptake, based on increased concentrations and content, which lead to greater shoot dry weights. At the 24 g rate, however, vector analysis, as interpreted by Timmer and Stone (1978) and Timmer and Armstrong (1987a), indicated that N was "toxic" to growth at both sampling points due to reductions in shoot growth despite increases in N concentration. Vector analysis has been used previously to identify "toxicities" from N associated with high fertilization rates in red pine (*Pinus resinosa* Ait.) (Timmer and Armstrong 1987a).

This helps to explain the significant growth reductions noted in seedlings grown under 24 g of CRF.

At both samplings, mean concentrations of P tended to decrease with fertilizer rate (Table 4.3). This effect has been observed for Douglas-fir fertilized with N in the field (Gill and Lavender 1983; Roth and Newton 1996) and nursery (van den Driessche 1980). It is difficult to find a satisfactory explanation for this response. Visser et al. (1993) found that when N fertilizer was applied primarily as NH_4^+, less P was taken up than when N fertilizer was applied as NO_3^- and attributed this to acidification of the rhizosphere when N was taken up as NH_4^+. Vector analysis showed reduced P concentration and content for all CRF treatments 4 months following sampling (Figure 4.11). However, P concentration at the 8 g rate was equal to that of the control after 8 months. Because shoot dry weight was greater in the 8 g rate compared to the control, P content (concentration x shoot dry weight) was also greater. Since P is highly immobile in the soil, it can be difficult to obtain a P response from fertilization if root development is reduced relative to shoot growth. The higher mean root volume and similar shoot:root volume as compared to other treatments (Table 4.2) helps to explain the positive P response at 8 g, as roots could effectively extract P from the soil and supply it to the foliage.

4.5.4 Chlorophyll Fluorescence

Chlorophyll fluorescence parameters were consistently higher in needles sampled from the middle of the plant as compared to the top of the plant (Table 4.5). Researchers have reported higher photosynthetic rates in current year foliage as compared to foliage from previous seasons (Hom and Oechel 1983; Teskey et al. 1984; Kajimoto 1990; Oleksyn et al. 1997). Needles sampled from seedlings in this experiment, however, were all from the same growth flush. Actively growing foliage generally exhibits a low photosynthetic capacity and high respiration rate (Radoglou and Teskey 1997). In a study with loblolly pine (*Pinus taeda* L.), the highest photosynthetic rates occurred when the foliage was 90%+ expanded (Radoglou and

Teskey 1997). This helps explain the reduced chlorophyll fluorescence of needles that were still actively elongating prior to the December 2000 sampling. It is interesting, however, that differences in chlorophyll fluorescence among needles in different positions were still apparent when seedlings were dormant in December 2000 and February 2001. This response illustrates the importance of developing a methodology to sample needles for chlorophyll fluorescence from a specific part of the plant, regardless of seedling dormancy status.

Maximal fluorescence (Fms) and quantum yield were higher for seedlings in all CRF treatments 3 months following transplant as compared to controls (Table 4.6). This coincided with the time period at which the majority of CRF had released (Figure 4.2). Increased nutrient availability for seedlings fertilized with CRF may have resulted in higher photosynthetic capacities at this point. Birchler et al. (2001) found that chlorophyll fluorescence (Fv/Fm) of nursery-grown Douglas-fir seedlings fertilized with N and K in fall was consistently higher than unfertilized seedlings. Lower rates of chlorophyll fluorescence due to N deficiencies have been previously reported for wheat (Shangguan et al. 2000), beans (Lima et al. 1999), and tomatoes (Guidi et al. 1998). Chlorophyll fluorescence parameters were not significantly different among treatments at later sampling points, however. Though not statistically significant, mean values for quantum yield were lowest for the 24 g CRF treatment, possibly due to fertilizer inhibitions acting to reduce photosynthetic capacities.

4.6 Conclusions

Despite improvements in forest tree seedling fertilization due to the advent of CRF, this study demonstrated that fertilizer rates can reach excessive levels and adversely affect seedling development. This is due to rapid release of nutrient contents from the fertilizer prills, even under well-irrigated conditions. CRF manufacturers must continue to develop this technology in order to provide gradual and consistent release of nutrients. Using concepts of exponential fertilization (Timmer and Armstrong 1987b; Timmer 1997), CRF may be applied with periodic increases in application rates that

coincide with the developmental stages of the plant. This will help to nutrient uptake and plant growth, while minimizing negative effects associated with excessive CRF rates.

When nutrients are limiting to seedling growth, application of CRF at ideal rates will generally improve productivity. Determining the ideal CRF composition, application timing or rate, and release character is difficult, however, and depends on a complex interaction of factors including seedling morphology/physiology and environmental growing conditions (e.g. soil type, water content, sunlight, temperature).

Localized CRF placement beneath the root system affected root architectural development with roots tending to proliferate above the fertilizer with increasing rate. At excessively high CRF rates, root penetration into lower soil zones will be severely restricted. This illustrates the importance of using conservative CRF rates and mixing fertilizer uniformly in soil media when growing seedlings in containers if the goal is to produce seedlings with desirable root architecture capable of meeting seedling needs. Though the use of pots and the fact that water was never limiting limits conclusions regarding root development when outplanted with CRF in the field, the same type of restrictions in root penetration at high CRF rates could occur when seedling roots grow through a layer of CRF applied to the planting hole. This would have implications regarding drought resistance in areas characterized by hot, dry summers during which seedlings must access subsurface water to resist drought stress with root systems that developed during spring.

Chlorophyll fluorescence was able to detect differences in photosynthetic capacities at the most active time of CRF nutrient release. It is essential to sample foliage for chlorophyll fluorescence in a systematic manner to avoid variations in data based on the developmental stage of foliage. Chlorophyll fluorescence may provide an inexpensive, non-invasive, and non-destructive means to detect changes in seedling physiological development based on fertilization.

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Chapter Five

Tolerance of Field-Fertilized Douglas-fir Seedlings in the Oregon Coast Range to Moisture Stress

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5.1 Abstract

The objective of this experiment was to determine how differences in initial seedling root volume and field fertilization affect seedling growth, root architectural development, and moisture stress. Coastal Douglas-fir (*Pseudotsuga menzieseii* (Mirb.) Franco) seedlings (1+1) were planted into a site on the eastern edge of the Oregon Coast Range in January 2000 and excavated in December 2000. Seedlings were divided into two initial root volume categories (R1: 8-13 cm^3 and R4: 23-35 cm^3) and either unfertilized or fertilized with 60 g Simplot Polyon® 19-6-12 controlled-release fertilizer (CRF) in the planting hole. Regardless of initial root volume, fertilized seedlings became significantly more water stressed than unfertilized seedlings during summer, reaching a minimum mean (± 0.1 SE) pre-dawn plant xylem pressure potential (XPP) of −1.9 MPa, compared to −1.3 Mpa for unfertilized seedlings. Rates of morning and afternoon stomatal conductance were significantly greater for unfertilized seedlings at the end of September. Shoot:root dry weight was significantly higher for fertilized seedlings and was inversely correlated ($R^2 = 0.54$) with XPP. Lateral root length, active root tips, and tap root dry weight were significantly less for fertilized seedlings. Root volume and lateral root dry weight of R4 fertilized seedlings was not significantly greater than unfertilized R1 seedlings following excavation. No localized root proliferation was found in four vertical soil zones relative to the placement of CRF as root development of fertilized seedlings was inhibited in all soil zones. Poor root growth of fertilized seedlings, probably associated with the buildup of salts due to the release of excessive fertilizer nutrients into the soil solution, increased seedling moisture stress, restricted photosynthesis, and led to reductions in aboveground morphological development. Criteria for successful field fertilization must consider the anticipated drought level of the site to attain a positive seedling response.

5.2 Introduction

The success of reforestation operations may be improved if seedlings remain on nearly the same growth trajectory following outplanting into the field that they were on in the nursery. Controlled-release fertilizers (CRF) offer a potential means to enhance seedling field performance substantially and interest in their application has increased in recent years (Haase and Rose 1997). With a single application, CRF will supply seedlings with improved nutrition for an entire growing cycle following application. The ultimate goal of CRF research has been to develop a product that delivers nutrients at a rate matching plant demand, thus improving crop yield and minimizing the loss of nutrients due to leaching (Hauck 1985; Goertz 1993).

Controlled-release fertilizers vary in terms of their nutrient formulations, estimated times for nutrient release, and prill coatings. The thickness and composition of CRF coatings generally determine the rate of nutrient release (Hauck 1985). Some of the first CRF were sulfur-coated urea (SCU). In most cases, polymer-coated CRF have replaced SCU because they provide a more gradual and consistent pattern of nutrient release (Goertz 1993). Nutrient release of most polymer-coated CRF is determined by the diffusion of water through the semi-permeable membrane (Goertz 1993). This process is accelerated at higher soil temperatures, with soil water content providing little influence on release (Kochba et al. 1990). Types of coatings include polymeric-resins, polyurethane, and polyolefin. These materials provide different rates and patterns of nutrient release among comparable CRF types (Huett and Gogel 2000) with the most rapid N and K release often occurring soon after application (Huett 1997a; Huett 1997b). Rapid nutrient release from CRF has the potential to negate positive benefits from field fertilization due to excessive leaching (Huett and Morris 1999) or high concentrations of fertilizer salts.

Reports of seedling growth responses to fertilization at outplanting vary considerably (van den Driessche 1997). Conflicting results are likely due to the variable experimental combinations of fertilizer formula, application rate, placement, site characteristics, planting stock, and silvicultural treatments. Nonetheless, positive responses to seedling field fertilization have been reported for Douglas-fir (*Pseudotsuga*

menziesii (Mirb.) Franco) (Austin and Strand 1960; Rothacher and Franklin 1964; Brix and Ebell 1969). Carlson (1981) found that application of Agriform® and Osmocote® CRF to the root zone of 1-0 plug Douglas-fir at the time of planting increased shoot and root growth relative to controls following two growing seasons. Significant height growth responses after several growing seasons were reported for Douglas-fir fertilized at the time of planting, with rate of N being more important than fertilizer type or release rate (van den Driessche 1988). Application of a slow-release NPK fertilizer at the time of outplanting improved growth of both containerized and bareroot white spruce (*Picea glauca* Moench Voss.) and Engelmann spruce (*Picea engelmannii* Parry ex Engelm.) (Burdett et al. 1984). Height, diameter, and stem volume of field-fertilized western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) was significantly greater than controls after 3 yr (Arnott and Burdett 1988). Broadcast application of N and P following site preparation improved productivity and root development of outplanted loblolly pine (*Pinus taeda* L.) through five growing seasons (Haywood et al. 1997). Fertilization of black spruce (*Picea mariana* (Mill.) B.S.P.) seedlings with NPK fertilizer improved shoot and root development as compared to controls (Paquin et al. 1998).

Other experiments have demonstrated the interaction between fertilization and the conditions of the outplanting site. Field fertilization increased growth of ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.) on a nutrient poor site, but had no effect on a fertile site (Gleason et al. 1990). Powers et al. (1996) reported improved growth of field-fertilized ponderosa pine seedlings only when adequate weed control was applied. Without adequate vegetation control, field fertilization may negatively affect seedling growth due to the fertilization of competing vegetation (Brockley 1988; Roth and Newton 1996). Fertilizer placement may also be important and many researchers have suggested applying fertilizer directly to the planting hole to facilitate nutrient uptake (Austin and Strand 1960; Carlson 1981; Carlson and Preisig 1981; Gleason et al. 1990; Rose et al. 1991), while negative experimental results have been reported for broadcast field fertilization. After 7 yr, survival had decreased by 17% for white spruce fertilized with 40 g of 8-6-12 Osmocote CRF at the base of each seedling (Simpson and Vyse 1995). Broadcast application of 220 kg/ha urea prill (46-0-0) resulted in no Douglas-fir

seedling growth response with adequate weed control and a negative response in the absence of weed control (Roth and Newton 1996).

Field fertilization in the planting hole could have a significant influence on seedling root development during the critical establishment period following outplanting. This may, in turn, affect the ability of seedlings to tolerate moisture stress. Shoot growth following planting is generally limited at first by water and then nutrients (Burdett et al. 1984). When bareroot seedlings are lifted from the nursery, a significant portion of roots, particularly fine roots can be lost (Nambiar 1980). Although there is evidence that large initial seedling root volumes may reduce moisture stress and subsequent transplant shock (Haase and Rose 1993; Rose et al. 1997), survival of newly planted seedlings is largely dependent on the rapid extension of roots, which reestablish root-soil contact and absorb water to reduce stress from transpirational water loss (Ritchie and Dunlap 1980; Sands 1984). Without efficient water extraction, seedlings continue to transpire, resulting in a condition of physiological drought, which contributes to transplant shock (Rietveld 1989).

Depending on the conditions of the outplanting site, seedlings may initially increase root growth relative to shoot growth (Ledig et al. 1970). This decreased shoot:root ratio allows a greater soil volume to be exploited with relatively less transpirational demand from the shoot (Imo and Timmer 1992b; McMillin and Wagner 1995). Fertilization at outplanting when soils are wet, however, may act to increase shoot:root ratios relative to unfertilized seedlings as high nutrient availability and sufficient water limit the need for root expansion while stimulating shoot growth (Imo and Timmer 1992a; Imo and Timmer 1992b; Tan and Hogan 1997). Reduced root expansion relative to shoot associated with field fertilization could result in increased water stress when soils dry (Gilman 1990).

Fertilization in the planting hole may also alter root architectural development because roots tend to proliferate in areas of high nutrient supply, as observed in numerous controlled experiments (Drew and Saker 1975; Coutts and Philipson 1976; Friend et al. 1990). Few field experiments have investigated root proliferation in response to localized nutrient sources, particularly with CRF. No localized root proliferation was associated with the placement of CRF in the root zone of Douglas-fir

(Carlson and Preisig 1981) or western hemlock (Carlson 1981). However, root penetration into subsoil zones could be limited if roots proliferate in areas of high nutrient concentration within the planting hole. Root growth and subsoil penetration may also be restricted by the diffusion of fertilizer salts into the soil solution, especially at high application rates. "Toxicities" to conifer seedling roots associated with excessive fertilization have long been recognized (Austin and Strand 1960). Conifers are particularly sensitive to high salt concentrations (Landis 1989) and excessive fertilizer salts can kill root apical meristems due to the buildup of toxic ion concentrations and osmotic effects (Drew 1975), further contributing to moisture stress.

Conifer roots generally grow in seasonal patterns associated with soil temperature (McMichael and Burke 1998), the availability of soil moisture (Comeau and Kimmins 1989; Nguyen and Lamant 1989), and the growth of the shoot (Krueger and Trappe 1967; Drew and Ledig 1980). Root growth of Douglas-fir and ponderosa pine generally begins in late-winter when soil temperatures increase and continues until bud-break in early spring (Cleary et al. 1978). Shoot growth then becomes the dominant sink for carbohydrates, limiting root growth (McMillin and Wagner 1995) until the cessation of shoot growth and fall moisture stimulates a second surge of root growth (Cleary et al. 1978). Seedlings must resist summer drought with root systems that developed during spring as moisture stress restricts root elongation (Becker et al. 1987). Thus, summer drought stress may be more severe for fertilized seedlings if shoot growth is enhanced relative to root growth during spring. Application of fertilizer to the planting hole may also limit tolerance to moisture stress if roots grow preferentially in upper soil horizons or root penetration into lower soil zones is restricted due to soil solution toxicities.

This experiment closely investigated the root architectural and physiological development of a sub-sample of seedlings in four treatments used in a larger 20-treatment factorial experiment (four initial root volume categories x five fertilization rates). We hypothesized that relative to the unfertilized treatment, field fertilization would result in (i) differences in whole-plant morphological development and allocation of biomass to above and below-ground tissues, (ii) decreased subsoil penetration and localized root proliferation relative to the placement of CRF, and (iii) increased

moisture stress and lower rates of stomatal conductance, which would be less pronounced in the higher initial root volume treatment.

5.3 Materials and Methods

5.3.1 Plant Material

Douglas-fir seedlings (seedlot 262, Western Forest Tree Seed Council, State of Oregon Tree Seed Zones) were grown for 2 yr (1+1) using standard nursery practices at Weyerhaeuser's Aurora, OR nursery until lifting in December 1999. Following lifting, seedlings were graded to operational specifications and placed in cold storage at 3°C. Several days before outplanting, seedlings were washed free of soil and measured for root volume (RV) by water displacement (Burdett 1979). Each seedling was then numbered, tagged, and returned to cold storage. Seedlings were divided into four categories based on their initial RV. Seedlings examined for this study were from the smallest (R1) (8-13 cm^3, mean 11.2) and largest (R4) (23-35 cm^3, mean 27.0) initial RV categories.

5.3.2 Treatments

Seedlings were either unfertilized or fertilized with approximately 60 g of a blended Simplot Polyon® CRF containing equal amounts of three fertilizers with differing release rates (Table 5.1). Simplot Polyon® CRF consists of prills containing water-soluble nutrients encapsulated within a polyurethane coating. Fertilizer was applied to the bottom of the approximately 25 cm planting hole, covered by a 1-2 cm layer of soil, and the seedling was planted.

Table 5.1. Nutrient composition (%) of the three Simplot Polyon® CRF used to create the composite fertilizer used in the experiment.

	Fertilizer Type			
	CRF#1 (3-4 month release)	CRF#2 (5-6 month release)	CRF#3 (8-9 month release)	CRF Composite
Nutrient	Composition (%)			
Nitrogen	19.00	19.00	18.00	18.67
NH_4	8.20	8.20	7.74	8.05
NO_3	10.80	10.80	10.26	10.62
P (P_2O_5)	5.00	6.00	6.00	5.67
K (K_2O)	12.00	12.00	12.00	12.00
Mg	1.00	0.90	0.90	0.93
S	1.80	1.70	1.70	1.73
Fe	0.45	0.45	0.45	0.45
Mn	0.200	0.190	0.190	0.193
Mo	0.009	0.009	0.009	0.009
Zn	0.056	0.055	0.050	0.054

5.3.3 Field Site

The outplanting site was in Oregon State University's Dunn Experimental Research Forest (44° 43' lat., 123° 20' long.). This site is on the eastern edge of the Coast Range and is characterized by mild winters with heavy precipitation and hot, dry summers. The 38-ha site was clearcut in 1998 and slash was piled and burned shortly thereafter. The aspect of the site was east, slopes ranged from 5-10%, and the soil was a deep, well-drained silty-clay loam. Seedlings were planted on January 19, 2000 at a 3 m x 3 m spacing. Immediately following planting, Vexar™ tubing was installed to protect seedlings from animal damage.

Aggressive chemical vegetation control was implemented to reduce sources of variation. Pre-plant (August 30, 1999) vegetation was controlled on the site using metsulfuron (Escort®, 0.05 kg/ha), sulfometuron (Oust®, 0.21 kg/ha), glyphosate (Accord®, 4.67 l/ha), and imazapyr (Arsenal®, 0.44 l/ha). Subsequent control included

hexazinone (Velpar®, March 28, 2000, 7.00 l/ha), clopyralid (Transline®, June 9, 2000, 0.73 l/ha), atrazine (March 30, 2001, 4.93 kg/ha), and triclopyr (Garlon 4®, March 30, 2001, 2.91 l/ha). Minimal vegetation emerged during the course of the experiment and observations indicated that there were no differences in competing cover between treatments.

5.3.4 Measurements

Seedlings were measured for height (groundline to base of terminal bud) and diameter at root collar soon after planting (February 2000). These measurements were again recorded in July, September, and December 2000.

Seedlings were sampled for pre-dawn xylem pressure potential (XPP) at four dates (July 3, July 31, August 31, and September 27). Four seedlings from each treatment replication were sampled on July 3 and five seedlings from each treatment replication were sampled at all other dates. A lateral branch from the lower half of the terminal shoot was cut for each sampled seedling. Within 5 min, a measurement of XPP using a pressure bomb (Model#600, PMS Instruments, Corvallis OR) (Cleary and Zaerr 1980) was recorded.

Five seedlings from each treatment replication were sampled for stomatal conductance (Model#1600, Li-Cor Inc., Lincoln NE) on September 28 and 29, 2000. Two blocks were sampled on the first day and the remaining two blocks on the second day. The same lateral branch on the lower half of the terminal shoot was sampled for morning (0800-1000) and afternoon (1300-1500) conductance. The sampled foliage was then placed into cold storage at 3°C. To adjust gas exchange measurements for variations in leaf area, the leaf area of each foliage sample was assessed using a video image recorder (Model#WV-CD20, Panasonic®, USA) and AgVision software (Decagon Devices, Pullman WA). The stomatal conductance measurement was then divided by the measurement of leaf area to provide a corrected value for stomatal conductance, which was used for analysis.

On December 8, 2000, the 80 seedlings used for PMS and stomatal conductance were excavated with care to preserve the root system. Seedlings were immediately placed into cold storage at 3°C. Within 7 d, seedlings were measured for height from cotyledon scar to base of terminal bud, diameter at root collar, tap root length, shoot/root volume, and shoot dry weight.

Root architectural development was assessed based on the vertical distribution of roots within the soil profile. Because seedlings were sampled soon after excavation, were dormant when lifted, and had relatively well-lignified root structures, it was assumed that roots held approximately the same vertical architectural orientation that was present in the soil. Seedlings were clipped at the cotyledon scar and again 5.0 cm below this point, which represented the position at which lateral roots tended to initiate. The remaining roots were then sliced into four 7.5-cm sections (S1-S4), with roots infrequently extending into the deepest section (S4). Roots in each section were then divided into tap and lateral roots, with the tap root never extending beyond S2. Within each root section, the number of active root tips (white tips > 1 mm in length) and first order lateral root length (cm) were assessed. Root sections were then dried at 70°C for 24 hr and dry weights of tap and lateral roots were recorded for each section.

5.3.5 Experimental Design and Statistical Analysis

The experiment was a randomized complete block design with four factorial treatments (two initial RV x two fertilizer rates) and four blocks. Five seedlings were sampled from each treatment within a block, for a total of 80 seedlings in the experiment. The experimental unit was the group of seedlings within treatment replication and the sampling unit was the individual seedling.

Data were subjected to analysis of variance (ANOVA) for a randomized complete block design with factorial treatments. Tests for normality and constant variance were performed to ensure the validity of the assumptions of the ANOVA and no transformations were necessary. If a significant RV x fertilizer rate interaction was detected ($p < 0.05$ in F test), Fisher's Protected Least Significant Difference procedure

was used to identify significant differences ($\alpha \leq 0.05$) among the four treatments. In the absence of a significant interaction, the main effects (RV and fertilizer rate) were analyzed to determine if significant differences were present ($p < 0.05$ in F test). Regression analyses were used to determine the relationship between shoot:root dry weight and measurements of XPP. Orthogonal contrasts were used to determine the statistical significance of higher-order regression models for explaining variability associated with the data but only linear relationships were significant. The mean values of the four replicates for each treatment were used in the regression and an adjusted R^2 value was determined to indicate the fit of the model. SAS® software (SAS Institute Inc., Cary, NC, USA) was used for analysis of all data.

5.4 Results

5.4.1 Xylem Pressure Potential and Stomatal Conductance

All seedlings became more moisture stressed over time during summer (Figure 5.1). There were no root volume x fertilizer interactions for XPP at any sampling point (July 3, p=0.2397; July 31, p=0.7447; August 31, p=0.4934; September 27, p=0.9921). The XPP of fertilized seedlings was more negative (more drought stressed) than unfertilized seedlings on July 3 (p=0.0113), July 31 (p=0.002), and August 31 (p=0.0006) (Figure 5.1a), which was during a period of no rainfall. In early September, approximately 5 cm of rain fell on the site and mean XPP of fertilized seedlings tended to be lower than unfertilized seedlings at the September 27 sampling, though not statistically significant (p=0.0851) (Figure 5.1). There was no significant difference in XPP at any sampling between the two RV categories. However, mean XPP of seedlings in the largest RV category (R4) was generally lower than those in the smallest RV category (R1) and the effect was nearly statistically significant on August 31 (mean -13.2 vs. -10.9 MPa, p=0.0583) (Figure 5.1b).

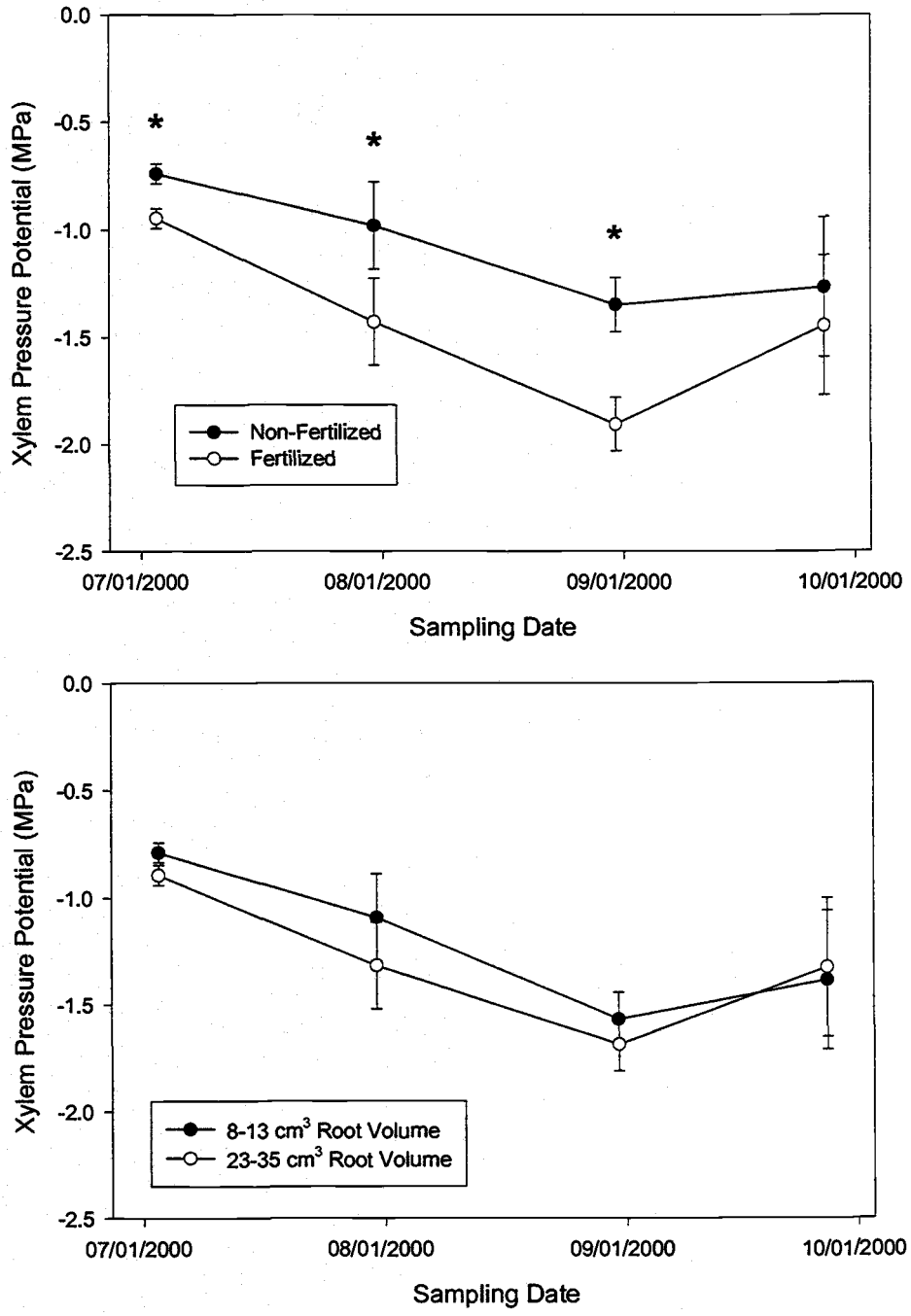

Figure 5.1. Mean values and standard error of pre-dawn xylem pressure potential for (a) fertilized and unfertilized seedlings (averaged over initial root volume treatments) and (b) R1 (8-13 cm^3) and R4 (23-35 cm^3) initial root volume treatments (averaged over fertilizer rate treatments) at four sampling points. For each chart and at each sampling, * denotes that treatments were significantly different at $\alpha = 0.05$ using Fisher's Protected Least Significant Differences Procedure.

No RV x fertilizer rate interaction was detected for stomatal conductance in September for the morning (p=0.4019) or afternoon (p=0.6714) sampling. Rates of stomatal conductance were higher for unfertilized seedlings than fertilized seedlings during both the morning (mean 37.6 vs. 15.7 mmol $m^{-2} s^{-1}$, p=0.0052) and afternoon (mean 18.9 vs. 9.1 mmol $m^{-2} s^{-1}$, p=0.0126) (Figure 5.2). There were no differences in conductance between R1 and R4 during the morning (p=0.4313) or afternoon (p=0.6911).

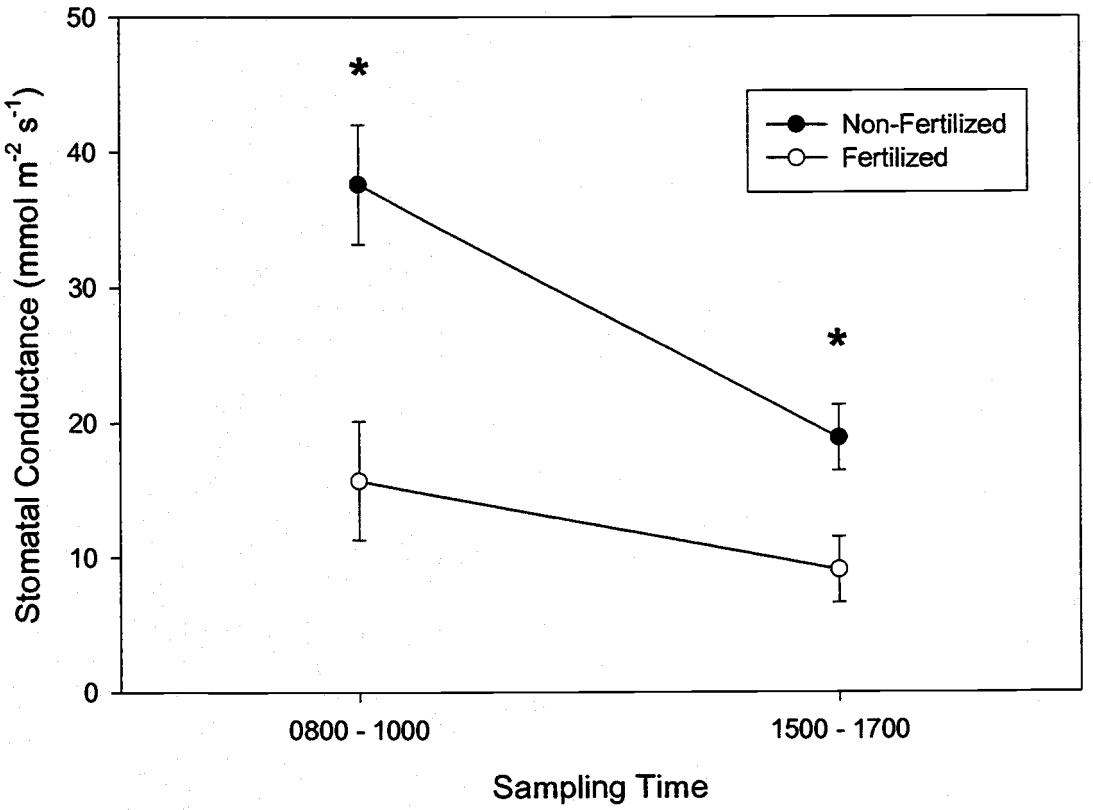

Figure 5.2. Mean values and standard error of stomatal conductance for fertilized and unfertilized seedlings (averaged over initial root volume treatments) sampled during morning and afternoon hours on September 28 and 29, 2000. At each sampling time, * denotes that treatments were significantly different at $\alpha = 0.05$.

5.4.2 Whole Plant Morphology

No significant RV x fertilizer rate interaction was detected for diameter growth at any sampling. Diameter growth was not different among fertilizer treatments in July (p=0.5524) or September (p=0.1934), but was greater for unfertilized seedlings in December (p=0.0300) (Figure 5.3). Height growth did not differ among fertilizer treatments in July (p=0.1129), September (p=0.3916), or December (p=0.8860) (data not shown).

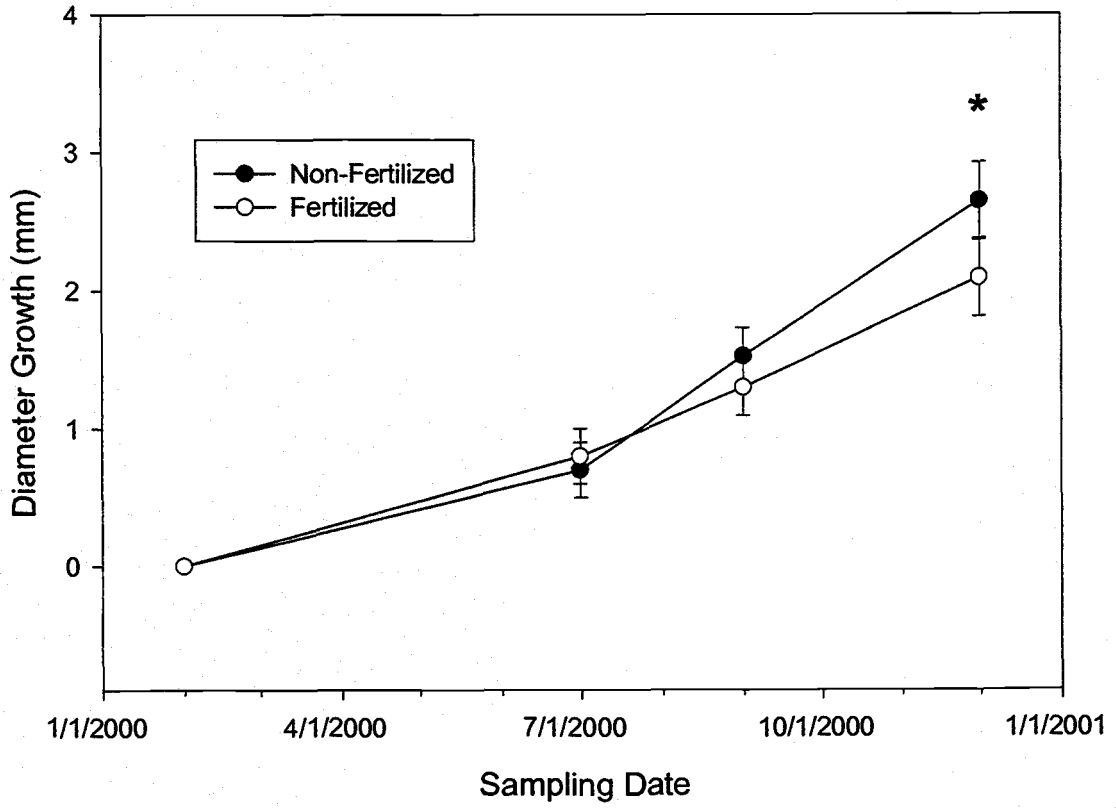

Figure 5.3. Mean values and standard error for diameter growth of fertilized and unfertilized seedlings (averaged over initial root volume treatments) at three sampling points. At each sampling point, * denotes that treatments were significantly different at $\alpha = 0.05$.

No RV x fertilizer rate interaction was detected for diameter at root collar (p=0.8164), height (p=0.5217), shoot:root volume (p=0.9654), shoot:root dry weight (p=0.4037), or height:diameter (p=0.8626) following excavation in December 2000. Diameter at root collar of unfertilized seedlings was greater than that of fertilized seedlings (p=0.0270) (Table 5.2). Shoot:root volume (p=0.0243) and shoot:root dry weight (p=0.0100) were greater for fertilized than unfertilized seedlings (Table 5.2). Shoot:root volume was also greater for R4 than R1 (p=0.0421), though shoot:root dry weight was not (p=0.6610) (Table 5.2). Mean height:diameter was greater in fertilized seedlings, though not statistically significant (p=0.0670) (Table 5.2). Although mean shoot volume and shoot dry weight of unfertilized seedlings tended to be greater than fertilized seedlings, these differences were not significant (p=0.0516 and p=0.0991, respectively). There were no differences for height (p=0.4245).

Table 5.2. Mean values and standard error for seedling morphology of non-fertilized and fertilized seedlings following excavation in December 2000. For each parameter, means followed by the same letter in a row did not differ significantly at $\alpha \leq 0.05$.

	Non-fertilized	Fertilized	Standard Error
Height (cm)	36.0 a	37.1 a	1.0
Diameter (mm)	8.26 a	7.82 b	0.25
Height:Diameter	4.4 a	4.8 a	0.2
Tap Root Length (cm)	13.6 a	12.6 a	0.5
Shoot Volume (cm^3)	35.3 a	29.6 a	2.9
Root Volume (cm^3)[1]	27.1	18.5	2.7
Shoot:Root Volume	1.32 b	1.62 a	0.10
Shoot Dry Weight (g)	12.4 a	11.0 a	1.17
Root Dry Weight (g) [1]	9.0	6.1	0.8
Shoot:Root Dry Weight	1.43 b	1.85 a	0.09

[1]A significant fertilizer x root volume interaction occurred and prevented statistical analysis of this main treatment effect.

Although means for root volume and root dry weight tended to be greater for unfertilized than fertilized seedlings at excavation (Table 5.2), a RV x fertilizer rate

interaction was detected for root volume (p=0.0429) and root dry weight (p=0.025). There was no RV x fertilizer rate interaction (p=0.708) for initial root volume at the time of planting and initial root volume was similar among fertilized and unfertilized seedlings (p=0.2029), though greater for R4 than R1 (p<0.0001) (Figure 5.4). At excavation, however, root volume was greatest for the unfertilized R4 treatment while the root volume of the fertilized R4 treatment was not statistically different than the unfertilized R1 treatment (Figure 5.4). Although the mean root volume of unfertilized R1 was greater than the fertilized R1, these treatments did not differ statistically (Figure 5.4).

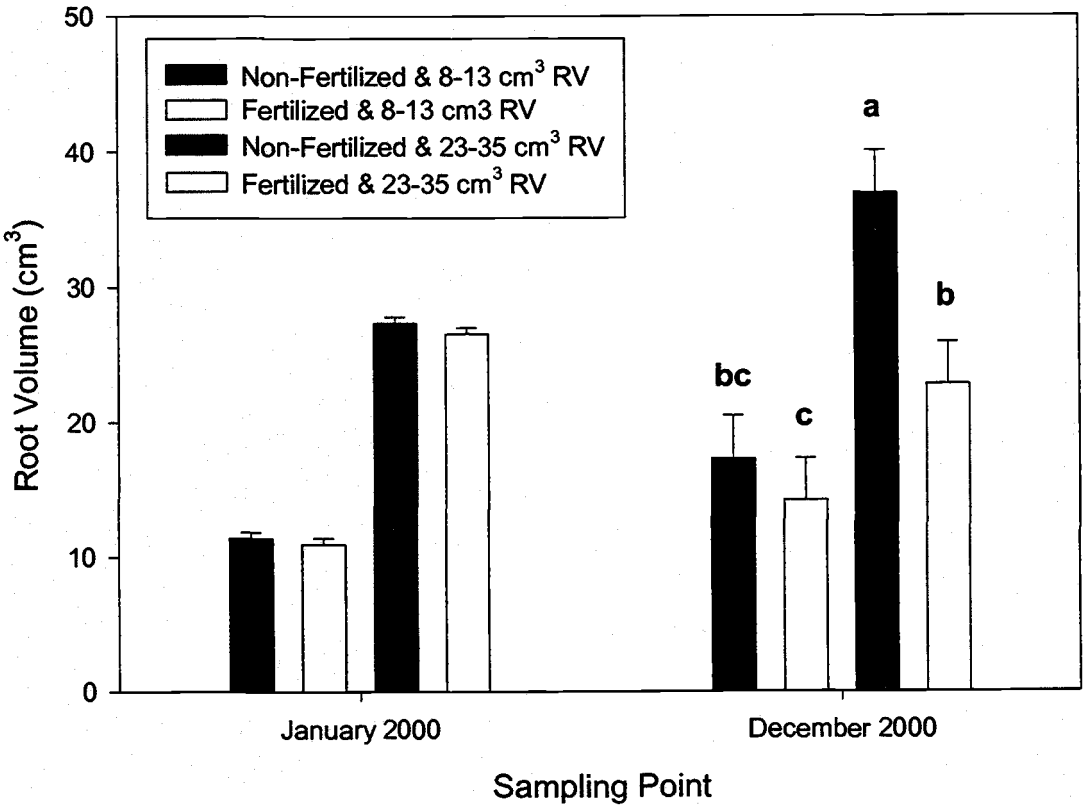

Figure 5.4. Mean initial seedling root volume and standard error at time of planting (February 2000) and following excavation (December 2000). In January 2000, RV treatments differed but fertilizer treatments did not differ at $\alpha = 0.05$. At the December 2000 sampling, treatments with the same letter did not differ significantly at $\alpha = 0.05$.

A regression analysis of shoot:root dry weight vs. XPP sampled on July 3, 2000 showed a inverse linear relationship (p=0.0008, adjusted $R^2 = 0.54$) (Figure 5.5). Differences in the grouping of data points representing unfertilized and fertilized seedlings reflected the significantly greater shoot:root dry weight and significantly lower XPP of fertilized seedlings (Figure 5.5).

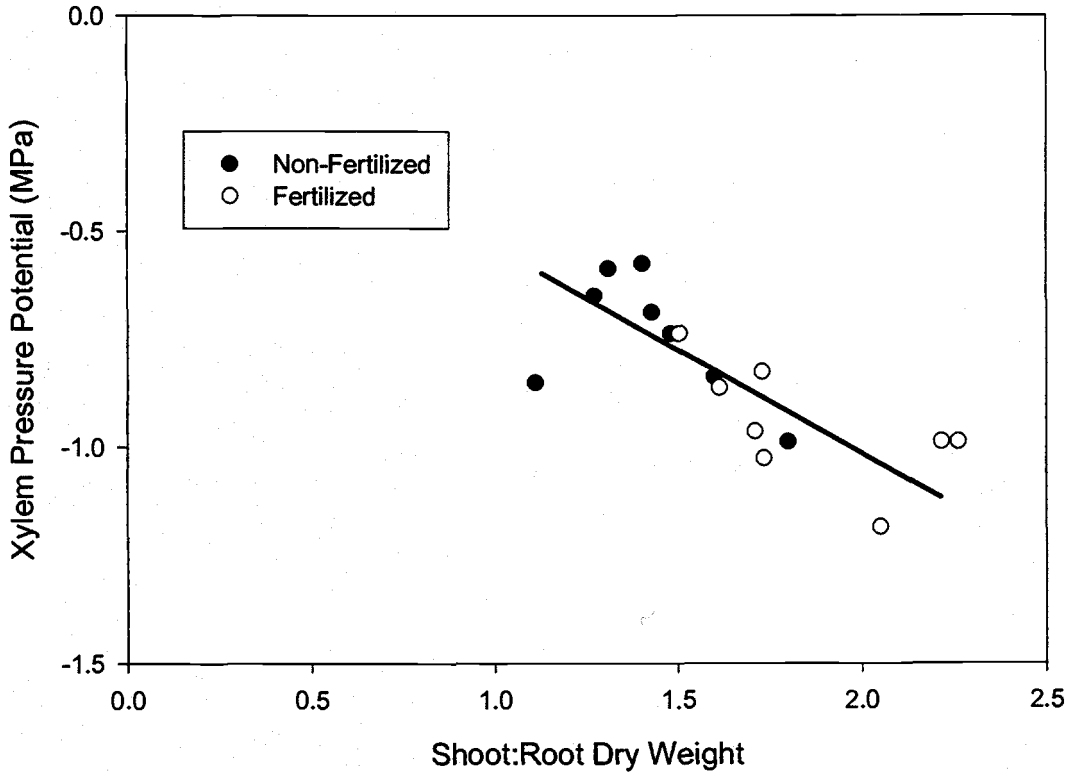

Figure 5.5. Regression of shoot:root dry weight on xylem water potential sampled on July 3, 2000 for fertilized and unfertilized seedlings. Regression equation is: Xylem Water Potential = 0.191 + 0.398 (Shoot DW/Root DW); Adjusted $R^2 = 0.54$.

5.4.3 Root Architecture

No RV x fertilizer rate interactions were observed for the total number of active root tips (p=0.5028) or active roots within any soil zone (S1, p=0.6392; S2, p=0.6446;

S3, p=0.3136; S4, p=0.8713). The mean number of active root tips tended to be greater for unfertilized than fertilized seedlings in all root zones (Table 5.3). Differences were established in the upper (S1) (p=0.03130) and S3 (p=0.0497) soil zones, though not in S2 (p=0.1113) or S4 (p=0.1948). The total number of active roots in all sections was greater for unfertilized than fertilized seedlings (p=0.0327) (Table 5.3).

Table 5.3. Mean values and standard error for root architecture parameters of fertilized and unfertilized seedlings following excavation in December 2000. For each parameter, means followed by the same letter in a row did not differ significantly at $\alpha \leq 0.05$.

	Unfertilized	Fertilized	Standard Error
S1 Number of Active Root Tips	137 a	101 b	13
S2 Number of Active Root Tips	126 a	94 a	14
S3 Number of Active Root Tips	54 a	30 b	8
S4 Number of Active Root Tips	11 a	4 a	4
Total Number of Active Root Tips	328 a	229 b	33
S1 First Order Lateral Root Length (cm)	94 a	88 a	6
S2 First Order Lateral Root Length (cm)	95 a	83 a	8
S3 First Order Lateral Root Length (cm)	36 a	23 a	7
S4 First Order Lateral Root Length (cm)	11 a	3 a	3
Total First Order Lateral Root Length (cm)	236 a	197 b	20
S1 Tap Root Dry Weight (g)	1.28 a	0.88 b	0.09
S2 Tap Root Dry Weight (g)	0.044 a	0.005 b	0.008
Total Tap Root Dry Weight (g)[1]	2.98	2.36	0.20
S1 Lateral Root Dry Weight (g)[2]	3.15	2.15	0.26
S2 Lateral Root Dry Weight (g)[2]	2.13	1.28	0.22
S3 Lateral Root Dry Weight (g)	0.64 a	0.31 a	0.12
S4 Lateral Root Dry Weight (g)	0.11 a	0.03 a	0.03
Total Lateral Root Dry Weight (g)[2]	6.03	3.76	0.59

[1]Includes portion of tap root that was excluded in root zone analysis.
[2]A significant fertilizer x root volume interaction prevented analysis of this main treatment effect.

No RV x fertilizer rate interactions were observed for first-order lateral root length (p=0.9963) or root length within any soil zone (S1, p=0.5608; S2, p=0.9293; S3,

p=0.7420; S4, p=0.7337). Means for total first-order lateral root length tended to be greater in all soil zones for unfertilized than fertilized seedlings, (S1, p=0.3359; S2, p=0.0658; S3, p=0.0630; S4, p=0.1277) and total first-order lateral root length was greater in unfertilized than fertilized seedlings (p=0.0185) (Table 5.3). Tap root length did not differ among fertilizer treatments (p=0.1473), though mean tap root length was approximately 1.0 cm greater for unfertilized than fertilized seedlings (Table 5.2).

No RV x fertilizer rate interactions were observed for total tap root dry weight (p=0.1127) or tap root dry weight in any soil zone (S1, p=0.2428; S2, p=0.1836). Tap root dry weight was greater for unfertilized than fertilized seedlings in S1 (p=0.0061) and S2 (p=0.0296) (Table 5.3). Total tap root dry weight, which included the 2.5-cm segment excluded from root zone analyses, was also greater for unfertilized than fertilized seedlings (p=0.0017) (Table 5.3). Means for lateral root dry weight tended to be greater for unfertilized than fertilized seedlings in all soil zones, though not statistically significant in S3 (p=0.0679) or S4 (p=0.1207) (Table 5.3). A RV x fertilizer rate interaction was detected for lateral root dry weight in S1 (p=0.0168), S2 (p=0.0296), and total lateral root dry weight (p=0.0377). Similar to the treatment differences noted for root volume, unfertilized R4 seedlings had the greatest total lateral root dry weight, while that of the fertilized R4 and unfertilized R1 were not significantly different (Figure 5.6). Although the mean lateral root dry weight of unfertilized R1 was greater than the fertilized R1, these treatments did not differ statistically (Figure 5.6).

5.5 Discussion

5.5.1 Fertilization and Moisture Availability

The ability of seedlings to respond positively to field fertilization seems to be constrained by moisture availability. Nitrogen fertilization at outplanting reduced growth and survival rates under conditions of limited moisture availability in Douglas-

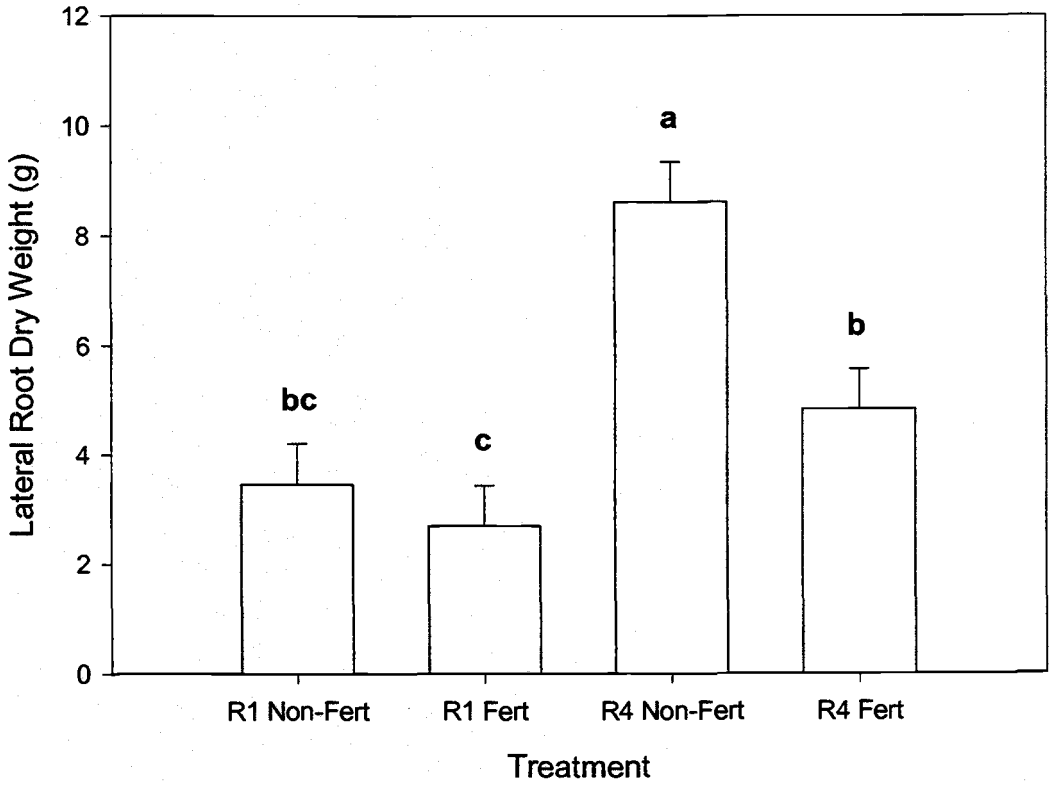

Figure 5.6. Mean lateral root dry weight and standard error for all treatments following excavation in December 2000. Treatments with the same letter did not differ significantly at $\alpha = 0.05$.

fir (Smith 1966) and Monterey pine (*Pinus radiata* D. Don) (Linder et al. 1987). Growth response of white pine (*Pinus strobus* L.) seedlings at various levels of fertilizer nutrients in solution was dependent on irrigation schedule, with mean seedling weight decreasing with reduced moisture to a greater extent in the full-strength solution than the half-strength solution (Schomacher 1969).

Poor performance of fertilized seedlings under low moisture may be associated with the influence of fertilization on drought resistance. Controlled experiments with loblolly pine (Pharis and Kramer 1964) and lodgepole pine (*Pinus contorta* Dougl. ex. Loud.) (Etter 1969) reported that seedlings in the lowest N treatments were most able to resist drought stress. Similarly, jack pine (*Pinus banksiana* Lamb.) seedlings under the

lowest N treatment were better suited to tolerate drought stress than seedlings in higher N treatments (Tan and Hogan 1997). Despite these reports, the role of N as a modifier of seedling morphology and physiology in relation to drought tolerance remains unclear (Tan and Hogan 1997). This experiment, however, demonstrated that there might be a complex interrelationship between fertilization, root proliferation, drought resistance, photosynthetic capacity, and whole-plant morphological development.

5.5.2 Influence of Fertilization on Root Development

Root extension and proliferation were clearly inhibited for fertilized seedlings as compared to unfertilized seedlings. This was likely due to the release of excessive fertilizer salts into the soil solution inhibiting root development. As fertilizer nutrients are released, the electrical conductivity (EC) of the soil solution increases, solute potential decreases, and osmotic effects may cause the death of root apical meristems. Conifers, and Douglas-fir in particular, are sensitive to high EC levels (Landis 1989). Tolerance to high salt concentrations varies with the age and size of root stock, generally increasing with age (Zekri 1993). Thus, young Douglas-fir seedling roots may be highly vulnerable to injury associated with excessive fertilization, which helps to explain the poor root development of fertilized seedlings in this study.

Root development for seedlings in the largest root volume category (R4) was more negatively affected by fertilization than those in the smallest root volume category (R1). This may have been due to increased root-fertilizer contact in the R4 fertilized treatment as compared to the R1 fertilized treatment. Additionally, this effect was probably magnified by the more rapid relative root growth rates of R4 unfertilized seedlings as compared to R1 unfertilized seedlings. Douglas-fir seedlings with large initial root volumes tend to better tolerate transplant shock following transplanting (Haase and Rose 1993) and may have more rapid relative growth rates compared to seedlings with smaller initial root volumes (Rose et al. 1991; Rose et al. 1997).

There were no distinct differences in root architectural development in vertical soil zones based on the position of CRF in the planting hole (Table 5.3). Although root

growth of both western hemlock (Carlson 1981) and Douglas-fir (Carlson and Preisig 1981) was improved with CRF application, no localized root proliferation within 12 soil zones was noted with respect to adjacent or in-hole fertilizer placement. In this experiment, however, the number of active root tips, first order lateral root length, and root biomass were generally lower in all soil zones for fertilized seedlings as compared to unfertilized seedlings. White root tips are anatomically suited for efficient ion uptake (Peterson et al. 1999) and tend to increase in nutrient-rich solutions (Coutts and Philipson 1977). The fertilizer was placed at a depth of approximately 25 cm in the soil and therefore generally associated with S3. A lack of increased numbers of active roots in S3 where the fertilizer was placed supports the conclusion that nutrient toxicities in the soil solution inhibited root proliferation. The same type of response was noted for lateral root length and biomass with no distinct proliferation by fertilized seedlings in any zone, which was again likely due to excessive nutrient concentrations in the soil solution. It is possible, however, that increased root proliferation in soil zones of CRF supply might have been observed at lower CRF application rates or in subsequent sampling years.

5.5.3 Influence of Root Development on Moisture Stress

Fertilized seedlings were significantly more moisture stressed than unfertilized seedlings during the summer. This may be at least partly explained by restrictions in root development. During the critical establishment period following outplanting, seedling growth is most limited by water availability (Burdett et al. 1984). Small seedlings have little capacity to store water due to the small ratio of sapwood volume to water transfer rate and the lack of other storage reservoirs (Newton and Preest 1988). Thus, to avoid drought stress, roots must rapidly extend through the soil profile to extract water for supply to actively transpiring shoots. Tan and Hogan (1997) found that N fertilization at high rates reduced tap root penetration in jack pine and suggested this would increase drought stress. Omi et al. (1991) found a significant correlation (R^2 = 0.65) between the number of new roots initiated by ponderosa pine seedlings and pre-

dawn leaf water potential. Leaf water potential of shortleaf pine (*Pinus echinata* Mill.) increased exponentially with new root projected surface area (Brissette and Chambers 1992). The significantly greater lateral root length of unfertilized seedlings allowed a greater volume of soil water to be exploited, reducing drought stress. Unfertilized seedlings also had significantly more active root tips, which are important sites for water uptake because there is anatomically less resistance to water passage (Peterson et al. 1999).

The increased shoot:root ratio of fertilized seedlings compared to unfertilized seedlings was also a stimulus for the increased drought stress of fertilized seedlings as evidenced by the inverse linear correlation between shoot:root dry weight and XPP. Increases in seedling shoot:root ratios in response to fertilization have been reported for Douglas-fir (Carlson and Preisig 1981; Shaw et al. 1998), Jeffrey pine (*Pinus jeffreyi* Grev. & Balf.) (Walker and Huntt 1992; Walker and Kane 1997), singleleaf pinyon pine (*Pinus monophylla* Torr. & Frem.) (Walker and Huntt 1992), jack pine (Tan and Hogan 1997), sycamore (*Platanus occidentalis* L.), and Sitka spruce (*Picea sitchensis* (Bong.) Carr.) (Mackie Dawson et al. 1995). Under conditions of high nutrient availability and no water stress, seedlings tend to allocate more resources toward shoot development and less to roots because belowground resources are sufficient for growth (Marschner 1995). Thus, high nutrient availability during spring may have stimulated shoot growth without the need for compensatory root growth when soils were wet. Based on the actual mean loss of rooting volume in R4 fertilized seedlings from time of planting until excavation (26.6 vs. 22.8 cm^3), it seems more likely that high concentrations of fertilizer salts in the soil prevented root elongation by killing root tips. Under conditions of high nutrient availability when soil moisture was still adequate, the shoots of fertilized seedlings were still able to elongate and this led to the increased shoot:root ratios.

There were no significant differences in XPP for seedlings in different initial root volume treatments and mean XPP was consistently lower (more negative) for seedlings in R4. Hermann (1964) postulated that a well-developed root system was more important to Douglas-fir seedling survival than the balance between shoot and root. Rose et al. (1997) suggested that improved survival after 8 growing seasons of

ponderosa pine seedlings with larger initial root volumes on a harsh, dry site was due to greater access of larger root systems to limited water and nutrients. Atkinson (1987), however, stressed that the balance between the shoot mass and root length is more significant than the development of the root system alone. The results from this experiment indicated that initial root volume in itself did not confer drought resistance and the allometric relationship between the root and shoot was more important.

Increased drought stress of fertilized seedlings may also have been influenced by changes in the chemical properties of the soil solution. Because the release of polymer-coated CRF is primarily dependent on soil temperature (Kochba et al. 1990), fertilizer nutrients continued to release into the soil solution during summer when seedlings were not actively growing. Since adequate water to leach nutrients from the soil was unavailable, fertilizer salts built up in the soil solution. This may have lowered the osmotic potential of the soil solution to the point where it actually forced water out of the plant and into the soil, intensifying drought stress. This type of physiological drought associated with high solute concentrations can occur even when soil moisture is at field capacity (Kozlowski 1987).

5.5.4 Influence of Drought on Whole-Plant Morphology

Diameter growth of fertilized seedlings was similar to that of unfertilized seedlings prior to summer, but was significantly less by December. Fertilized seedlings also had a smaller mean shoot volume/dry weight as compared to unfertilized seedlings following excavation. Reduced growth rates were due, at least in part, to drought stress. Mean (± 0.1 SE) pre-dawn XPP of fertilized seedlings reached –1.9 MPa for fertilized seedlings at the end of August, as compared to –1.3 MPa for unfertilized seedlings. Mid-day XPP values were likely even lower because a greater leaf-atmosphere vapor pressure deficit during the day increases transpirational demand from the plant. Although healthy, established Douglas-fir trees can survive drought stress to –11 MPa, photosynthesis typically begins to decline at –1 MPa (Brix 1979).

As XPP decreases, stomatal conductance also declines (Meinzer 1982). Murphy and Ferrell (1982) reported a seasonal decline in stomatal conductance of Douglas-fir seedlings of nearly 50% when XPP reached −1.5 MPa. Fertilized seedlings in this experiment had significantly lower rates of stomatal conductance than unfertilized seedlings during both morning and afternoon samplings. The relatively low stomatal conductance for seedlings in either treatment at this point illustrates the severity of drought stress on the site. However, because photosynthesis is directly related to stomatal conductance (Hinckley et al. 1978), unfertilized seedlings were able to photosynthesize at higher rates than fertilized seedlings under these droughty conditions. Fertilized seedlings were probably unable to balance the energy costs associated with respiration with gains from photosynthesis. This helps to explain the smaller whole-plant morphology noted for fertilized seedlings.

Two potential limitations to this study exist. First, the chemical preparation of the site with metsulfuron and sulfometuron may negatively affect Douglas-fir seedling growth due to the persistence of herbicide residues in the soil (Cole and Newton 1989; Vizantinopoulos and Lolos 1994). However, significant time passed (5 months) between the point of application and planting. Seedlings remained dormant for at least 2.5 additional months (7.5 months since application). Herbicide persistence is dependent upon soil type and interaction of water/temperature (Vizantinopoulos and Lolos 1994) and during this time, the high clay and organic matter content of the site acted to bind the herbicide and initiate breakdown, reducing potential for seedling injury. Douglas-fir was not damaged following site preparation with imazapyr at higher application rates than those used in this experiment (Belz and Nishimura 1989). Few experiments have investigated effects of metsulfuron and sulfometuron applied during site preparation on subsequent performance of Douglas-fir seedlings, though Coate (2000) reported no damage with either herbicide applied at operational rates. Additionally, seedling survival on both experiments exceeded 94% following two growing seasons. Finally, these herbicides were applied as evenly as possible throughout the entire site, minimizing any chance for treatment confounding. This type of potential "confounding" is an inherent risk in the establishment of any field experiment. Many factors across a site (e.g. soil type, mechanical site preparation,

microsite variations, presence of mycorrhizal mats, emergence of competing vegetation, etc.) cannot be completely controlled. In this instance, it appears likely that the relative responses to treatments will be proportional to those observed where such residues are absent, hence the findings are likely to be valid.

The second potential limitation is that vegetative cover percentages for the different treatments were not quantified and may have added variability to XPP. Observationally, however, vegetative control was highly effective on the site and there were no differences between treatments in emergence of competition. Additionally, previous studies across a range of sites in the Pacific Northwest involving fertilization and vegetation control with Douglas-fir have failed to quantify a difference in competing cover between fertilized and non-fertilized treatments with planting hole fertilization (Rose and Ketchum (*In Press*)). Finally, a major portion of the variability in XPP was associated with the increased shoot:root ratio of fertilized seedlings. Increased vegetative competition immediately following planting is more likely to be associated with reduced shoot:root ratios as compared to weed-free conditions (Perry et al. 1985) . Thus, it is unlikely that increased drought stress of fertilized seedlings was associated with higher rates of vegetative competition for these treatments.

5.6 Conclusions

Fertilization at the time of outplanting in this experiment negatively affected seedling diameter growth following summer drought and led to reductions in aboveground morphology. Root development was severely restricted in fertilized seedlings, presumably due to the excessive concentrations of fertilizer salts in the soil solution. High moisture stress was observed in fertilized seedlings. This was explained by the combination of greater shoot:root ratios, the limited ability of seedlings to exploit water from the soil profile associated with a reduction in first-order lateral root length and active root tips, and changes in soil osmotic concentration. In an effort to conserve water, fertilized seedlings had lower rates of stomatal conductance and were, therefore,

unlikely to photosynthesize at the rate of unfertilized seedlings, which may have acted to negatively affect morphological development.

Based on these results, it is clearly important to match the level of field fertilization with the anticipated degree of moisture stress on the site. The 60 g fertilization rate used in this experiment altered seedling root morphology such that the ability of seedlings to resist drought stress was inhibited. It is best to err on the side of conservative CRF application rates, particularly on droughty sites. Continued evolution of CRF technology to produce a nutrient release mechanism that is dependent on microbial activity, which corresponds more closely with soil moisture and active plant growth (e.g. water-insoluble nitrogen), may improve fertilization response on moisture-limited sites. Consideration should also be given to applying fertilizer 1-2 yr following planting when seedling root systems have established. Although roots did not proliferate in vertical soil zones of CRF application in this experiment, it may be wise to apply fertilizer deep in the subsoil where moisture availability is higher or to use a different method (i.e. placement, timing) of fertilizer application. On sites where drought is extreme, it may be necessary to avoid field fertilization entirely.

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Chapter 6. Dissertation Synthesis

6.1 Dissertation Hypothesis

Though the importance of vigorous root system development to reforestation success has often been alluded to, roots are difficult to measure and there is little information available on their early growth (Mackie Dawson et al. 1995). The primary objective of this dissertation was to gain a better understanding of how the Douglas-fir seedling root system may be manipulated during early growth and to examine the influence that changes in root architecture might have on seedling performance. This was accomplished through a series of nursery and field experiments designed to modify soil physical and chemical properties, examine subsequent whole-plant morphological and physiological responses, and assess root architectural development via destructive harvest. Based on the results of these experiments, the overall dissertation hypothesis that cultural treatments would modify root architecture and that these changes would influence seedling morphological and physiological development was modified slightly for acceptance.

The accepted hypothesis was that nursery and field cultural treatments modified seedling root architectural development and that these changes, in conjunction with the development of the shoot, influenced aspects of seedling physiological development. Root architecture was manipulated in the nursery and field, particularly with the use of controlled-release fertilizers (CRF). Additionally, modifications in root architecture and resulting changes in shoot:root ratios were linked to differences in seedling physiological status such as xylem pressure potential (XPP), stomatal conductance, and nutrient uptake. These variations in physiological development helped to explain differences in whole-plant seedling morphological development, though a direct correlation between root architecture and changes in seedling morphology was not established.

6.2 Synthesis of Experiments

The results described in Chapter 2 indicated that the use of soil amendments during nursery transplant changed root architectural development during nursery growth to some degree, but differences were not pronounced at lifting. Subsequent benefits associated with nursery treatments on field establishment were also somewhat inconclusive, though no negative results were detected. Planting-hole fertilization with CRF, however, resulted in distinct differences in field growth over two seasons. As often reported in field fertilization (Austin and Strand 1960; Brix and Ebell 1969; Carlson and Preisig 1981; McClarnon 1999), above-ground growth was stimulated during the first year following planting. Interestingly, substantial reductions in growth occurred during the second growing season, resulting in smaller seedlings two years following planting. To help identify a causal mechanism for this effect, subsequent chapters closely investigated root system response to locally-applied CRF and the influence of root architecture on whole-plant morphological and physiological development.

Chapters 3 and 4 were designed to better understand nutrient release mechanisms of CRF and the effects of localized CRF supplies on Douglas-fir root architectural development over time. These experiments were both established in a controlled greenhouse environment where soil moisture was never limiting. In each experiment, CRF was applied as a single layer beneath the transplanted root system and seedlings were destructively harvested at multiple samplings over time. In Chapter 3, the hypothesis that differences in nutrient release technology among the CRF types would affect seedling development over time was supported by the temporal variations in seedling growth, chlorophyll fluorescence, and nutrient uptake. Differences in shoot:root biomass allocation were detected at the end of the experiment among the CRF types and this was well-correlated with the foliar concentration of soil-immobile P ($R^2 = 0.51$). Implications for the importance of understanding differences in nutrient release among CRF types with regard to soil electrical conductivity (EC), nutrient leaching, and dormancy were discussed.

In Chapter 3, roots proliferated in upper soil zones, above the locally-applied CRF, with increasing fertilizer rate, similar to previous research involving the localized application of nutrient solutions (Drew 1975; Drew and Saker 1975; Coutts and Philipson 1976; Friend et al. 1990). However, the CRF rates in this experiment did not prevent roots from penetrating into lower soil zones. Additionally, negative effects on morphological development or toxicities associated with excessive nutrients at the higher CRF rates were not observed. Thus, an additional greenhouse experiment was installed to investigate potential negative effects on seedling development associated with higher rates of CRF.

In Chapter 4, a wider range of CRF rates were applied using the same methodology from Chapter 3 and samplings were executed more frequently. Based on active root tips, root proliferation after two months in the soil zone above the CRF again increased with fertilizer rate ($R^2 = 0.51$). The hypothesis that root penetration into lower soil zones would be restricted at successively higher CRF rates was supported after six months ($R^2 = 0.72$) as few roots penetrated into the lowest soil zone at the highest rate. I know of no previous experiments documenting this effect on seedling root architecture with the use of locally-applied CRF. At six months, soil EC was significantly greater in the zone of locally-applied CRF for all fertilized seedlings as compared to unfertilized seedlings and it was hypothesized that EC exceeded safe levels at the highest CRF rates, killing elongating root apical meristems. Seedling morphological development also varied among CRF rates relative to the control, with performance improving at the lowest CRF rate and negative effects identified at the higher CRF rates. At the highest CRF rate, nitrogen consistently reached toxic levels (Timmer and Stone 1978; Timmer and Armstrong 1987) relative to the control. Phosphorus uptake was enhanced at the lowest CRF rate but depleted at the highest CRF rate. Implications regarding the influence of higher CRF rates during field fertilization on root subsoil penetration and subsequent tolerance of seedlings to drought stress were discussed.

Based on results from Chapters 3 and 4, a field experiment was established to carefully examine the influence of initial root volume and field fertilization on seedling morphological and physiological development during the establishment period

following outplanting. The major objectives of Chapter 5 were to determine if the same type of localized root proliferation and restriction in root penetration observed in Chapters 3 and 4 also occurred under field conditions and what effect changes in root architectural development might have on drought resistance, stomatal conductance, and growth. The hypotheses that fertilization at a relatively high CRF rate would restrict root penetration and intensify drought stress were supported. Rates of stomatal conductance also decreased for fertilized seedlings. I know of no previous experiments reporting reduced xylem pressure potential or stomatal conductance associated with field fertilization. Because stomatal conductance is well-correlated with photosynthesis, respiration costs for fertilized seedlings may have exceeded photosynthetic inputs during the summer drought, helping to explain the proportionally smaller aboveground morphological development relative to non-fertilized seedlings.

Despite suggestions in previous literature (Haase and Rose 1993; Rose et al. 1997), initial root volume in itself did not confer drought resistance during the first summer following planting. Instead, the allometric relationship between the shoot and root appeared to be more important. An increase in shoot:root dry weight, which was significantly greater for fertilized seedlings, explained much of the variation associated with decreasing XPP ($R^2 = 0.54$). In Chapter 5, root development of fertilized seedlings in all vertical soil zones was negatively affected relative to non-fertilized seedlings and no localized root proliferation was found relative to the CRF layer as in Chapters 3 and 4. Though overall root growth was enhanced, Carlson (1981) and Carlson and Preisig (1981) also found no localized root proliferation associated with CRF placement in Douglas-fir and western hemlock. The possibility that localized root proliferation might have been detected at lower CRF rates, was discussed.

In Chapter 2, a separate fertilizer release experiment was established in the field and showed that nutrients continued to release into the soil solution during the summer drought. This is typical of polymer-coated CRF, as release is governed primarily by temperature with moisture having little influence on release (Kochba et al. 1990). Thus, in each field experiment (Chapters 2 and 5) it was hypothesized that the release of fertilizer nutrients into the soil solution when water was scarce acted to increase soil EC in the root zone. Little research regarding EC levels and conifer seedling roots has been

reported, though it has been suggested that Douglas-fir is sensitive to high EC levels (Landis 1989). Elevated EC levels created a difference in osmotic concentration between the soil solution and root that killed elongating root tips, restricting root growth. Poor root growth then led to increased drought stress and decreased whole-plant growth relative to unfertilized seedlings. Implications regarding the impact of these results on seedling field fertilization programs were discussed in Chapters 2 and 5.

As detailed in Chapter 1, because of the large number of statistical hypotheses that were tested, it is likely that some of the significant results that were reported were Type I errors. This was due to the exploratory nature of the work and the large number of response variables involved in the statistical analyses of cultural treatment effects on root architectural development. Thus, the reader is cautioned to be aware of this potential limitation and to understand that this is an inherent risk related to exploratory studies of seedling root architecture. Future research directed toward testing specific hypotheses about specific responses in this area will help to confirm or reject potential effects of silvicultural treatments on root architectural development that were measured in this study.

6.3 Conclusions

This dissertation demonstrated that cultural treatments may act to manipulate the architecture of the Douglas-fir seedling root system in the nursery and field, particularly with the use of CRF. The effects of CRF on plant morphological and physiological development vary and depend on a complex interaction of factors including plant developmental stage, CRF nutrient release technology, and environmental growing conditions. When nutrients are limiting and water is sufficient, polymer-coated CRF will likely improve seedling productivity when applied at ideal rates. When CRF are applied locally, however, seedling roots may proliferate above the fertilizer source and root penetration into subsoil zones may be restricted at excessively high CRF rates.

Reductions in root elongation and restrictions in root penetration at high CRF rates are due to the release of excessive fertilizer salts into the soil solution, which act to

decrease osmotic potential to the point where elongating root apical meristems are killed. This can limit the ability of roots to access water as soils dry and may increase seedling susceptibility to moisture stress. Additionally, continued CRF nutrient uptake when soils are wet may cause rapid shoot growth relative to root growth and restrict the formation and elongation of new root tips. This may lead to nutrient imbalances, severe summer drought stress, decreased photosynthetic capacities, and reduced whole-plant growth rates.

Continued development of CRF technology to produce a mechanism of nutrient release that coincides closer with plant developmental status may be needed. Currently, it appears that conservative CRF rates, the use of CRF with moisture-dependent release characteristics (e.g. IBDU, Ureaform), or avoiding field fertilization entirely would help to reduce the potential for negative results associated with seedling fertilization on drought-prone sites.

Several primary conclusions can be identified from this dissertation. First, it appears that root architectural development is a criterion that should be considered when designing methods to improve reforestation success. Second, intensive measurements of root system development, though tedious, time-consuming, and generally requiring the destructive harvest of the plant, may provide a good assessment of seedling response to cultural treatments. Finally, to improve seedling establishment in reforestation operations, silvicultural treatments should be constructed to promote optimal shoot and root growth.

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Appendices

Appendix 1. Summary data for parameters related to Chapter 2.

Appendix 1. Table 1. Summary report with mean values and standard errors (SE) for morphological parameters measured in Aug. 1999 for the drier moisture experiment.

	Soil Amendment Treatment							
	Control	Low Manure	High Manure	Low Peat	High Peat	Low Verm.	High Verm.	SE
S1 Root Tips	2.8	0.8	1.3	4.1	4.8	2.9	1.8	1.2
S2 Root Tips	18.2	18.5	13.5	13.7	18.8	17.1	14.9	3.5
S3 Root Tips	5.9	8.6	6.4	4.5	9.2	8.6	5.8	1.9
Total Tips	26.9	27.9	21.3	22.3	32.8	28.7	22.5	5.3
S1 Laterals	2.1	0.7	1.1	2.0	2.8	1.8	1.3	0.7
S2 Laterals	11.4	9.5	8.3	9.8	10.8	10.0	11.0	1.1
S3 Laterals	4.6	5.7	6.3	5.7	5.7	6.8	5.7	1.0
Total Laterals	18.1	15.8	15.6	17.5	19.3	18.5	18.0	1.0
S1 Root Length	22.5	6.4	11.2	27.9	37.3	21.5	17.8	8.7
S2 Root Length	128.9	103.8	101.2	113.5	139.2	135.9	136.5	16.1
S3 Root Length	39.2	50.4	56.9	47.8	57.3	64.8	53.4	9.9
Total Length	190.6	160.6	169.3	189.3	233.8	222.3	207.7	21.0
S1 Tap Dry Wt.	0.23	0.19	0.25	0.21	0.24	0.22	0.22	0.02
S2 Tap Dry Wt.	0.14	0.15	0.15	0.13	0.13	0.12	0.15	0.02
S3 Tap Dry Wt.	0.03	0.03	0.03	0.03	0.02	0.03	0.03	0.01
Total Tap Dry Wt.	0.40	0.37	0.43	0.37	0.39	0.37	0.40	0.05
S1 Lat. Dry Wt.	0.06	0.01	0.02	0.06	0.11	0.05	0.03	0.02
S2 Lat. Dry Wt.	0.32	0.21	0.27	0.28	0.37	0.31	0.36	0.06
S3 Lat. Dry Wt.	0.09	0.10	0.11	0.09	0.12	0.11	0.11	0.02
Total Lat Dry Wt.	0.46	0.32	0.40	0.44	0.60	0.47	0.50	0.08

Appendix 1. Table 2. Summary report with mean values and standard errors (SE) for morphological parameters measured in Jan. 2000 for the drier moisture experiment.

	Soil Amendment Treatment							
	Control	Low Manure	High Manure	Low Peat	High Peat	Low Verm.	High Verm.	SE
S1 Root Tips	5.5	2.5	2.7	2.7	4.7	1.5	1.7	1.4
S2 Root Tips	29.2	15.8	15.9	16.8	11.9	20.7	20.0	4.5
S3 Root Tips	7.2	9.0	6.4	9.8	4.7	12.7	9.8	2.0
Total Tips	41.9	27.3	25.0	29.3	21.3	34.8	31.4	5.2
S1 Laterals	2.2	1.5	1.7	2.3	2.1	1.3	1.9	0.8
S2 Laterals	12.4	9.0	8.8	11.2	10.9	10.2	13.4	1.7
S3 Laterals	3.5	5.8	4.3	8.5	6.3	7.5	10.1	1.8
Total Laterals	18.1	16.3	14.8	21.9	19.3	19.0	25.4	2.7
S1 Root Length	24.3	16.9	9.9	22.1	27.3	14.8	44.7	11.8
S2 Root Length	157.2	108.8	86.7	148.2	121.6	146.1	154.6	20.6
S3 Root Length	39.1	57.6	38.3	86.0	62.4	78.7	71.3	9.3
Total Length	220.5	183.3	134.9	256.3	211.3	239.5	270.6	26.4
S1 Tap Dry Wt.	0.26	0.24	0.23	0.28	0.22	0.25	0.28	0.03
S2 Tap Dry Wt.	0.16	0.15	0.15	0.18	0.13	0.17	0.17	0.02
S3 Tap Dry Wt.	0.02	0.03	0.02	0.05	0.02	0.05	0.04	0.01
Total Tap Dry Wt.	0.43	0.42	0.40	0.51	0.37	0.47	0.48	0.05
S1 Lat. Dry Wt.	0.08	0.04	0.03	0.04	0.06	0.03	0.06	0.02
S2 Lat. Dry Wt.	0.49	0.27	0.23	0.38	0.28	0.35	0.34	0.07
S3 Lat. Dry Wt.	0.12	0.13	0.09	0.19	0.11	0.17	0.17	0.03
Total Lat Dry Wt.	0.69	0.44	0.34	0.62	0.44	0.55	0.57	0.08

Appendix 1. Table 3. Summary report with mean values and standard errors (SE) for morphological parameters measured in Aug. 1999 for the wetter moisture experiment.

	Soil Amendment Treatment							
	Control	Low Manure	High Manure	Low Peat	High Peat	Low Verm.	High Verm.	SE
S1 Root Tips	13.9	2.3	13.3	15.9	18.6	17.1	10.8	4.8
S2 Root Tips	50.0	57.5	76.3	57.9	60.4	51.7	58.3	7.8
S3 Root Tips	16.8	23.6	19.4	16.7	18.3	26.3	22.5	4.9
Total Tips	80.7	83.4	109.0	90.5	97.3	95.0	91.7	10.1
S1 Laterals	3.1	0.8	2.0	2.3	3.3	3.5	1.8	0.9
S2 Laterals	11.3	11.2	13.1	11.4	13.8	10.3	11.5	1.4
S3 Laterals	4.75	6.25	5.2	4.8	5.8	5.8	7.1	0.9
Total Laterals	19.08	18.2	20.3	18.6	22.9	19.7	20.4	1.7
S1 Root Length	51.58	38.0	30.4	34.9	96.8	59.9	30.5	26.2
S2 Root Length	194.3	193.3	231.2	191.3	220.0	182.0	189.2	26.8
S3 Root Length	63.8	89.7	71.4	78.2	72.0	91.6	94.8	13.3
Total Length	309.7	291.0	333.0	304.3	388.8	333.5	314.5	33.6
S1 Tap Dry Wt.	0.66	0.63	0.73	0.74	0.89	0.72	0.76	0.07
S2 Tap Dry Wt.	0.47	0.48	0.56	0.54	0.63	0.54	0.56	0.06
S3 Tap Dry Wt.	0.08	0.10	0.08	0.11	0.10	0.12	0.13	0.02
Total Tap Dry Wt.	1.21	1.21	1.37	1.38	1.61	1.38	1.45	0.14
S1 Lat. Dry Wt.	0.45	0.05	0.15	0.23	0.59	0.42	0.31	0.15
S2 Lat. Dry Wt.	1.72	1.43	1.64	1.55	1.88	1.42	1.66	0.30
S3 Lat. Dry Wt.	0.46	0.53	0.48	0.56	0.41	0.66	0.81	0.08
Total Lat Dry Wt.	2.63	2.00	2.26	2.33	2.88	2.50	2.79	0.30

Appendix 1. Table 4. Summary report with mean values and standard errors (SE) for morphological parameters measured in Jan. 2000 for the wetter moisture experiment.

	Soil Amendment Treatment							
	Control	Low Manure	High Manure	Low Peat	High Peat	Low Verm.	High Verm.	SE
S1 Root Tips	16.8	11.5	22.5	22.5	14.5	11.4	9.7	4.6
S2 Root Tips	55.0	55.8	58.8	59.2	59.6	56.3	65.4	6.2
S3 Root Tips	19.6	20.4	15.4	16.0	13.5	21.7	17.5	2.6
Total Tips	91.4	87.8	96.7	97.7	87.6	89.3	92.6	8.3
S1 Laterals	3.3	2.4	4.0	2.8	3.9	1.9	1.8	0.9
S2 Laterals	11.7	12.4	10.8	13.5	13.8	12.8	13.0	1.7
S3 Laterals	4.5	4.9	4.4	5.4	3.9	4.3	4.7	0.9
Total Laterals	19.4	19.8	19.3	21.8	21.7	19.0	19.5	1.9
S1 Root Length	52.5	31.1	51.2	63.7	44.8	32.2	33.0	14.7
S2 Root Length	175.6	183.8	151.6	217.8	203.8	218.8	203.5	28.2
S3 Root Length	58.3	60.7	56.3	64.3	49.8	69.1	67.3	10.9
Total Length	286.3	275.5	259.1	345.8	297.8	320.1	303.8	31.5
S1 Tap Dry Wt.	0.61	0.60	0.75	0.80	0.63	0.62	0.69	0.09
S2 Tap Dry Wt.	0.41	0.44	0.48	0.56	0.44	0.46	0.48	0.08
S3 Tap Dry Wt.	0.07	0.10	0.06	0.11	0.06	0.09	0.08	0.02
Total Tap Dry Wt.	1.08	1.13	1.29	1.47	1.13	1.17	1.24	0.17
S1 Lat. Dry Wt.	0.32	0.21	0.37	0.48	0.30	0.16	0.20	0.10
S2 Lat. Dry Wt.	1.40	1.34	1.47	1.70	1.58	1.49	1.64	0.30
S3 Lat. Dry Wt.	0.57	0.45	0.42	0.48	0.36	0.48	0.45	0.08
Total Lat Dry Wt.	2.29	2.00	2.34	2.61	2.24	2.13	2.29	0.34

Appendix 2. Summary data for parameters related to Chapter 4.

Appendix 2. Table 1. Mean values and standard error (SE) for morphological parameters of seedlings grown under different rates of CRF at four sampling points. For each parameter and within each sampling point, means followed by the same letter in a row did not differ significantly at $\alpha \leq 0.05$.

	Fertilizer Rate				
	0 g	8 g	16 g	24 g	SE
Height Growth					
Aug-00	9.2	10.4	10.3	10.4	0.60
Oct-00	16.1ab	18.5a	14.9bc	12.2c	1.07
Dec-00	16.0b	18.2a	17.0ab	13.7c	0.70
Feb-01	17.8	20.4	17.6	16.6	1.07
Diameter Growth					
Aug-00	1.25	1.42	1.49	1.37	0.07
Oct-00	2.46c	3.00a	2.85ab	2.53bc	0.12
Dec-00	3.26b	3.83a	3.89a	3.06b	0.16
Feb-01	2.96	3.52	3.45	3.07	0.21
Shoot Volume					
Aug-00	5.8	6.0	6.4	6.3	0.47
Oct-00	13.6a	15.7a	13.6a	10.5b	0.93
Dec-00	16.0ab	18.4a	13.8b	10.5c	0.99
Feb-01	15.0	16.4	15.5	13.2	2.54
Root Volume					
Aug-00	3.10a	2.66ab	2.22bc	1.83c	0.20
Oct-00	5.07ab	5.91a	4.66bc	4.12c	0.30
Dec-00	8.91ab	10.2a	8.22b	6.14c	0.61
Feb-01	8.33	11.3	10.6	6.69	1.35
Shoot:Root Volume					
Aug-00	1.97c	2.36bc	2.94b	3.66a	0.22
Oct-00	2.90	2.91	3.22	3.04	0.18
Dec-00	1.84	1.86	1.81	1.92	0.14
Feb-01	1.71	1.42	1.57	1.97	0.99
Shoot Dry Weight (g)					
Aug-00	0.66	0.72	0.78	0.8	0.07
Oct-00	2.1ab	2.35a	2.22a	1.81b	0.12
Dec-00	3.04ab	3.61ab	2.91bc	2.34c	0.21
Feb-01	3.32	3.97	3.82	3.1	0.47
Root Dry Weight (g)					
Aug-00	0.37	0.4	0.37	0.34	0.02
Oct-00	0.90ab	1.03a	0.81b	0.76b	0.05
Dec-00	1.43ab	1.71a	1.39b	1.01c	0.10
Feb-01	1.51	2.19	2.02	1.36	0.24

Appendix 2. Table 2. Mean values and standard error (SE) for root morphological parameters of seedlings grown under different rates of CRF at four sampling points. For each parameter and within each sampling point, means followed by the same letter in a row did not differ significantly at $\alpha \leq 0.05$.

	Fertilizer Rate				
	0 g	8 g	16 g	24 g	SE
S1 Number of Active Root Tips					
Aug-00	18.8c	23.8bc	30ab	36.1a	2.4
Oct-00	31.7b	42.5a	47.1a	46.8a	3.4
Dec-00	44.8	63.4	75.4	84.5	10.0
Feb-01	63.5	65.7	68.2	71.4	11.2
S2 Number of Active Root Tips					
Aug-00	22.1	17.8	15.8	11.8	1.9
Oct-00	43.3a	31.0b	20.6c	14.8c	2.2
Dec-00	55.4a	44.7a	23.7b	14.7c	3.9
Feb-01	44.6	66.7	37.9	28	8.6
S3 Number of Active Root Tips					
Aug-00	23.6a	9.92b	3.9c	1.1c	1.5
Oct-00	43a	16.3b	9.7c	1.4d	2.2
Dec-00	35.6a	26.4a	6.1b	0.8b	3.8
Feb-01	33.1	41.2	17	1.4	8.9
Total Number of Active Root Tips					
Aug-00	64.5	51.5	49.7	49.0	4.4
Oct-00	118.0a	89.9b	77.4bc	63.0c	5.6
Dec-00	135.8	135	105.2	100.0	13.5
Feb-01	141.2	174	122.7	100.8	22.2
S1 Lateral Root Length (cm)					
Aug-00	72.5	66.3	70.8	76.2	4.5
Oct-00	52.5bc	59.2ab	61.3a	51.7bc	2.6
Dec-00	65.6	71.8	72.3	76.1	4.0
Feb-01	59.3	53.6	58.4	59.8	2.9
S2 Lateral Root Length (cm)					
Aug-00	79.8a	45.8b	25.2c	20.1c	2.0
Oct-00	65.7a	48.4b	27.3c	20.9c	2.7
Dec-00	70.8a	52b	28.5c	17.7c	4.4
Feb-01	49.8	51.9	41.6	25.2	6.4
S3 Lateral Root Length (cm)					
Aug-00	59.9a	27.3b	8.04c	2.4c	2.4
Oct-00	56.7a	32.8b	12.1c	2.9d	2.7
Dec-00	51.8a	36.8a	12.5b	1.7b	6.2
Feb-01	35.5	42	31	4.5	7.9
Total Lateral Root Length					
Aug-00	212.2a	139.5b	104c	98.7c	5.9
Oct-00	174.8a	140.4b	100.7c	75.5d	4.8
Dec-00	188.2a	160.6a	113.3b	95.6b	11.8
Feb-01	144.6	147.5	131.1	89.4	13.9

Appendix 2. Table 2. (Continued)

	Fertilizer Rate				
	0 g	8 g	16 g	24 g	SE
S1 Number of F.O. Laterals					
Aug-00	12.6	12.4	13.3	13.8	0.7
Oct-00	10.7	11.3	12.3	11.3	0.4
Dec-00	13.5	14.3	14.8	15.6	1.0
Feb-01	11.2	10.2	11.6	11.6	0.6
S2 Number of F.O. Laterals					
Aug-00	11.2a	8.8b	6.3c	5.7c	0.5
Oct-00	10.3a	9.5a	6.9b	5.9b	0.5
Dec-00	12.8a	10b	7.5c	5.9c	0.7
Feb-01	8.77	9.3	8.4	6.7	1.0
S3 Number of F.O. Laterals					
Aug-00	8.5a	3.8b	1.4c	0.4c	0.4
Oct-00	9a	5.5b	2.1c	1.1c	0.5
Dec-00	10a	7.3a	2.3b	0.4b	1.2
Feb-01	5.9	7	5	0.9	1.3
S1 Tap Root Dry Weight (g)[1]					
Aug-00	0.03a	0.05b	0.04b	0.04b	0.002
Oct-00	0.11	0.11	0.10	0.09	0.010
Dec-00	0.16	0.15	0.18	0.12	0.020
Feb-01	0.16	0.23	0.2	0.18	0.020
S1 Lateral Root Dry Weight (g)					
Aug-00	0.10	0.12	0.14	0.14	0.01
Oct-00	0.22b	0.31a	0.29a	0.27a	0.02
Dec-00	0.35b	0.55a	0.52a	0.45ab	0.04
Feb-01	0.47	0.66	0.66	0.52	0.07
S2 Lateral Root Dry Weight (g)					
Aug-00	0.08	0.08	0.07	0.06	0.007
Oct-00	0.17b	0.2a	0.14bc	0.13c	0.012
Dec-00	0.32a	0.32a	0.21b	0.12c	0.021
Feb-01	0.25	0.47	0.41	0.22	0.076
S3 Lateral Root Dry Weight (g)					
Aug-00	0.082a	0.059a	0.018b	0.006b	0.008
Oct-00	0.197a	0.144b	0.064c	0.035c	0.016
Dec-00	0.241a	0.261a	0.116b	0.003b	0.039
Feb-01	0.16	0.344	0.248	0.03	0.064
Total Lateral Root Dry Weight (g)					
Aug-00	0.26	0.26	0.23	0.2	0.02
Oct-00	0.58ab	0.66a	0.49bc	0.43c	0.03
Dec-00	0.94ab	1.13a	0.84b	0.57c	0.08
Feb-01	0.92	1.47	1.31	0.77	0.18

[1]Tap root rarely extended into S2 or S3 and means for these sections are not reported.

Appendix 2. Table 3. Parameter estimates, adjusted R^2 values, and model p-values for polynomial contrasts related to root morphology two months following transplant.

	Intercept	Rate	Rate^2	Adj. R^2	Model p-value
Root Volume	3.08908	-0.05308		0.4837	0.0006
S1 Active Root Tips	18.425	0.7276		0.511	0.0001
S2 Active Root Tips	21.8	-0.41042		0.4345	0.0003
Log (S3 Active Root Tips+1)	3.11	-0.17269	0.0024	0.9468	0.0001
S1 Lateral Root Dry Wt.	0.10267	0.00161		0.1919	0.0186
Log (S3 Lateral Root Dry Wt. + 0.01)	-2.48402	-0.07966		0.8420	0.0001
Total Lateral Root Length	211.8125	-11.0182	0.26335	0.8664	0.0001
Shoot:Root Dry Weight	1.72667	0.02399		0.2799	0.0046
Shoot:Root Volume	1.88368	0.07092		0.6000	0.0001

Appendix 2. Table 4. Parameter estimates, adjusted R^2 values, and model p-values for polynomial contrasts related to root morphology four months following transplant.

	Intercept	Rate	Rate^2	Adj. R^2	Model p-value
Height Growth	16.49694	0.27844	-0.01964	0.283	0.0304
Shoot Volume	13.79874	0.33973	-0.02012	0.2286	0.0252
S1 Active Roots	34.72917	0.61979		0.1667	0.0272
S2 Active Roots	41.85417	-1.19792		0.7504	0.0001
Log (S3 Active Roots+1)	3.58327	-0.15118	0.00107	0.8856	0.0001
Log (S3 Lateral Root Dry Wt. + 0.01)	-1.79252	-0.087		0.6485	0.0001
Total Number of Active Roots	114.1458	-2.22135		0.5383	0.0001
Total Lateral Root Length	30.01528	-0.50156		0.4943	0.0001

Appendix 2. Table 5. Parameter estimates, adjusted R^2 values, and model p-values for polynomial contrasts related to root morphology six months following transplant.

	Intercept	Rate	Rate^2	Adj. R^2	Model p-value
Height Growth	16.09389	0.410	-0.022	0.3629	0.0034
Diameter Growth	3.26051	0.120	-0.005	0.3603	0.0035
Shoot Volume	16.37153	0.287	-0.023	0.4493	0.0007
Root Volume	9.0719	0.181	-0.013	0.2041	0.0350
Shoot Dry Weight	3.11725	0.073	-0.005	0.3109	0.0077
Root Dry Weight	1.4564	0.043	-0.003	0.2911	0.0106
Log (S2 Active Roots + 1)	4.05168	-0.06124		0.7520	0.0001
Log (S3 Active Roots + 1)	3.60197	-0.13648		0.8007	0.0001
Log (S1 Lateral Root Dry Wt. + 0.01)	-1.29817	0.09498	-0.00337	0.2263	0.0260
Log (S2 Lateral Root Dry Wt. + 0.01)	-1.20731	0.01566	-0.00261	0.5944	0.0001
Log (S3 Lateral Root Dry Wt. + 0.01)	-1.67841	0.03177	-0.00598	0.7249	0.0001
Total Lateral Root Length	188.2097	-4.01198		0.4651	0.0001