Growth and Nutrient Allocation of Douglas-fir Seedlings: Response to Varying Ammonium : Nitrate Ratios and to Different Methods of Nursery Fertilizer Application

By

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ABSTRACT

Regeneration of Douglas-fir following harvesting has proven difficult on many sites in British Columbia. As a result, alternative nursery practices are being explored to improve seedling growth. Using two experiments I aimed to identify an improved nutritional program for Douglas-fir seedlings. The objective of the first experiment was to identify the optimum nitrogen (N) source ratio for Douglas-fir between two inorganic sources of N, ammonium (NH_4^+) and nitrate (NO_3^-). Seedlings were grown in controlled environments in aeroponic culture with solutions containing 0:100, 20:80, 40:60, 60:40, 80:20 or 100:0 NH_4^+:NO_3^- ratios. Growth and nutrient allocation was observed for 45 days. Different NH_4^+ and NO_3^- ratios resulted in significant differences in growth and nutrient allocation. Seedlings grown in solutions containing abundant and relatively equal portions of NH_4^+ and NO_3^- were characterized by the greatest relative growth rates, greatest biomass and stable internal nitrogen concentrations. Seedlings grown in solutions containing high NH_4^+ (80:20 and 100:0 NH_4^+:NO_3^-) concentrations were characterized by lower relative growth rates, less biomass, lower internal nutrient concentrations and lower rates of photosynthesis and root respiration compared to seedlings with less NH_4^+ (20:80, 40:60 and 60:40 NH_4^+:NO_3^-). Seedlings appeared to take up a greater proportion of NH_4^+ than NO_3^- from solution.

The objective of the second experiment was to examine the implications of two nursery fertilization regimes for growth and nutrient dynamics. Seedlings were grown in a nursery with nutrients added at a constant rate (conventional fertilization) or at an exponentially increasing rate of 2% day^{-1} (exponential nutrient loading). At the time of planting, half of the conventionally fertilized seedlings were planted with slow release fertilizer packets. Growth and nutrient allocation was observed for two years following planting. In the field experiment, although exponential nutrient loading applied 25% more N in the nursery compared to the conventional regime, no benefits in growth or nutrient allocation were found. Two years after planting, there were no significant differences in height, root collar diameter or total dry mass between seedlings grown under the different nursery fertilizer regimes. In contrast, seedlings planted with additional fertilizer consistently outperformed seedlings grown with exponential nutrient loading, with greater height, root collar diameter and dry mass. Two growing seasons after planting there were no significant differences among treatments in whole-plant N concentrations.

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CHAPTER 1. GENERAL INTRODUCTION

Study species: Ecology and background information

Douglas-fir (*Pseudotsuga menziesii*), a member of the family Pinaceae, is considered one of the world's most important and valuable timber tree species (Hermann and Lavender 1990). Worldwide, there are eight species in the genus *Pseudotsuga*, two of which are native to western North America (Farrar 1995). In British Columbia, there are two varieties of Douglas-fir, the coastal variety (*Pseudotsuga menziesii* var. *menziesii* (Mirb.) Franco) and the interior, or Rocky Mountain, variety (*Pseudotsuga menziesii* var. *glauca* (Beissn.) Franco).

In North America, Douglas-fir ranges from a latitude of 19° N in central Mexico to 55° N in the central interior of British Columbia (Hermann and Lavender 1990) (Figure 1.1). The coastal variety of Douglas-fir has a 2200 km range along the Pacific coast to a southernmost latitude of 34° 44' N, while the interior variety has a range extending almost 4500 km from the interior of British Columbia, through the Rocky Mountains to Mexico (Hermann and Lavender 1990). Generally, the upper elevation limit for Douglas-fir in central British Columbia is 760 m, but that rises to 1250 m on Vancouver Island. The principle factors limiting distribution tend to be temperature in the north and precipitation in the south (Hermann and Lavender 1990).

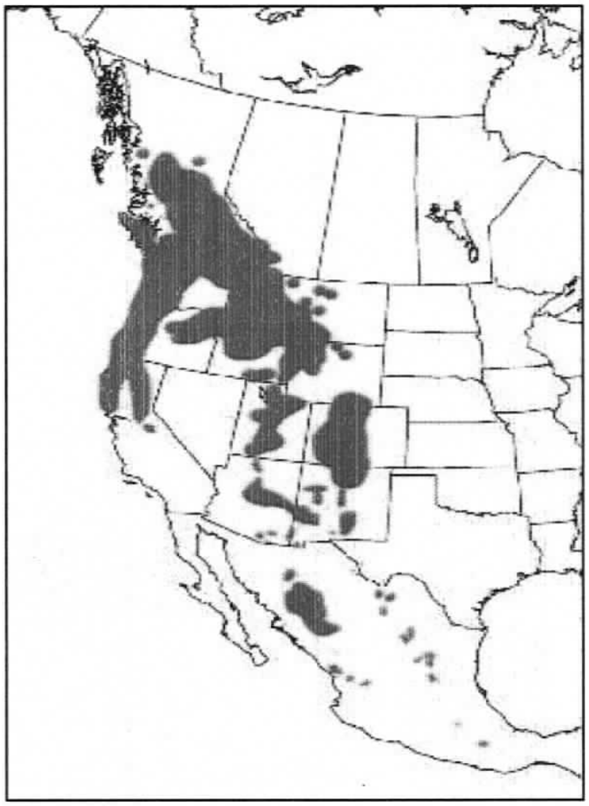

Figure 1.1. Douglas-fir range map (from Hermann and Lavender 1990). Douglas-fir ranges from central interior British Columbia in the north to central Mexico in the south.

The two varieties of Douglas-fir grow under climatically different conditions. Interior Douglas-fir is generally exposed to colder winters, shorter frost free periods and larger diurnal fluctuations in temperature when compared to coastal Douglas-fir (Hermann and Lavender 1990). Annual precipitation for interior Douglas-fir in British Columbia varies from 560 to 1020 mm, most of which occurs in the form of snow. Frost-free days average from 60 to 120 days per year. In contrast, coastal Douglas-fir receives between 760 and 3400 mm of annual precipitation, most of which is in the form of rain. Frost-free days range from 195 to 260 (Hermann and Lavender 1990). Average temperatures where interior Douglas-fir grows are 14 to 20 °C in July and –7 to 3 °C in

January. In contrast, average temperatures where coastal Douglas-fir is found are much warmer, 20 to 27 °C in July and –2 to 3 °C in January (Hermann and Lavender 1990).

Douglas-fir can be found on a variety of sites, but grows best on deep, well-drained, well aerated soils. Poorly drained or compacted sites and limestone soils provide poor growing conditions (Farrar 1995). Douglas-fir forests mainly occur on sites with pH 5 and 6; however, surface soils tend to be moderately more acidic (Hermann and Lavender 1990). In British Columbia, soils tend to be of glacial origin, and are often characterized by low total nitrogen (N) concentrations (Hermann and Lavender 1990).

Coastal Douglas-fir is considered an early successional species often regenerating after forest fires. Over time, coastal Douglas-fir is generally replaced by species such as Sitka spruce (*Picea sitchensis* Parry ex Engelm.), western hemlock (*Tsuga heterophylla* (Raf.) Sarg.), amabilis fir (*Abies amabilis* (Dougl. ex Loud.)), western red cedar (*Thuja plicata* Don ex D. Don) and grand fir (*Abies grandis* (Dougl. ex D.Don) (Farrar 1995). Interior Douglas-fir can be both an early successional and climax species. After early succession, interior Douglas-fir is generally replaced by white spruce (*Picea glauca* (Moench) Voss) and subalpine fir (*Abies lasiocarpa* (Hook.) Nutt.) (Alexander 1988).

The regeneration of Douglas-fir has been highly variable, especially following harvesting (Hermann and Lavender 1990). Regeneration of the coastal variety has been more successful on mesic to sub-hydric sites, however much difficulty still remains in attaining adequate regeneration on drier sites (Hermann and Lavender 1990). Successful regeneration of interior Douglas-fir usually occurs on early successional sites, however less regeneration success occurs on sites where Douglas-fir has been a climax species (Ryker 1975).

Old-growth Douglas-fir timber is characterized by fine grain, exceptional strength and durability, which allows a wide variety of commercial uses. Mature trees are often characterized by long straight trunks and minimal taper, which yield a high volume of clear wood. These traits have created high demand for Douglas-fir for architectural purposes as well as industrial purposes where long lasting timbers are required (Hermann and Lavender 1990).

Nitrogen nutrition

Arguably, the most important nutrient in plant nutrition is nitrogen (N). As one of the key macronutrients, N is required in the greatest abundance and often is the nutrient most limiting to growth (Crawford and Glass 1998, Forde and Clarkson 1999). Available N in the forest floor primarily originates from the recycling of organic matter (Chapin 1991). Nitrogen is available for uptake by plants in several forms including the inorganic forms, ammonium (NH_4^+) and nitrate (NO_3^-), and organic forms, such as amino acids (Crawford and Glass 1998). Plant uptake of amino acids has traditionally been thought to be limited by competition with microorganisms (Chapin et al. 1993), resulting in the majority of plant N being taken up in inorganic forms.

Soil inorganic N availability is mainly dependant on the mineralization of organic N to NH_4^+ by microorganisms and the subsequent conversion to NO_3^- (Haynes and Goh 1978). The total amount of N, and the ratio of NH_4^+ to NO_3^-, depends largely on the quantity and quality of N input, and on the balance of ammonification, nitrification, immobilization and denitrification processes (Haynes and Goh 1978). These processes are further influenced by several factors, including the accumulation of organic matter, soil pH and soil temperature (Rice and Pancholy 1972, Haynes and Goh 1978, Lodhi 1978). Both NH_4^+ and NO_3^- uptake can be limited by sub-optimal conditions of pH (van den Driessche 1971, 1978, Rygiewicz et al. 1984a,b) and temperature (Haynes and Goh 1978, Clarkson et al. 1986, Gessler et al. 1998). Furthermore, variations in relative concentrations of N and N form in the soil play a critical role in plant growth and physiology.

Steady state nutrition

Steady state nutrition has been identified as a key requirement for plant nutrition studies (Ingestad and Lund 1986). By definition, steady state requires seedlings to be grown with constant internal nutrient concentrations, free from nutrient stress (Ingestad and Lund 1986). Steady state corresponds with the competitive natural environment, where vegetation naturally grows at an exponential rate and nutrient uptake occurs at a rate similar to the plant growth rate (Timmer 1997). This allows plant internal nutrient concentrations to remain stable, thereby preventing nutrient stress. Steady state theory follows the concept that plants grow under conditions of constant N supply, rather than decreasing N over time. Constant N concentrations are typically seen in natural conditions (Ingestad 1982), whereas decreasing N levels are typically seen in nursery situations when only one concentration of N is applied to seedlings over time. Growth following steady state nutrition techniques allows seedlings to become better adapted to constant N uptake, rather than contending with situations of nutrient deficiency.

The situation is similar for seedlings grown in nutrient solution. As nutrient uptake occurs, nutrients must be replaced into the system to maintain steady state conditions (Ingestad and Lund 1986). If nutrients are taken up and not replaced, seedlings will, over time, start to display symptoms of nutrient deficiency. Similarly, growth of seedlings under sub-optimal conditions (i.e. toxic NH_4^+:NO_3^- ratios) may result in seedlings exhibiting decreasing internal nutrient concentrations over time, rather than steady state nutrition.

Steady state is achieved when the internal nutrient concentration, the relative uptake rate (R_U), and the relative growth rate (R_G) remain constant over time (Ingestad and Lund 1986):

$$R_G = (\ln W_2 - \ln W_1)/(t_2 - t_1), \tag{1.1}$$

where W is plant biomass, t is time, and W_1 and W_2 are plant biomasses at time t_1 and t_2, respectively (Ingestad and Lund 1986).

In steady state nutrition, the relative uptake rate (R_U), must equal the relative growth rate (R_G), and must be matched by an equal relative addition rate (R_A) in order to maintain R_U, and R_G in an unchanging state (Ingestad and Lund 1986). Therefore,

$$R_A = R_U = R_G \tag{1.2}$$

Nitrogen nutrition and forest succession

Below ground environmental conditions may play as large a role in forest succession patterns as above ground conditions. Factors such as NH_4^+ and NO_3^- availability are currently causing speculation as to the role they play in the transition of plant species over time (Lavoie et al. 1992, Kronzucker et al. 1997, Kronzucker et al. 2003). Ammonium and NO_3^- concentrations in the soil fluctuate after disturbances such as fire, windthrow or harvesting (Vitousek et al. 1982, Lavoie et al. 1992). Soils are typically characterized by a rapid flush of NO_3^- after disturbance, followed by a gradual transition to increased NH_4^+.

This concept of temporal variations in NH_4^+ and NO_3^- concentrations has recently been coupled with the diversity of plant responses to variations in inorganic N sources (Kronzucker et al. 1997, Kronzucker et al. 2003). Kronzucker et al. (2003) postulated that over time, trees have become adapted to the nitrogenous characteristics of their native habitats. Consequently, these adaptations to specific N sources have led to an increase in competitive ability on sites with preferred N sources and reduced competitive abilities on sites with sub-optimal N sources (Kronzucker et al. 2003). Min et al. (1999) found that trembling aspen (*Populus tremuloides* (Michx.)) and Douglas-fir, defined by Kronzucker et al. (2003) as two early successional species, both exhibited poor regulation over NH_4^+ uptake which resulted in excessive accumulation of NH_4^+ in the root cytosol. Alternatively, white spruce, a late successional conifer, has shown a clear preference for NH_4^+ (Kronzucker et al. 1995 a, b), yet exhibits much lower NH_4^+ concentrations in the root cytosol therefore, indicating greater regulation over NH_4^+ uptake and efflux.

If the hypothesis outlined by Kronzucker et al. (2003) is correct, and NH_4^+ uptake in early successional species (including Douglas-fir) is excessive and insufficiently regulated, it could be expected that enhanced uptake of NH_4^+ may not necessarily be translated to growth.

Optimal nutrient regimes for Douglas-fir seedlings

Developing an optimal nutrient regime for Douglas-fir could provide forest managers with an enhanced tool for improving regeneration of this species following harvesting. Regeneration of Douglas-fir, especially in the interior, has proven challenging in British Columbia (Newsome 1997). Difficult environmental conditions have often been linked to the sub-optimal growth of plants (Millard 1996), such as Douglas-fir (Newsome 1997); however, improved nutritional management may help offset these conditions.

The objective of this experiment was to improve Douglas-fir nutrition through two approaches:

(I) By examining the effects of varying NH_4^+:NO_3^- ratios on the growth and nutritional characteristics of Douglas-fir, I hoped to identify the optimal N source ratio. Providing forest nursery managers with the optimal N source ratio will allow them to refine their nutrient formulations to best fit Douglas-fir and potentially improve growth following planting.

(II) By testing the benefits of alternative fertilizer regimes that aim to achieve steady state conditions in nursery grown seedlings, I hoped to refine techniques in BC conifer seedling nurseries to best suit Douglas-fir. Seedlings raised with exponentially increasing nutrient supply in the nursery and characterized by steady-state nutrient conditions, were planted in the field, and I compared their performance in the subsequent two years with seedlings grown using a conventional fertilization regime, planted either with or without additional fertilizer.

Together, these two experiments were used to develop an optimal nutritional program for Douglas-fir, not only by developing the optimal N source ratio for nutrient formulations, but by identifying the best method by which to apply these nutrients in the nursery. Rapid regeneration of Douglas-fir, immediately following harvest, is important from an operational forestry perspective. Growth of optimally conditioned, fast growing seedlings may improve regeneration following harvesting, reduce the time to stand establishment, and may reduce the need for environmentally sensitive activities such as on-site fertilization and herbicide application. Improving these aspects of regeneration has the potential to dramatically reduce silviculture related costs.

No known studies of this nature have been conducted on Douglas-fir. Several studies have examined nutrient uptake from different N sources (e.g Krajina et al. 1973, Gisjman 1990 a, b, Kamminga-van Wijk and Prins 1993), however few studies have linked this uptake to growth, and no known studies have linked it to nursery fertilization regimes. Although several studies have examined the benefits of exponential nutrient loading (e.g. Malik and Timmer 1995, Salifu and Timmer 2001, 2003, Hawkins et al. 2005), no field studies are known to have been conducted on interior Douglas-fir. This experiment was intended to establish a comprehensive nutritional regime for Douglas-fir.

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CHAPTER 2. GROWTH AND NUTRIENT DYNAMICS OF DOUGLAS-FIR SEEDLINGS RAISED WITH VARYING RATIOS OF AMMONIUM AND NITRATE

INTRODUCTION

Mineral nutrient supply plays a critical role in plant growth and development. Nutrient supply can alter plant growth by affecting survival, morphology, growth rates, photosynthesis and disease resistance (Brown et al. 1996). Understanding the physiological and morphological response of seedlings to key mineral nutrients will enhance the ability of forest nursery managers to create optimally conditioned seedlings for the regeneration of forested ecosystems.

Nitrogen (N) is the macronutrient needed in the greatest quantity and is a major factor limiting plant growth in the field (Vitousek and Howarth 1991, Forde and Clarkson 1999). In most forested ecosystems, inorganic N is available to conifers in the forms of ammonium (NH_4^+) and nitrate (NO_3^-) (Gessler et al. 1998). Inorganic soil N availability is mainly dependant on the mineralization of organic N by microorganisms (Haynes and Goh 1978), with the distribution and abundance of these N sources varying both spatially and temporally (Turner et al. 1993, Laverman et al. 2000). The total amount of N, and the ratio of NH_4^+ to NO_3^- depends largely on the quantity and quality of organic N input, and on the balance of ammonification, nitrification, immobilization and denitrification processes in the soil (Haynes and Goh 1978). In well-aerated soils nitrification can be so rapid that NH_4^+ concentrations can be limited, while in acidic or waterlogged soils nitrification is inhibited and NH_4^+ accumulates (Crawford and Forde 2002).

The abundance and ratio of NH_4^+ to NO_3^- vary in natural ecosystems, with concentrations fluctuating greatly following disturbance (Lavoie et al. 1992).

Disturbance can cause changes in soil aeration, soil temperature, soil moisture and forest floor composition. These factors can affect soil mineralization processes and thus the concentrations of NH_4^+ and NO_3^-. Most undisturbed boreal and sub-boreal coniferous forests are characterized by acidic and cold soils that typically have slow rates of organic matter decomposition and slow rates of nitrification (Lavoie et al. 1992). However, immediately following disturbances, such as clearcut harvesting, there tends to be an increase in soil NO_3^- pools (Vitousek et al. 1979, Bradley 2001), due to changes in the soil environment that favour the presence of autotrophic nitrifiers and increased amounts of NH_4^+ available for conversion to NO_3^- (Vitousek et al. 1979, Paavoliainen and Smolander 1998). As a result, early successional stands tend to be characterized by higher NO_3^- concentrations while late successional stands tend to be characterized by higher NH_4^+ concentrations (Lavoie et al. 1992, Ste-Marie and Pare 1999).

The elevated NO_3^- concentrations in soil following disturbance may result in some plant species that are adapted to high NH_4^+ concentrations becoming poor competitors (Tilman 1987, Jobidon et al. 1989). Kronzucker et al. (1997) hypothesized that niche partitioning of optimum N sources may be a major factor driving succession, and that the ability of early seral species to utilize greater concentrations of NO_3^- may be an effective competitive strategy. Early successional species may be good competitors for NO_3^-, thus disturbed sites may become dominated by these species (Kronzucker et al. 1997). Late successional species, such as white spruce (*Picea glauca* (Moench) Voss) have been demonstrated to take up much greater amounts of NH_4^+ while early successional species, such as trembling aspen (*Populus tremuloides* (Michx.)), tend to take up greater quantities of NO_3^- (Kronzucker et al. 2003).

The adaptation of many boreal conifers to reduced forms of N, such as NH_4^+, may lead to difficulties in regenerating these species following disturbance (Lavoie et al. 1992), and species better adapted to high NO_3^- conditions may be best suited for regeneration following disturbance (Kronzucker et al. 1997). Kronzucker et al. (2003) hypothesized that, as an early successional species, Douglas-fir is better adapted to high NO_3^- conditions, and thus grows poorly in situations of high NH_4^+ concentrations. Min et al. (2000) found Douglas-fir had a high capacity for NO_3^- uptake even at low external concentrations, and could maintain adequate cytoplasmic NO_3^- concentrations for optimal rates of reduction at low external NO_3^- concentrations. Kronzucker et al. (2003) postulated that it is not the inability to take up high levels of NH_4^+, but rather the inability of Douglas-fir to assimilate high levels of NH_4^+ that leads to reduced growth on this N source.

Ammonium and NO_3^- uptake depend on a number of distinct transport systems. Ammonium uptake depends on both a high affinity transport system (HATS) and a low affinity transport system (LATS), while NO_3^- is taken up through two HATS and one LATS (Glass et al. 2002). High affinity transport systems work under conditions of low external concentrations (µM), while LATS operate under conditions of high external (mM) concentrations (King et al. 1993).

Ammonium and NO_3^-, are both directly absorbed by roots of plants, however the uptake process is distinctly different between the two ions. Ammonium absorption depends on carrier-mediated NH_4^+ unitransport, a system that facilitates transport through the plasma membrane without the movement of another ion (Epstein and Bloom 2005). Due to the electro-chemical nature of NH_4^+ a cell cannot sustain a continual influx of

NH_4^+, without counter-ion flow to restore electro-neutrality (Epstein and Bloom 2005). Following uptake, cells assimilate the NH_4^+, which generates a H^+ ion that lowers the cytoplasmic pH and stimulates H^+ efflux from the cell (Serrano 1989). This release of H^+ ions can lower the pH of the rhizosphere (Gisjman 1990b), which may be a cause of NH_4^+ toxicity (Kronzucker et al. 2003). Because the slightly acidic nature of NH_4^+ can also lower the cell's internal pH, it must be quickly converted to amino acids to avoid internal NH_4^+ toxicity immediately after uptake (Epstein and Bloom 2005).

Uptake of NO_3^- is more complex than NH_4^+ uptake and requires greater metabolic energy (Bloom et al. 1992). Plant cell cytoplasm typically contains a negative electrical potential and a higher NO_3^- concentration with respect to its exterior, therefore passive movement of NO_3^- across the plasma membrane does not readily occur (Epstein and Bloom 2005). Both electrical and chemical potentials oppose NO_3^- influx, thus metabolic energy must be expended during NO_3^- uptake (Glass and Siddiqi 1995, Bloom et al. 1992). Uptake occurs in the outer cell layers of the root by active transport processes mediated by H^+ co-transport (Crawford and Forde 2002). Unlike the lowering of pH that occurs in the rhizosphere of NH_4^+ fed plants, the pH of the rhizosphere tends to increase with NO_3^- fed plants due to both the co-transport of H^+ ions and NO_3^- ions out of solution and the release of OH^- ions during NO_3^- assimilation (Gisjman 1990 a, b). Unlike NH_4^+, which must be assimilated immediately after uptake, NO_3^- can be mobilized in the xylem for transport to the shoot, can be stored in the vacuole or can be immediately reduced via NO_3^- reductase (Campbell 1999, Crawford and Forde 2002). Rather than immediate conversion to amino acids, as is the case with NH_4^+, NO_3^- must be reduced, either in the roots or the leaves to NH_4^+ by two enzymes: NO_3^- reductase and nitrite reductase

(Smirnoff et al. 1984). As a result of these complex processes, NO_3^- absorption and assimilation requires substantially more metabolic energy than NH_4^+ absorption and assimilation (Bloom et al. 1992).

Understanding the relative contribution of each of these N sources to seedling growth may provide valuable information for understanding the underlying mechanisms of Douglas-fir seedling nutrition. In past experiments, Douglas-fir has shown preferential uptake of NH_4^+ in some studies (van den Driessche 1978, Kamminga-van Wijk and Prins 1993), but of NO_3^- in other cases (Krajina et al. 1973, van den Driessche 1978). Several studies have looked at N source uptake, and some have related this to subsequent growth (van den Driessche 1978, Krajina et al. 1973, Bedell et al. 1999). Bedell et al. (1999) found whole seedling biomass, relative growth rate (RGR), and root and shoot elongation all improved with NH_4^+ nutrition, compared to NH_4NO_3 or NO_3^- nutrition, when grown on conditions ranging in pH from 3.9 to 5.2. Alternatively, Krajina et al. (1973) found greatest growth on NO_3^- compared to NH_4NO_3 or NH_4^+ nutrition, with a pH of 5.5.

All studies conducted on NH_4^+ and NO_3^- nutrition in Douglas-fir have been limited to 100% NH_4^+, 100% NO_3^-, or a single ratio of NH_4NO_3, but no known studies have attempted to determine the optimum N source ratio for Douglas-fir which requires growing seedlings using a range of NH_4^+:NO_3^- ratios. Knowing the optimum N source ratio may provide forest managers with enhanced tools for improving regeneration.

By growing seedlings in highly controlled aeroponic environments with varying ratios of NH_4^+:NO_3^-, this study relates nutrient uptake patterns with growth and nutrient dynamics in Douglas-fir seedlings. While maintaining temperature, pH, and light intensity constant, seedlings were grown at NH_4^+:NO_3^- ratios of 100:0, 80:20, 60:40, 40:60, 20:80 and 0:100, and the growth, nutrient and physiological responses were measured. I hypothesized that the different NH_4^+:NO_3^- ratios would result in significant differences in seedling growth and internal N concentrations.

MATERIALS AND METHODS

Biotronic units

All seedlings were grown in specialized growth units called Biotronic units (BIOTRONIC, Uppsala, Sweden) (Figure 2.1). The Biotronic units are an aeroponic plant culture system in which the roots of each individual plant are sprayed constantly with nutrient solution, allowing plants free access to available nutrients. As nutrients are removed from the system, changes in solution conductivity are noted by sensors in the Biotronic units, recorded by an external computer, and additional nutrients added automatically to maintain a constant supply of nutrients in the system. Solution conductivity, temperature, pH and nutrient additions were all monitored hourly for the duration of the experiment. The container that holds the nutrient solution is black to prevent growth of bacteria. Before the start of each experiment, units were cleansed with 70% ethanol for 4 hr to prevent growth of bacteria. Similarly, the foam plugs used to hold the seedlings in place were monitored for growth of algae or bacteria and were replaced regularly to reduce nutrient uptake by other organisms.

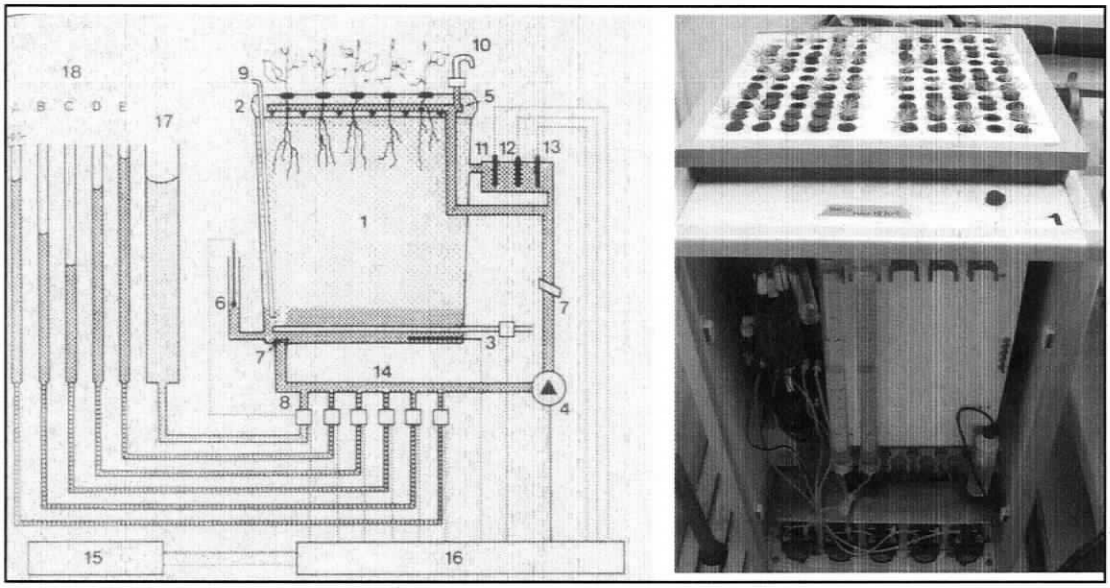

Figure 2.1. Design and appearance of the Biotronic growth units. **(Left:)** Schematic diagram of the Biotronic growth unit (1) plastic container with nutrient solution spray, (2) cover with holes for seedlings, (3) cooling coil, (4) pump for circulation of nutrient solution, (5) tubes with nozzles for spray of culture solution, (6) solution level sensor, (7) filters, (8) magnetic valve for addition of distilled water, (9) aeration nozzle, (10) sampling tube, (11) pH sensor, (12) conductivity sensor, (13) temperature sensor, (14) magnetic valve for nutrient addition, (15) PC computer with (16) interface, (17) burette for distilled water, (18) burette for nutrient stock solutions. Modified from Ingestad and Lund (1979). **(Right:)** Image of Biotronic growth unit used in these experiments.

Seedling culture

Coastal Douglas-fir seed from high elevation (seedlot 40462 (BC Ministry of Forests) 123° 58' longitude, 48° 41' latitude, 725 m) and low elevation (seedlot 3023 (Canadian Forest Service) 49° 16' longitude, 124° 16', 75m) sources were obtained. To stimulate germination, seeds were imbibed for 24 hours, surface sterilized for five minutes in 3% hydrogen peroxide, and thoroughly rinsed with distilled water before being placed in moist conditions at 4 °C for 21 days for stratification. Following the stratification period, seeds were placed in a Conviron germination chamber (Conviron, Winnipeg, Canada) set for an 8 hour light period at 30 °C and a 16 hour dark period at 20 °C. Seeds were allowed to germinate and were removed from the chamber when root lengths reached approximately 3 cm. Seedlings were placed in foam collars in a Biotronic unit containing pre-treatment solution (Table 2.1).

Table 2.1. **Concentrations (μM) of all chemicals in pre**-treatment nutrient solutions. Biotronic units contained 10 L of solution. Germinants were placed into pre-treatment solutions until they reached an approximate fresh mass of 0.07 g.

Chemical formula	Concentration (μM)	Chemical formula	Concentration (μM)
K_2HPO_4	214.00	$FeNO_3*9H_2O$	2.53
$Mg(NO_3)_2*6H_2O$	183.48	$Mn(NO_3)_2*4H_2O$	1.46
K_2SO_4	147.37	$Na_2B_4O_7$	0.93
KNO_3	143.52	$Zn(NO_3)_2*4H_2O$	0.18
HNO_3	143.41	$CuCl_2*2H_2O$	0.09
$Ca(NO_3)_2*4H_2O$	91.70	$Na_2MoO_4*2H_2O$	0.01
KH_2PO_4	6.50		

Seedlings remained in pre-treatment for approximately 10 days, at which time they were removed and placed randomly into Biotronic units containing treatment solutions. Before being placed in the Biotronic unit, each seedling was rinsed free of nutrient solution and weighed for fresh mass. Seedlings were placed in the Biotronic units containing 5 L of nutrient solution, with one seedling per slot. A minimum of one slot was left empty between seedlings to avoid shading. The Biotronic units were placed in a walk-in Conviron growth chamber (Conviron, Winnipeg, Manitoba) set for a 16 hour photoperiod with 500 $\mu mol\ m^{-2}\ s^{-1}$ photosynthetic photon flux density (PPFD). Relative humidity and temperature were maintained at 50% and 20 °C, respectively. A pH of 4.0 was maintained throughout the experiment, with adjustments made by single drops of 1M

NaOH as required. Conductivity of the nutrient solution was maintained between 90-100 µSe in order to ensure seedlings had free access to nutrients.

Nutrient solutions

All seedlings were grown in nutrient solution containing a macronutrient ratio of 100 N: 45 K: 15 P: 1 Ca: 2 Mg: 9 S. Seedlings were subjected to one of six different treatments containing varying ratios of NH_4^+ and NO_3^-: 100:0, 80:20. 60:40, 40:60, 20:80 and 0:100 (Tables 2.2 – 2.7). Each NH_4^+:NO_3^- treatment consisted of two separate solutions to prevent precipitation, one solution contained the N, P, K, and S sources, and the second contained the Ca, Mg, and micronutrients.

Each treatment was replicated two times, with three randomly selected treatments being conducted concurrently, resulting in four experimental runs. Seedlings were harvested on days 16, 25 and 45 in three of four of the experimental runs. Seedlings in the first experimental run were harvested earlier as information on initial growth patterns was limited. Careful examination of seedling growth curves indicated delaying harvest schedules would provide increased growth information. As a result, seedlings from the first experimental run have been removed from analysis.

Table 2.2. Chemical composition of solutions used within the Biotronic units for the 0:100 NH_4^+: NO_3^- treatment containing 5 L of solution.

Chemical formula of Solution A	Concentration (µM)	Chemical formula of Solution B	Concentration (µM)
HNO_3	282.50	$Ca(NO_3)_2*4H_2O$	6.30
$NaNO_3$	50.20	$Mg(NO_3)_2*6H_2O$	12.30
KH_2PO_4	48.40	$FeNO_3*9H_2O$	2.53
K_2SO_4	42.20	$Mn(NO_3)_2*4H_2O$	0.91
KNO_3	10.50	$Na_2B_4O_7$	0.47
		$Zn(NO_3)_2*4H_2O$	0.12
		$CuCl_2*2H_2O$	0.04
		$Na_2MoO_4*2H_2O$	0.01
		HNO_3	0.53

Table 2.3. Chemical composition of solutions used within the Biotronic units for the 20:80 NH_4^+: NO_3^- treatment containing 5 L of solution.

Chemical formula of Solution A	Concentration (µM)	Chemical formula of Solution B	Concentration (µM)
HNO_3	191.90	$Ca(NO_3)_2*4H_2O$	6.30
NH_4NO_3	140.80	$Mg(NO_3)_2*6H_2O$	12.30
KH_2PO_4	48.40	$FeNO_3*9H_2O$	2.53
K_2SO_4	42.20	$Mn(NO_3)_2*4H_2O$	0.91
KNO_3	10.50	$Na_2B_4O_7$	0.47
		$Zn(NO_3)_2*4H_2O$	0.12
		$CuCl_2*2H_2O$	0.04
		$Na_2MoO_4*2H_2O$	0.01
		HNO_3	0.53

Table 2.4. Chemical composition of solution used within the Biotronic units for the 40:60 NH_4^+: NO_3^- treatment containing 5 L of solution.

Chemical formula of Solution A	Concentration (µM)	Chemical formula of Solution B	Concentration (µM)
NH_4NO_3	282.50	$Ca(NO_3)_2*4H_2O$	6.30
HNO_3	50.30	$Mg(NO_3)_2*6H_2O$	12.30
KH_2PO_4	48.40	$FeNO_3*9H_2O$	2.53
K_2SO_4	42.40	$Mn(NO_3)_2*4H_2O$	0.91
KNO_3	10.50	$Na_2B_4O_7$	0.47
		$Zn(NO_3)_2*4H_2O$	0.12
		$CuCl_2*2H_2O$	0.04
		$Na_2MoO_4*2H_2O$	0.01
		HNO3 (66%)	0.53

Table 2.5. Chemical composition of solution used within the Biotronic units for the 60:40 NH_4^+: NO_3^- treatment containing 5 L of solution.

Chemical formula of Solution A	Concentration (µM)	Chemical formula of Solution B	Concentration (µM)
NH_4NO_3	257.90	$Ca(NO_3)_2*4H_2O$	6.30
NH_4Cl	85.40	$Mg(NO_3)_2*6H_2O$	12.30
KH_2PO_4	48.40	$FeNO_3*9H_2O$	2.53
K_2SO_4	42.20	$Mn(NO_3)_2*4H_2O$	0.91
KCl	5.20	$Na_2B_4O_7$	0.47
		$Zn(NO_3)_2*4H_2O$	0.12
		$CuCl_2*2H_2O$	0.04
		$Na_2MoO_4*2H_2O$	0.01
		HNO_3	0.53

Table 2.6. Chemical composition of solution used within the Biotronic units for the 80:20 NH_4^+: NO_3^- treatment containing 5 L of solution.

Chemical formula of Solution A	Concentration (µM)	Chemical formula of Solution B	Concentration (µM)
NH_4Cl	228.10	$Ca(NO_3)_2*4H_2O$	6.30
NH_4NO_3	114.90	$Mg(NO_3)_2*6H_2O$	12.30
KH_2PO_4	48.40	$FeNO_3*9H_2O$	2.53
K_2SO_4	42.20	$Mn(NO_3)_2*4H_2O$	0.91
KCl	5.30	$Na_2B_4O_7$	0.47
		$Zn(NO_3)_2*4H_2O$	0.12
		$CuCl_2*2H_2O$	0.04
		$Na_2MoO_4*2H_2O$	0.01
		HNO_3	0.53

Table 2.7. Chemical composition of solution used within the Biotronic units for the 100:0 NH_4^+: NO_3^- treatment containing 5 L of solution.

Chemical formula of Solution A	Concentration (µM)	Chemical formula of Solution B	Concentration (µM)
NH_4Cl	362.90	$CaCl_2*2H_2O$	1.30
K_2HPO_4	41.90	$MgSO_4*7H_2O$	4.10
K_2SO_4	29.80	$Fe(III)Cl_3$	1.69
$NaH_2PO_4*2H_2O$	8.30	$MnCl_2*4H_2O$	0.72
KCl	11.70	$Na_2B_4O_7$	0.47
KH_2PO_4	0.40	$ZnCl_2$	0.63
		$CuCl_2*2H_2O$	0.04
		$Na_2MoO_4*2H_2O$	0.01

Measurements

Seedlings were grown in treatment solution for a period of 45 days, with intermittent harvests to provide information on relative growth rate (RGR), nutrient allocation, biomass allocation, net photosynthesis and root respiration. Total seedling fresh biomass, root collar diameter (RCD), primary root length, shoot fresh mass, stem length, stem fresh mass, and the number of main roots were measured on individual seedlings at each harvest.

Net photosynthesis and root respiration were measured at Harvest 2 on experimental runs 2 and 3, providing physiological information on each of the six treatments. Photosynthesis and root respiration were measured on six seedlings per treatment per seedlot using a LCA4, closed system, dual gas exchange analyser (Analytical Development Company Ltd., Hoddesdon, Hertfordshire, UK). For all measurements, air flow through the chamber was set at 4 m s^{-1}, with air inside the chamber being mixed by a small fan. Reference air was drawn from a large external bag to maintain constant CO_2 concentrations. To measure photosynthesis, shoot systems were placed inside the chamber. To measure root respiration, seedlings were removed from the Biotronic units, roots were coiled, bound with a twist-tie and placed in the cuvette which was covered with an aluminum foil lid to keep the roots dark. Carbon dioxide flux data was recorded after the difference between CO_2 concentration of the reference air and the chamber air had stabilized (5-10 min). For each seedling, CO_2 differential, H_2O differential (millibars), PPFD (μmol m^{-2}), and temperature (^{o}C) was recorded.

Following harvest, seedlings were dried at 70 °C for 72 hours, after which dry stem mass, dry root mass and dry needle mass were measured. Nutrient analysis was conducted for all seedlings, however due to their small size, samples were bulked in order to produce enough material for nutrient analysis. Seedlings that were obtained from Harvest 1 were bulked into groups of 12 seedlings for roots, needles and stems. For Harvests 2 and 3, seedlings were bulked into groups of 4 seedlings, and divided into roots, needles and stems. Each of the bulked samples was ground to a fine powder using the Wig-L-Bug Amalgamator (model 3110-3A, Cresent Dental MFG Co., Illinois, USA). Ground samples were stored in 1 mL glass vials, where the samples were then dried for 24 hours at 70 °C and packaged into 6 – 8 mg samples in 10.0 mm diameter tin capsules. Capsules were analyzed for N content using a FlashEA 1112 Elemental Analyser (ThermoQuest Corp., Italy).

Seedling relative growth rate (RGR) was calculated by:

$$\mathrm{RGR} = (\ln W_2 - \ln W_1)/(t_2 - t_1), \tag{2.1}$$

where W is plant biomass, t is time, and W_1 and W_2 are plant biomasses at time t_1 and t_2, respectively (Ingestad and Lund 1986).

Total RGR was calculated from the time seedlings were placed in treatment solutions until the final harvest (Harvest 3). In addition, RGR was calculated between each harvest for each individual treatment.

Statistical analysis

A two-way factorial analysis of variance was used to test the significance ($P \leq 0.05$) of nutritional treatment on the growth parameters of the two seedlots at each harvest. The following general linear model was used (Quinn and Keough 2002):

$$Y_{ijk} = \mu + \alpha_i + \beta_i + (\alpha\beta)_{ij} + \varepsilon_{ijk} \qquad (2.2)$$

where;

Y_{ijk} = is the value of the *k*th seedling from the combination of the *i*th treatment and the *j*th seedlot,

μ = is the overall mean,

α_i = is the effect of the *i*th treatment across all seedlots,

β_i = is the effect of the *j*th seedlot across all treatments,

$(\alpha\beta)_{ij}$ = is the effect of the interaction of the *i*th treatment and the *j*th seedlot,

ε_{ijk} = is the random or unexplained error associated with the *k*th seedling from the combination of the *i*th treatment and the *j*th seedlot.

Specific root length (SRL) (cm g^{-1}) was log-transformed to meet the assumptions of the ANOVA, values presented in the figure represent untransformed data. Means were tested using Tukey's Honestly Significantly Different (HSD) test of hypothesis and Tukey's HSD test for unequal –N when required. Root to shoot ratios were tested using a Chi-squared analysis. All analysis was conducted in STATISTICA Version 6 (2003).

RESULTS

NH_4^+:NO_3^- uptake

Nitrogen uptake by seedlings increased with the proportion of NO_3^- applied (Table 2.8). Nutrient uptake, as inferred from the total N added over the experiment, was greatest in the 0:100 NH_4^+:NO_3^- treatment and steadily declined as NH_4^+ concentrations increased. Although total N uptake was greatest in solutions high in NO_3^- it was the NH_4^+ levels that were the most depleted in the final analysis of the solutions. As a result NH_4^+ appeared to be taken up preferentially compared to NO_3^- with the final NH_4^+:NO_3^- ratios always being low (Table 2.8).

Nitrification of some NH_4^+ may have resulted in slight decreases in NH_4^+ concentration, however minimal nitrification was expected due to sterilization techniques. This is further supported by the very low levels of NO_3^- found in the 100:0 NH_4^+:NO_3^- treatment.

Table 2.8. A comparison of NH_4^+:NO_3^- ratios at the start of the experiment versus NH_4^+:NO_3^- remaining in solution after 45 days of treatment.

Initial NH_4^+:NO_3^- ratio	0:100	20:80	40:60	60:40	80:20	100:0
Mean [NH_4^+] remaining (μM)	0.6	3.9	4.4	0.6	1.7	0.6
Mean [NO_3^-] remaining (μM)	110.6	82.2	70.0	2.7	1.1	1.0
Total remaining N (μM)	111.2	86.1	74.4	3.3	2.8	1.6
Final NH_4^+:NO_3^-	0:100	1:99	2:98	6:94	30:70	14:86
Total amount of N added over 45 days (mM)	9.08	8.64	7.70	3.88	2.76	0.05

Growth rates and biomass allocation

There were significant treatment effects ($P < 0.0001$) in RGR calculated after 45 days of treatment (Figure 2.2). Relative growth rate was greatest in the 60:40 $NH_4^+:NO_3^-$ treatment and lowest in the 80:20 and 100:0 treatments. There were significant differences between seedlots ($P = 0.030$) and no significant treatment by seedlot interaction effects. Variations in RGR from Harvest 1 to Harvest 2 and Harvest 2 to Harvest 3 differed among the six treatments (Figure 2.3). Both the 80:20 and the 100:0 $NH_4^+:NO_3^-$ treatments displayed relatively unstable RGR between the three harvests compared to the 0:100, 20:80, 40:60 and 60:40 $NH_4^+:NO_3^-$ treatments.

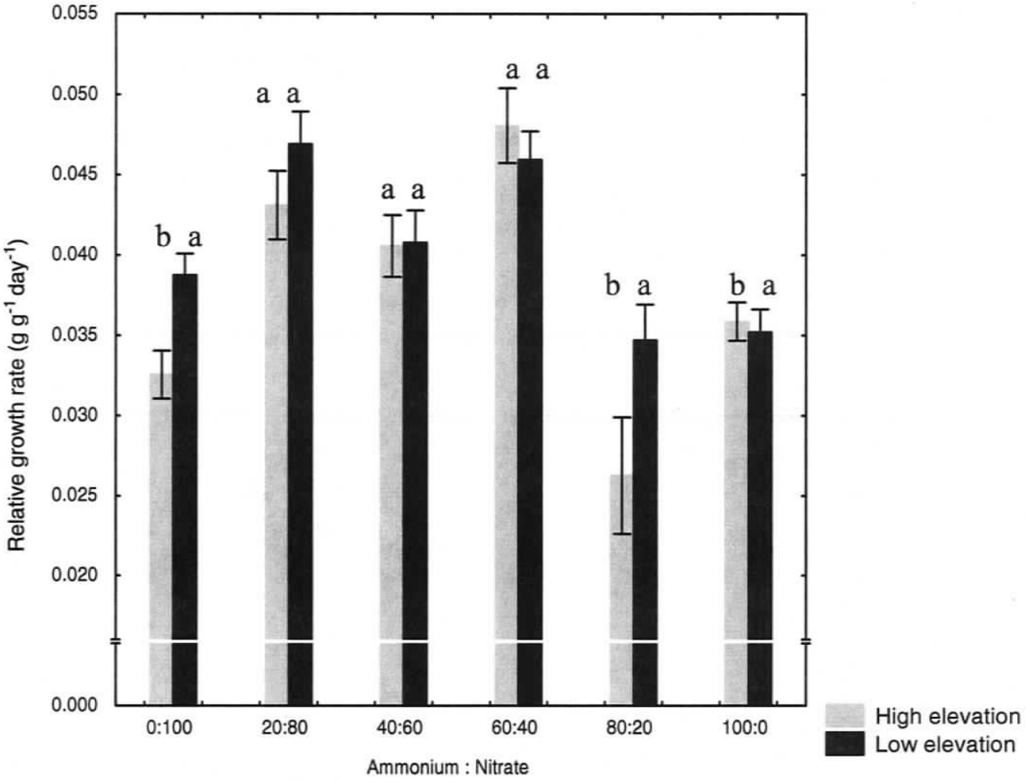

Figure 2.2. Relative growth rate (g g^{-1} day^{-1}) of Douglas-fir seedlings over 45 days of treatment. Lines indicate standard error of the mean. Bars marked with different letters denote significant differences between seedlots within treatments ($P \leq 0.05$). NH_4^+:NO_3^- treatments 0:100, 40:60 20:80 and 100:0 *n*=24; 80:20 and 60:40 NH_4^+:NO_3^- *n*=12.

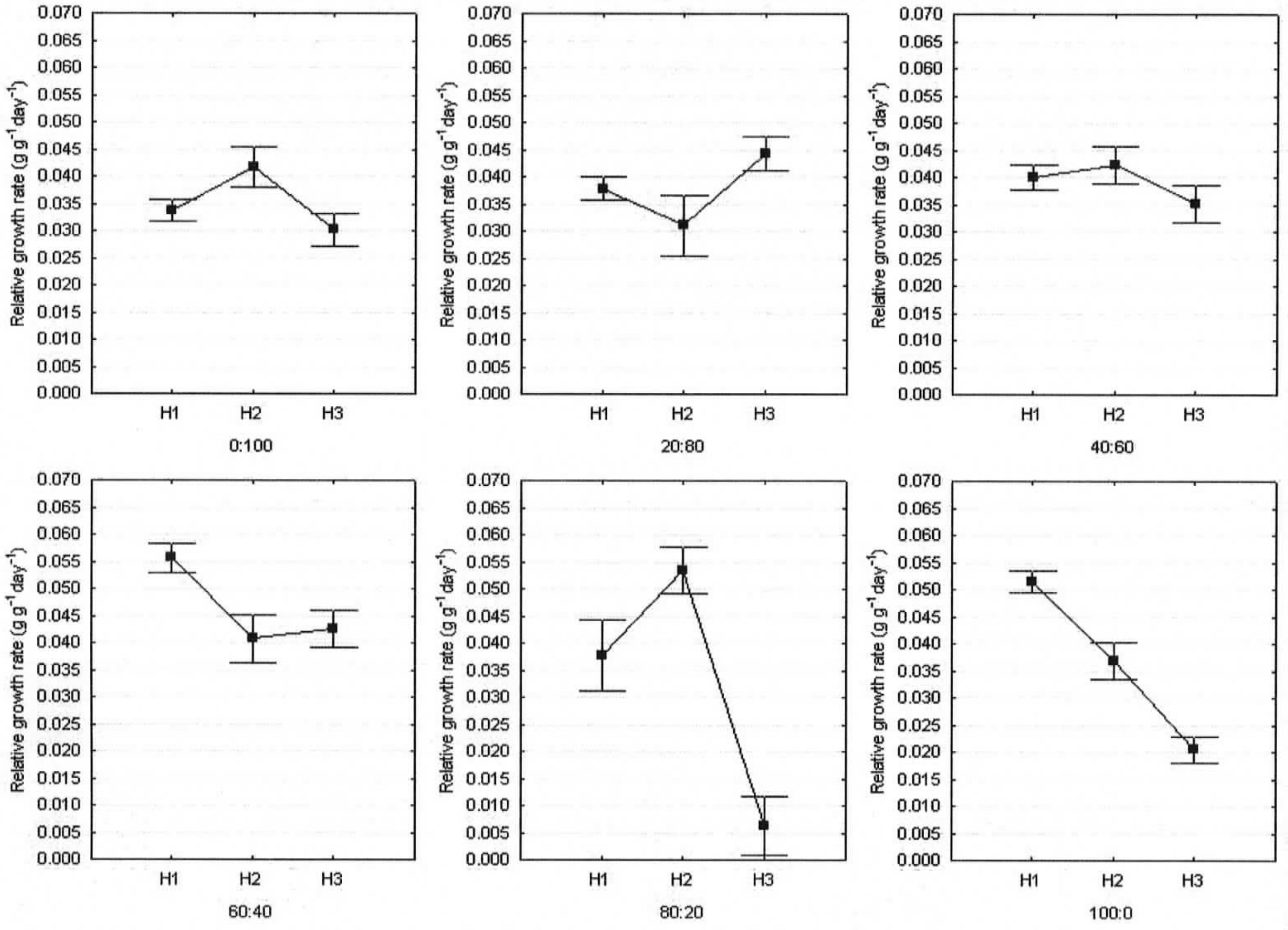

Figure 2.3. Douglas-fir seedling relative growth rate (g g^{-1} day^{-1}) at the end of the first, second and third growing periods at Harvest 1 (H1), Harvest 2 (H2) and Harvest 3 (H3). Lines indicate standard error of the mean. NH_4^+:NO_3^- treatments 0:100, 40:60 20:80 and 100:0 *n*=24; 80:20 and 60:40 NH_4^+:NO_3^- *n*=12.

Significant treatment differences existed in seedling dry biomass ($P < 0.0001$) (Figure 2.4). There were no differences between seedlots ($P = 0.587$) and no treatment by seedlot interactions ($P = 0.166$). Dry mass was greatest in the 20:80, 40:60 and 60:40 NH_4^+:NO_3^- treatments, where both N sources were abundant in solution. The seedlings grown in the 20:80, 40:60 and 60:40 NH_4^+:NO_3^- treatments allocated greater biomass to needles than the 0:100, 80:20 and 100:0 treatments, reflecting the difference in overall biomass (Figure 2.5).

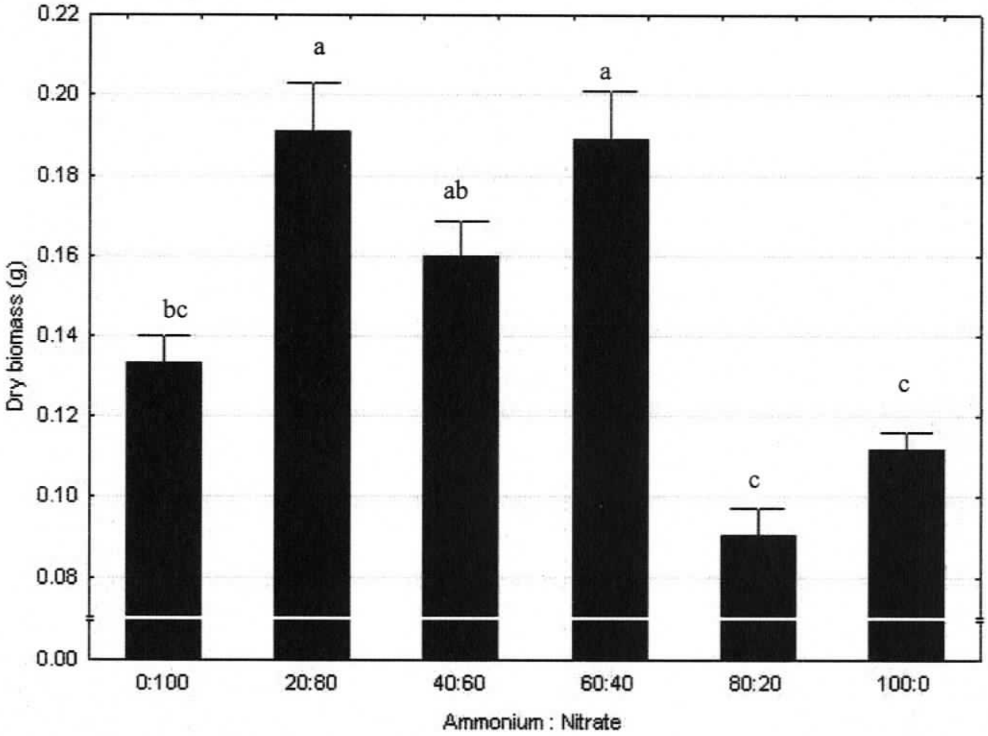

Figure 2.4. Dry biomass (g) of Douglas-fir seedlings after 45 days of treatment. Lines indicate standard error of the mean. Bars marked with different letters denote significant differences ($P \leq 0.05$). NH_4^+:NO_3^- treatments 0:100, 40:60, 20:80 and 100:0 *n*=48; 80:20 and 60:40 NH_4^+:NO_3^- *n*=24.

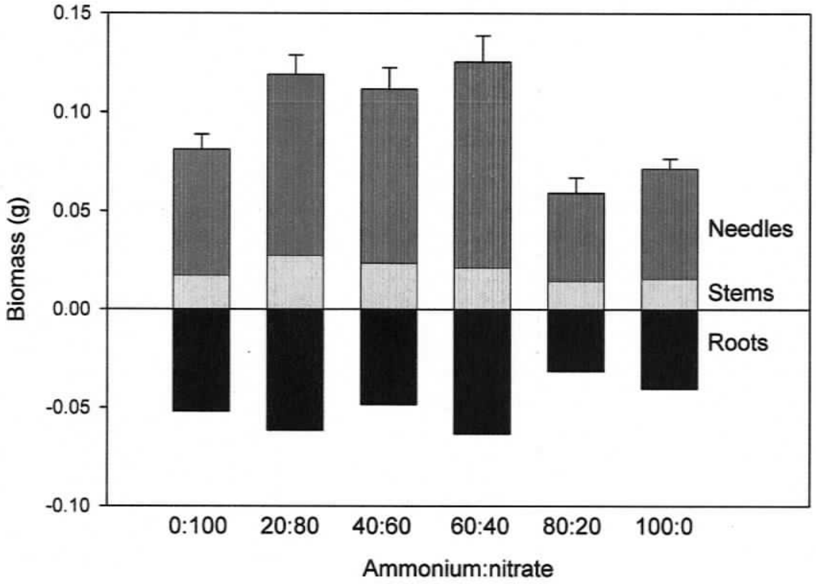

Figure 2.5. Dry biomass (g) allocation for Douglas-fir seedling after 45 days of treatment. Lines indicate standard error of the mean of total biomass. NH_4^+:NO_3^- treatments 0:100, 40:60, 20:80 and 100:0 *n*=48; 80:20 and 60:40 NH_4^+:NO_3^- *n*=24.

There were significant differences (P = 0.01) between root:shoot ratios of the treatments (Table 2.9). Seedlings grown in the 40:60 NH_4^+:NO_3^- treatment had the lowest root:shoot ratio, while the 0:100 treatment had the highest root:shoot ratio. There were significant differences (P =0.001) in specific leaf area (SLA) across the NH_4^+:NO_3^- treatments (Table 2.9). The smaller SLA in the 60:40 treatment indicates narrower, thicker needles, while higher SLA values in the 0:100 treatment indicate a broader thinner needle.

Table 2.9. Morphological effects (root:shoot and specific leaf area) of NH_4^+:NO_3^- treatment after 45 days. Values indicate mean ± S.E. Letters denote significant differences between treatments. NH_4^+:NO_3^- treatments 0:100, 40:60, 20:80 and 100:0 n=48; 80:20 and 60:40 NH_4^+:NO_3^- n=24.

	0:100	20:80	40:60	60:40	80:20	100:0
Root:Shoot (P = 0.01)	0.65 ± 0.02 a	0.52 ± 0.02 bc	0.47 ± 0.06 c	0.52 ± .02 bc	0.51 ± 0.04 bc	0.57 ± 0.02 b
SLA (cm g^{-1}) (P = 0.001)	87.59 ± 2.35 a	73.60 ± 2.04 ab	86.01 ± 8.50 ab	65.30 ± 1.98 b	86.66 ± 3.19 ab	71.70 ± 1.52 ab

Seedling morphology

Log specific root length (SRL) was significantly affected by NH_4^+:NO_3^- ratios (P < 0.0001) (Figure 2.6). There were significant differences in seedlots (P = 0.01) and there were no significant seedlot by treatment interactions. SRL increased with increasing NH_4^+ concentrations (Figure 2.6 and 2.7). High SRL indicates root systems that are typically thinner, either in individual root diameter, or with fewer or shorter laterals. Low SRL indicates root systems that are typically more robust with either larger diameter or increased growth of laterals.

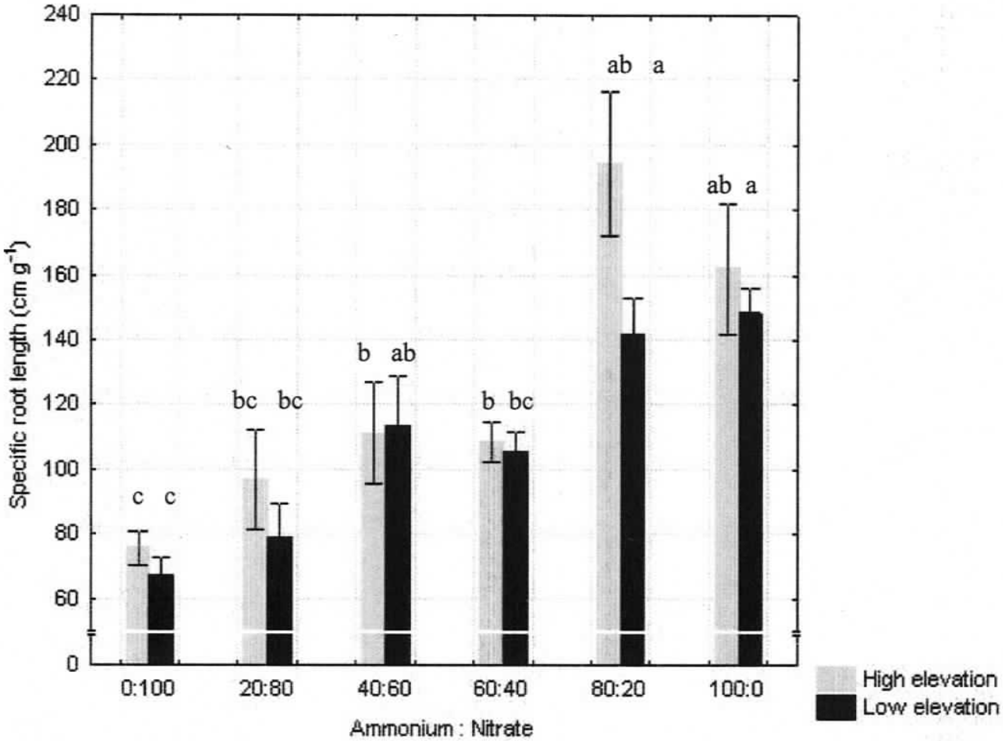

Figure 2.6. Specific root length (cm g^{-1}) of Douglas-fir seedlings after 45 days of treatment. Lines indicate standard error of the mean. Bars marked with different letters denote significant differences within seedlots ($P \leq 0.05$). NH_4^+:NO_3^- treatments 0:100, 40:60 80:20 and 100:0 *n*=24; 20:80 and 60:40 NH_4^+:NO_3^- *n*=12.

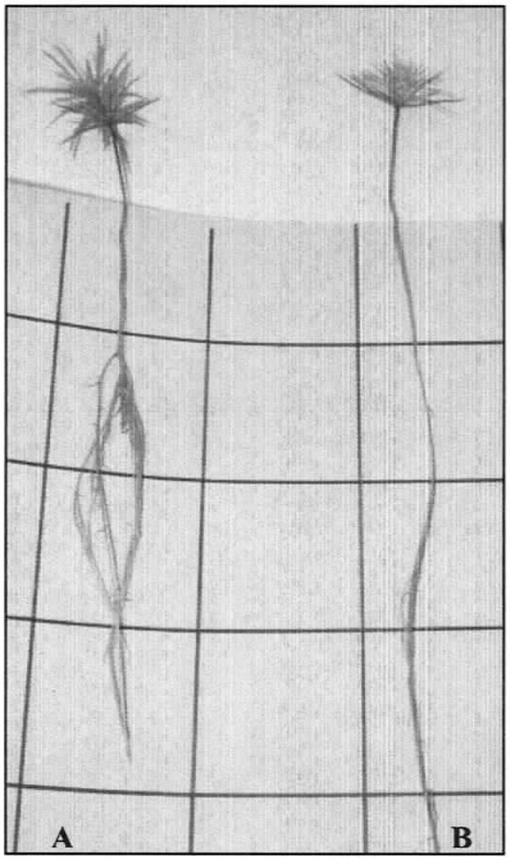

Figure 2.7. Douglas-fir seedlings grown for 45 days in **(A)** 0:100 NH_4^+:NO_3^- ratio or **(B)** 100:0 NH_4^+:NO_3^- ratio. The shorter overall root length but more robust root system in the 0:100 NH_4^+:NO_3^- treatment is reflected by the lower SRL (cm g^{-1}), while the longer thinner root system of the 100:0 NH_4^+:NO_3^- treatment is reflected by the higher SRL.

N concentrations

There were significant differences ($P < 0.0001$) in both whole plant nitrogen (wpN) content and concentration among treatments. Seedlings from the 20:80 and 40:60 NH_4^+:NO_3^- treatments had the greatest wpN content and seedlings from the 80:20 and 100:0 NH_4^+:NO_3^- treatments had the lowest wpN content at Harvest 3 (Figure 2.8). There were no significant differences in seedlot ($P = 0.988$) and no significant treatment by seedlot interactions ($P = 0.951$). There were also significant differences ($P < 0.0001$) in wpN concentrations among treatments with seedlings from the high NO_3^- treatments having the greatest wpN concentrations, and the high NH_4^+ treatments having the lowest wpN concentrations (Figure 2.9). For each seedling component, N concentration varied significantly among treatments ($P < 0.0001$). Needles, roots and stems of seedlings grown in the 0:100, 20:80 and 40:60 NH_4^+:NO_3^- treatments had greater N concentrations compared to the 60:40, 80:20 and 100:0 NH_4^+:NO_3^- treatments. On average, seedlings in the 0:100, 20:80 and 40:60 NH_4^+:NO_3^- treatments had a greater relative concentration of N in roots compared to needles, than in the 60:40, 80:20 and 100:0 NH_4^+:NO_3^- treatments. Seedling wpN concentrations varied over time from Harvest 1 to Harvest 3 in each of the treatments (Figure 2.10). Seedlings from the high NH_4^+ treatments (60:40, 80:20 and 100:0) exhibited a steep decline in wpN concentration from Harvest 1 to Harvest 3. Seedlings from the high NO_3^- concentrations (0:100, 20:80, and 40:60) displayed comparatively stable wpN concentrations over time, with no major decreases in internal N.

Nutrient uptake efficiency, or the amount of N that was taken up by seedlings and converted to internal N content varied by treatment. Nutrient uptake efficiency was 1.99%, 3.88%, 4.08% 7.80%, 68.75% and 28.46% for the 0:100, 20:80, 40:60, 60:40, 80:20 and 100:0 NH_4^+:NO_3^- treatments, respectively.

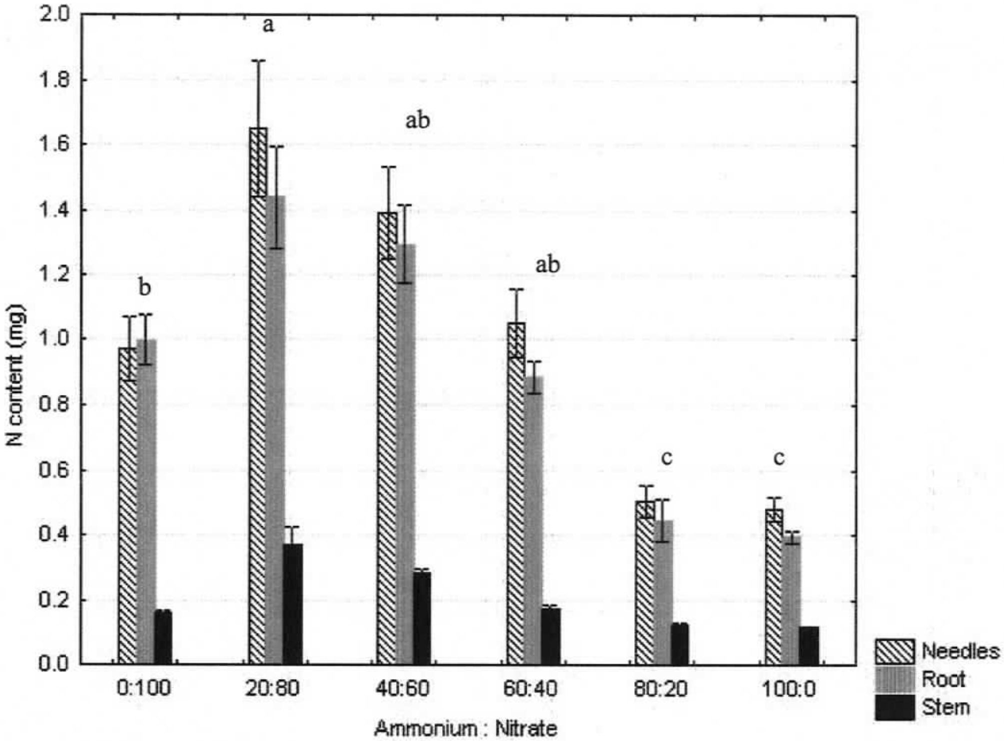

Figure 2.8. Nitrogen (N) content of Douglas-fir seedlings by component, needles, roots and stems. Seedlings were grown for 45 days in varying ratios of NH_4^+:NO_3^-. Lines indicate standard error of the mean. Letters denote significant differences ($P \leq 0.05$) in whole plant N content between treatments. NH_4^+:NO_3^- treatments 0:100, 40:60, 20:80 and 100:0 *n*=24; 80:20 and 60:40 NH_4^+:NO_3^- *n*=12.

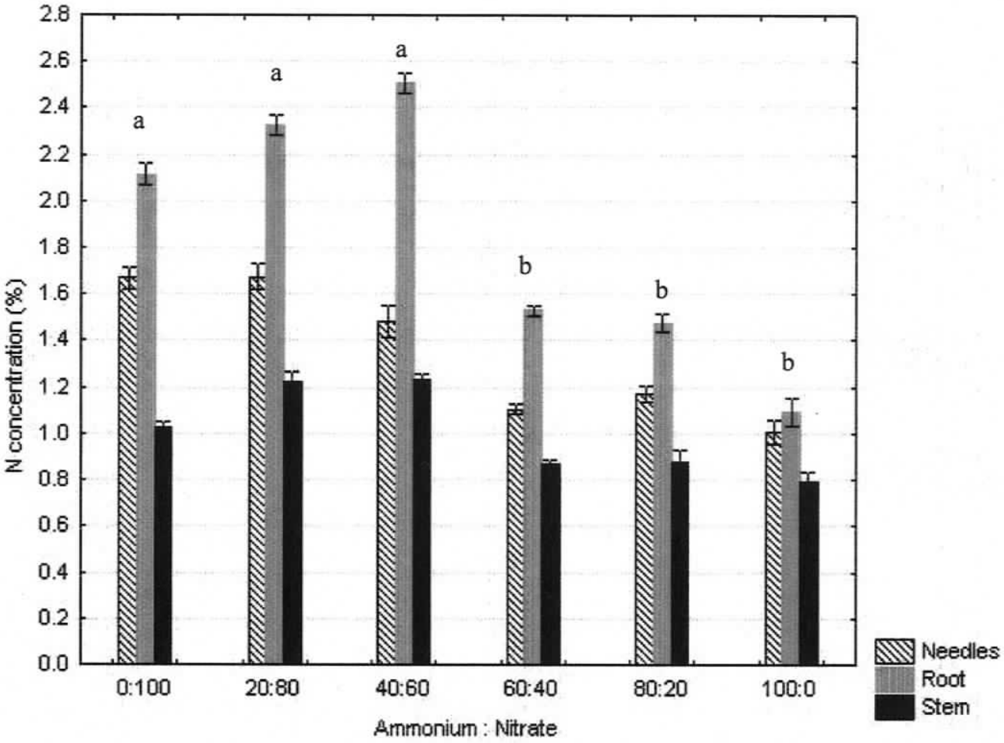

Figure 2.9. Nitrogen (N) concentration of Douglas-fir seedlings by component, needles, roots and stems. Seedlings were grown for 45 days in varying ratios of NH_4^+:NO_3^-. Lines indicate standard error of the mean. Letters denote significant differences ($P \leq 0.05$) in whole plant N content between treatments. NH_4^+:NO_3^- treatments 0:100, 40:60, 20:80 and 100:0 n=24; 80:20 and 60:40 NH_4^+:NO_3^- n=12.

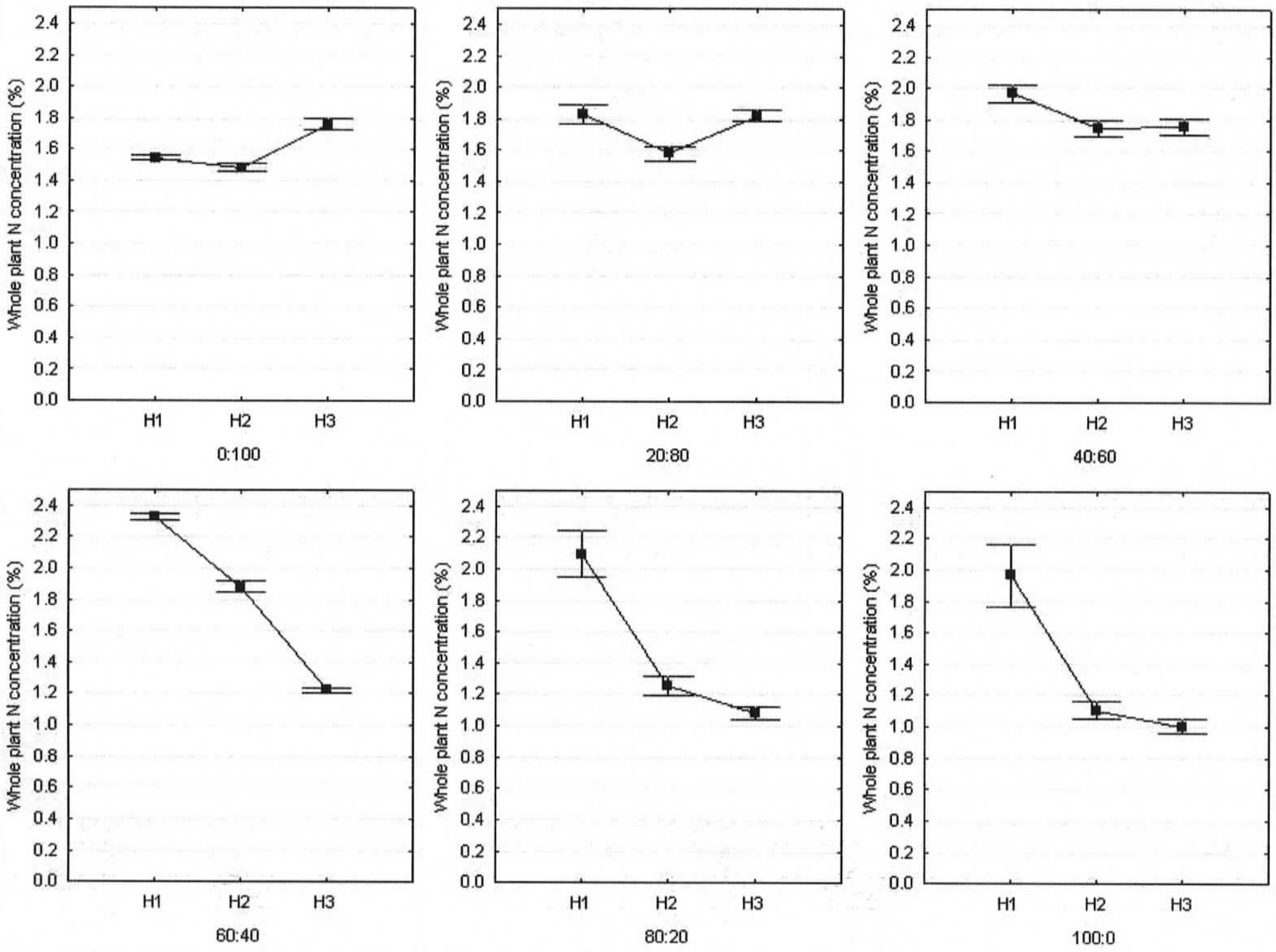

Figure 2.10. Douglas-fir seedling whole plant nitrogen (N) concentrations (%) over three harvests (H1 = Harvest 1, H2 = Harvest 2, H3 = Harvest 3). Lines indicate standard error of the mean. H1 *n*=4, H2 and H3 *n*=12.

Photosynthesis and root respiration

Photosynthetic rate, on average, declined with increased NH_4^+ concentrations, with the exception of the 60:40 NH_4^+:NO_3^- treatment (Figure 2.11). Root respiration also generally decreased with increasing NH_4^+:NO_3^- ratios. The 0:100 treatment had the highest levels of shoot photosynthesis and root respiration, on average, while the 100:0 treatment displayed the lowest levels.

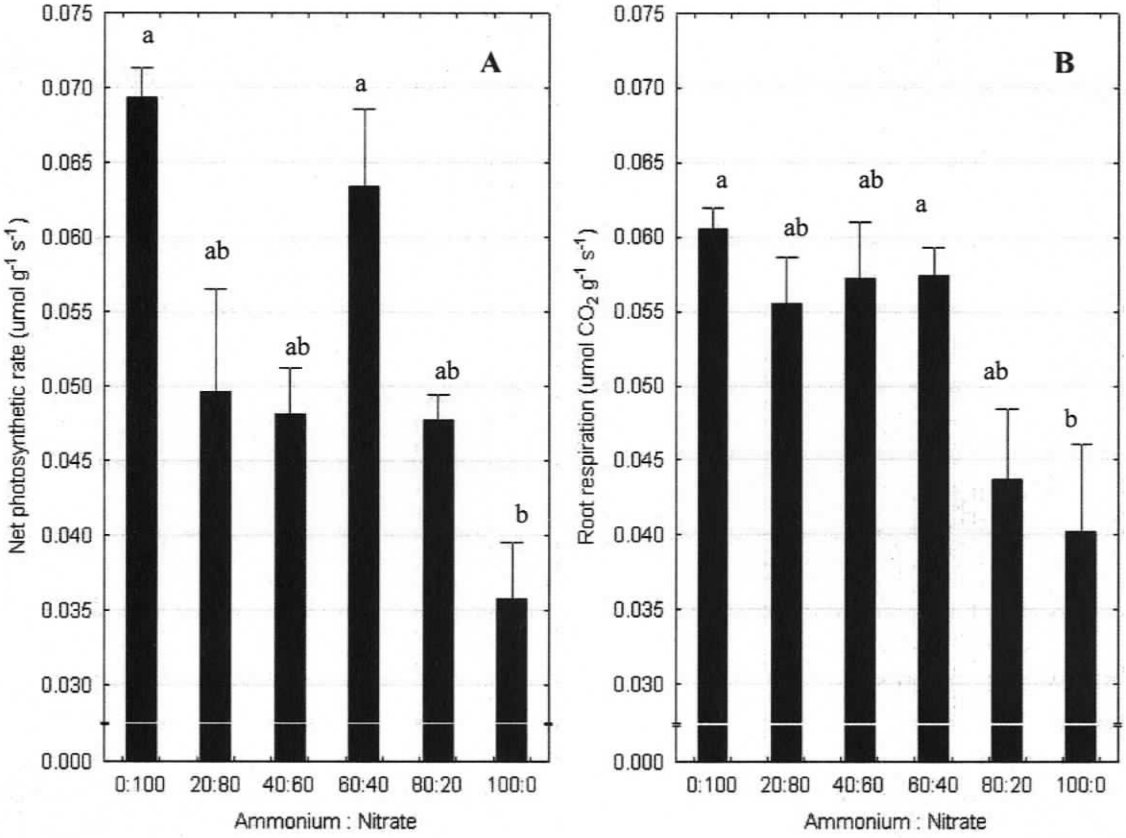

Figure 2.11. Net photosynthesis and root respiration rates in Douglas-fir seedlings. **(A)** Net photosynthetic rates of Douglas-fir seedlings after 25 days of treatment **(B)** Root respiration rates of Douglas-fir seedlings after 25 days of treatment. Lines indicate standard error of the mean. Bars marked with different letters denote significant differences ($P \leq 0.05$), $n = 12$.

DISCUSSION

NH_4^+ and NO_3^- uptake

Treatment solutions containing high NO_3^- concentrations (0:100, 20:80 and 40:60) had the greatest levels of nutrient uptake, while treatment solutions with high NH_4^+ (60:40, 80:20 and 100:0) had the lowest levels of nutrient uptake. The low levels of nutrient uptake in the 80:20 and 100:0 NH_4^+:NO_3^- treatments corresponded with low overall RGR, low total dry biomass and decreasing internal N concentrations, while the 60:40 NH_4^+:NO_3^- treatment was only characterized by decreasing internal N concentrations. The high level of nutrient uptake in the 0:100 treatment did not correspond with elevated growth, but seedlings in this treatment were characterized by lower RGR and total dry biomass. These findings indicate a reduction in overall uptake in the presence of high NH_4^+ concentrations, but also the need for a minimum concentration of NH_4^+ in solution to achieve optimal growth.

When both ions were available in solution, Douglas-fir exhibited a preference for NH_4^+ uptake compared to NO_3^- uptake. Although Douglas-fir seedlings appeared to take up NH_4^+ more quickly than NO_3^-, seedlings grown in solutions with the highest NH_4^+ concentrations produced neither greater growth nor greater biomass. Several similar studies have found NH_4^+ to be taken up in greater abundance than NO_3^- by Douglas-fir when both ions were available in solution (Boxman and Roelofs 1988, Rygiewicz and Bledsoe 1986, Kamminga-van Wijk and Prins 1993). The presence of NH_4^+ ions applied concurrently with NO_3^- in solution has been found to affect the uptake of NO_3^- by other tree species (Gessler et al.1998, Marchner et al. 1991, Ingestad 1979), as well as numerous herbaceous species (Lee and Drew 1989, King et al. 1993). Kamminga-van

Wijk and Prins (1993) found the presence of NH_4^+ in solution decreased the uptake rate of NO_3^- by Douglas-fir from 28 to 66% when NH_4^+ was present in as low a concentration as 10 μM. It has been postulated that the products of NH_4^+ and NO_3^- assimilation, such as amino acids, may be responsible for the down-regulation of NO_3^- uptake when NH_4^+ is supplied concurrently (Imsande and Touraine 1994). Alternatively, the greater capacity of seedlings to take up NH_4^+ may result from a lower affinity of NO_3^- for the binding sites at the plasma membrane of root cells or may reflect a lower capacity of the roots to metabolize NO_3^- (Marchner et al. 1991). In this study all six treatments appeared to have taken up a reduced proportion of NO_3^-, when both NH_4^+ and NO_3^- were available in solution. This may indicate Douglas-fir generally had a reduced ability for NO_3^- uptake when NH_4^+ was also present. Alternatively, NH_4^+ uptake may be favoured because of the lower energy cost associated with uptake and assimilation.

The rapid and preferential uptake of NH_4^+ by several coniferous species has raised questions about the ability of some species to regulate NH_4^+ uptake. This has led to speculation that these species may be less tolerant of sites with high NH_4^+ availability (Kronzucker et al. 1997, 2003). Kronzucker et al. (2003) postulated that it is not a species' ability to take up and acquire net NH_4^+, but rather its ability to translate this uptake into growth that determines survival on sites with high NH_4^+ concentrations. Kronzucker et al. (2003) found that the uptake capacity for NH_4^+ in early-successional species was excessive and insufficiently regulated, with only a small amount of the incoming N being channelled to metabolism and a high percentage of the remaining N being channelled towards efflux. In a study conducted on Douglas-fir, Britto and Kronzucker (2002) found that NH_4^+ was lost almost as rapidly as it was gained, indicating a poor adaptation to this N source, rather than a preference for NH_4^+. The high level of efflux can create toxic conditions for the plant by decreasing the rhizosphere pH by up to 2 units through the accompanying excretion of H^+ ions (Kronzucker et al. 2003). Likewise, toxic conditions inside the cell can develop by the lowering of cytoplasmic pH, from excessive NH_4^+ uptake (Kronzucker et al. 2003). Britto and Kronzucker (2002) postulated that the lack of regulation of NH_4^+ uptake and the reduced selectivity against NH_4^+ at the membrane indicate an impairment of the plant's nutrient uptake system, therefore rendering some species less fit for growth on sites with high NH_4^+ concentrations.

The relatively quick removal of NH_4^+ from solutions in our study, without increased growth, lends support to the theory of Kronzucker et al. (2003). In both the 80:20 and 100:0 NH_4^+:NO_3^- treatments, Douglas-fir appeared to take up all available NH_4^+ from solution, yet seedling growth appeared to be inhibited, perhaps by internal NH_4^+ toxicity. Additionally, high NH_4^+ treatments displayed rapid increases in solution conductivity that coincided with nutrient additions. These increases in conductivity could be the result of the rapid uptake and subsequent efflux of NH_4^+ from roots, similar to the phenomenon described by Kronzucker et al. (2003). It is important to note these sudden increases in solution conductivity did not occur in the high NO_3^- treatments, thereby supporting the theory that NH_4^+ is subject to rapid efflux from the plant rather than being assimilated into amino acids.

Relative growth rate and biomass allocation

Seedlings grown in the highest NH_4^+:NO_3^- ratios had significantly lower overall RGR, less biomass, lower wpN concentrations and lower rates of photosynthesis than seedlings grown with a greater proportion of NO_3^-. Seedling growth, in terms of overall RGR and biomass, was greatest when both NH_4^+ and NO_3^- were available in solution in abundant and relatively equal proportions. Although seedlings grown with NO_3^- only had relatively low overall RGR, internal N concentrations remained stable (at a significantly greater level) in all high NO_3^- treatments and photosynthesis rates were higher than for seedlings grown with high NH_4^+ concentrations. These factors indicate that internal nutrient stress was limited compared to seedlings grown in high NH_4^+ concentrations.

Growth implications of NH_4^+ versus NO_3^- as a N source is conflicting in the literature. For example, Krajina et al. (1973) found Douglas-fir had significantly greater growth, in terms of height and biomass, with NO_3^- nutrition, compared to NH_4^+ nutrition. Alternatively, several earlier studies indicate greater growth with NH_4^+ or NH_4^+ and NO_3^- nutrition (Bedell et al. 1999, van den Driessche 1971, Gisjman 1990b, Kamminga-van Wijk and Prins 1993). Variations among the studies most likely stem from differences in experimental conditions including pH, temperature and growing substrate. van den Driessche (1971) observed that Douglas-fir growth response to NH_4^+ decreased when pH dropped below 5.3, thus supporting the finding of our study of decreased growth with high NH_4^+ concentrations at relatively low pH levels.

The most profound morphological effect of the NH_4^+:NO_3^- treatments that I found was in the root systems. Root development displayed high levels of plasticity in response to the relative abundance of NH_4^+ and NO_3^-. Seedlings in the high NO_3^- treatment were characterized by robust root systems, typically shorter in overall length but with many long laterals (Figure 2.6). Lateral root elongation in the presence of high NO_3^- has been documented previously (Zhang and Forde 1998, Linkhor et al. 2002, Hodge 2004), however these studies found that lateral root elongation typically occurred in patches of high NO_3^- in otherwise heterogeneous nutrient conditions and were less likely to occur in conditions of uniformly high NO_3^-. Linkhor et al. (2002) postulated that this might be a growth response that allows optimal nutrient uptake in relatively nutrient poor areas. In addition to the variations in SRL, roots grown with high NO_3^- were observed to be much whiter in colour, with each individual root (lateral and main root) being much larger in diameter. Alternatively, seedlings grown with high NH_4^+ were characterized by long, thin root systems, with one main root and a number of short, thin laterals. Roots grown with high NH_4^+ were observed to be darker in colour, with the exception of the root tips. Similar root morphologies in Douglas-fir were identified by Gijsman (1990a). This discolouration of the root system that occurred in the high NH_4^+ treatments, with the exception of the actively growing tissues, may be indicative of NH_4^+ toxicity (Gisjman 1990a).

Seedling N concentrations

At the final harvest, seedling wpN concentration was greatest in the 40:60 NH_4^+:NO_3^- treatment, with a gradual decrease in wpN concentration as treatments reached extreme NH_4^+ or NO_3^- concentrations. Allocation of N between seedling components, needles, stems and roots however, displayed an interesting pattern. Needle N allocation was greatest in the 0:100 NH_4^+:NO_3^- treatment and gradually decreased with an increase in NH_4^+, supporting an observation by Marschner (1995) that as the supply of NO_3^- increases, a greater proportion of NO_3^- will be translocated to the shoot for assimilation. Root N concentrations followed a similar pattern with highest N levels in the 0:100 treatment, with a continual decrease in root N to the 100:0 NH_4^+:NO_3^-

treatment. In general, root N concentrations were high, potentially due to the presence of residues of nutrient solutions remaining from harvest. Although seedling roots were rinsed with distilled H_2O at the time of harvest there is potential that not all of the N from solution was completely removed from the roots. As a result patterns of N allocation to root systems should be interpreted with caution.

Whole plant N concentrations decreased in high NH_4^+ treatments (60:40, 80:20 and 100:0) from Harvest 1 to Harvest 3, while all treatments grown with high NO_3^- (0:100, 20:80 and 40:60) displayed relatively stable wpN concentrations (Figure 2.10). This decrease in seedling wpN concentration in high NH_4^+ treatments could be a reflection of NH_4^+ toxicity, and may provide an early warning of future growth decreases in the 60:40 NH_4^+:NO_3^- treatment, even though this treatment displayed the greatest overall RGR at the third harvest. Decreasing internal nutrient concentrations contradict the theory of steady state nutrition, and are reflective of nutrient stress (Ingestad and Lund 1986).

Nutrient uptake efficiency increased with increases in NH_4^+ concentration, with the exception of the 100:0 NH_4^+:NO_3^- treatment. This may indicate that NH_4^+ was more efficiently converted to internal N content compared to NO_3^-. This may be linked to the greater energy requirements associated with NO_3^- assimilation compared to NH_4^+ assimilation (Bloom et al. 1992).

Photosynthesis and root respiration

In many studies, photosynthesis is strongly correlated with N availability (Lambers et al. 1998), therefore treatments in which N was limited (due to a sub-optimal N source) were expected to have reduced photosynthetic rates. With the exception of the 60:40 NH_4^+:NO_3^- treatment, photosynthesis rates decreased with increasing NH_4^+

concentrations. This may be related to excessive and inadequate regulation of NH_4^+ uptake as described by Kronzucker et al. (2003). Unlike the 60:40 NH_4^+:NO_3^- treatment, the high rate of photosynthesis in the 0:100 NH_4^+:NO_3^- treatment did not correspond with elevated overall RGR or biomass accumulation. Although the 0:100 treatment had relatively low overall RGR and biomass the greater rate of photosynthesis did correspond with relatively high wpN concentrations at Harvests 2 and 3, unlike the 60:40 NH_4^+:NO_3^- treatment.

Root respiration was greatest in seedlings grown in the greatest NO_3^- concentrations and decreased with higher NH_4^+ concentrations. Nitrate uptake across the plasma membrane requires greater metabolic energy and also requires increased energy for assimilation compared to NH_4^+ uptake (Bloom et al. 1992). This may explain the elevated rates of root respiration. In barley, NH_4^+ absorption and assimilation accounted for 14% of the CO_2 catabolism, while under NO_3^- nutrition 5% of CO_2 catabolism was linked to NO_3^- absorption, 15% to NO_3^- assimilation and an additional 3% to the subsequent NH_4^+ assimilation (Bloom et al. 1992). Increased energy expenditures to take up NO_3^- appears to correspond with the increase in root respiration at proportionately high levels of NO_3^-.

pH

Fluctuations in the pH of nutrient solutions can affect plant growth (Haynes and Goh 1978, van den Driessche 1991). The presence of excess H^+ ions may affect root growth, membrane integrity and the rate of ion uptake (van den Driessche 1991). Changes in pH may induce dissociation of organic acids, resulting in changes in the overall external charge of the membrane (van den Driessche 1991). Changes in the charge of membrane molecules can alter their interactions, resulting in changes in lipid mobility and therefore membrane permeability (van den Driessche 1991). Furthermore, root respiration can be altered by changes in pH that affect H^+-ATPase activities, essentially increasing root respiration with decreasing pH to a point at which growth is no longer possible (Lambers et al. 1998).

Changes in rhizosphere pH have been linked to NH_4^+ and NO_3^- nutrition. Media containing NO_3^- tends to become more alkaline while media containing NH_4^+ tends to become more acidic due to the release of OH^- and H^+ ions, respectively as inorganic N is converted to organic N forms (Haynes and Goh 1978, Gisjman 1990 b). The release of OH^- and H^+ ions during plant N assimilation, can significantly change the pH of the rhizosphere compared to surrounding soil or solution. This may further affect nutrient uptake conditions (Marschner 1991, Gisjman 1990 a,b). Although rhizosphere conditions can be altered by NH_4^+ and NO_3^- nutrition, the aeroponic nature and pH buffering of this study should have minimized any rhizosphere effect.

Even though pH does affect NH_4^+ and NO_3^- uptake, studies examining Douglas-fir seedlings grown with NH_4^+, NO_3^- or NH_4^+:NO_3^- found that pH between 4.0 and 6.3 did not affect seedling growth (van den Driessche 1971). Although the pH level used in this study was relatively acidic (pH 4.0), similar studies on Douglas-fir found that the rate of uptake of both NH_4^+ and NO_3^- was constant over a pH range from 3.0 to 5.5 (Rygiewicz et al. 1984 a, b). With a greater range of pH, van den Driessche (1978) found Douglas-fir growing at pH 4.0 had greatest growth on NO_3^-, while on pH 7.0 Douglas-fir had greatest growth on NH_4^+.

Temperature

Ammonium and NO_3^- uptake varies with temperature of the root environment (Haynes and Goh 1978, Clarkson et al. 1986, Gessler et al. 1998). At low temperatures, nitrate uptake is much more inhibited than NH_4^+ uptake (Clarkson et al. 1986, Gessler et al. 1998), possibly due to the lower activity of the enzymic NO_3^- reducing system (Haynes and Goh 1978). Optimum NO_3^- uptake in Norway spruce (*Picea abies* (L.) Karst.) was found to occur at 25 °C, while at temperatures between 10 °C and 15 °C, NO_3^- uptake was reduced by 84 - 89%. In contrast, NH_4^+ uptake at 10 °C was only reduced by 27% of maximal uptake (Gessler et al. 1998). Seedlings grown in the Biotronic units had root and solution temperatures held constant at 20 °C, which may have influenced the relative uptake of NH_4^+ and NO_3^-. Based on the previous studies, uptake of NO_3^- may be enhanced at this temperature, as it is approaching optimal conditions for NO_3^- uptake.

Mycorrhizae

Mycorrhizal fungi benefit their associated hosts by increasing the plant's ability to capture water and nutrients (Raven et al. 2004). Mycorrhizal associations are widespread in the natural environment and play a key role in nutrient acquisition, especially for key elements of phosphorus, zinc, manganese and copper (Raven et al. 2004). This study was conducted without mycorrhizal associations. Determining seedling nutrient uptake patterns under non-mycorrhizal conditions allowed us to view the effects of varying N sources directly on seedling growth and development, increasing knowledge of seedling physiology without additional confounding effects. Ammonium and NO_3^- uptake may be altered in the presence of mycorrhizal associations, however, several studies have indicated that the down-regulation of high affinity influx systems under conditions of low

N availability may occur due to improved transfer of organic N sources from the mycorrhizae to the plant (Finlay et al. 1988, Ek et al. 1994). In a study on Norway spruce, it was found that mycorrhizal roots could absorb both NH_4^+ and NO_3^- (Finlay et al. 1989), but they tended to show a preference for NH_4^+ (Kronzucker et al. 1997, Kahr and Arveby 1986). The presence of mycorrhizal fungi on Douglas-fir may alter the patterns of NH_4^+ and NO_3^- uptake observed in this study. As always, studies conducted in controlled environments should be replicated in more natural environmental conditions before conclusive statements can be made.

Comments on experimental procedure

As has been demonstrated, factors such as pH, temperature and mycorrhizal associations can have profound implications on the relative uptake of NH_4^+ and NO_3^-. This study was limited by examination of N source uptake at a single temperature and narrow pH range. When examining the results of this study, consideration should be given to these limitations. The relative uptake of each N source will most likely be altered with variations in both temperature and pH. In order to be able to extrapolate these results beyond these pH and temperature ranges, future studies should be conducted incorporating a combined range of temperatures and pH levels.

Similarly, NH_4^+ and NO_3^- uptake can only be inferred from the relative loss of each ion from the nutrient solutions. Future studies incorporating the use of isotope labelled N may provide valuable information on uptake patterns and relative contribution of each ion to growth. Furthermore, conducting the experiments for longer periods of time may allow us to more clearly view the effects of NH_4^+ toxicity, especially for the treatments containing intermediate levels of NH_4^+. Closer examination of the ratios between 60:40 and 40:60 may be warranted to more clearly distinguish between the levels of optimal growth and NH_4^+ toxicity.

Conclusions

Douglas-fir produced the greatest growth, in terms of RGR and biomass, when both NH_4^+ and NO_3^- were available in near equal proportions in solution. Rapid decreases in RGR, biomass, N concentration and photosynthesis all occurred when NH_4^+ reached levels of 285 μM in solution. An NH_4^+ concentration of 214 μM and NO_3^- concentration of 149 μM, a pH of 4.0 and root temperature of 20 °C appeared optimal for growth as judged by RGR and biomass. However, the rapid decrease in wpN concentration in the 60:40 treatment from Harvest 1 to Harvest 3 indicates that NH_4^+ toxicity may have been developing in this treatment as well, but had yet to be reflected in RGR. If this is true, a slightly lower NH_4^+:NO_3^- ratio may be optimal. The optimal N source ratio should maintain stable internal nutrient concentrations within the seedling, achieving steady-state, thereby reducing internal nutrient stress.

Adjustments to the optimal NH_4^+:NO_3^- ratios will be required with changing pH. Solutions with high pH (up to 5.5) may be better able to take up NH_4^+, however the concentrations at which NH_4^+ becomes toxic at this level of acidity would need to be studied.

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CHAPTER 3. DOUGLAS-FIR SEEDLINGS GROWN UNDER EXPONENTIALLY LOADED OR CONVENTIONAL FERTILIZER REGIMES

INTRODUCTION

Regeneration of interior Douglas-fir has proven difficult in British Columbia (Newsome 1997). Factors such as limited nutrient availability (Millard 1996), poor planting stock (Simard et al. 1997), summer drought and frequent frost occurrence (Newsome 1997) have all been blamed for the sub-optimal regeneration of conifers. Improved nutritional management may increase seedling vigour and tolerance of difficult environmental conditions. Developing optimally conditioned nursery stock is therefore an important step in improving regeneration success.

BC conifer seedling nurseries have traditionally followed conventional fertilization regimes, adding constant rates of fertilizer to seedlings, regardless of their growth rate (Timmer 1997). This 'constant feed' model, consists of repeated nutrient applications at constant addition rates over the entire nursery period (Timmer 1997). The conventional regime typically results in nutrient additions that exceed the seedling's ability to take up nutrients at early stages, while at later stages, nutrient additions may be inadequate to meet the seedling's nutritional demands (Timmer 1997). This may result in seedlings leaving the nursery with sub-optimal internal nutrient contents, characterized by diluted internal nitrogen (N) concentrations (Timmer 1997), indicating nutrient stress. Alternative fertilization regimes attempt to increase internal nutrient concentrations of nursery stock prior to planting, thus enabling seedlings to better withstand difficult environmental conditions (Malik and Timmer 1995, Timmer 1997, Imo and Timmer 2001, Salifu and Timmer 2003). The key difference between alternative and conventional fertilizer regimes is the exponential addition of nutrients to seedlings versus the constant rate addition of nutrients, respectively. The exponential method aims to maintain constant internal nutrient concentrations within individual seedlings. This may result in an increased ability to retranslocate nutrients (Salifu and Timmer 2001, 2003), reduce the severity of planting shock (Salifu and Timmer 2003), and increase the ability of the seedlings to take up nutrients in competitive environments (Timmer 1997, Malik and Timmer 1998).

Alternative nursery fertilization regimes are built upon the concept of steady-state nutrition. Steady-state nutrition is achieved when internal nutrient concentrations remain constant over time (Ingestad and Lund 1986), and when the relative uptake rate of nutrients is controlled by an equal and constant relative addition rate (Ingestad and Agren 1995). By matching the exponential growth rate of seedlings with corresponding exponentially increasing nutrient additions, it is possible to achieve steady state, and thus reduce nutrient deficiencies within the seedling (Timmer 1997). This process is similar to the growth of natural regeneration in the field where root systems expand at exponential rates, naturally inducing a steady-state nutrient status (Ingestad and Lund 1986).

Exponential nutrient loading takes the concept of exponential fertilization one step further. This technique builds nutrient reserves in seedlings promoting the luxury consumption of nutrients through the use of higher fertilization rates over the entire nursery growing season (Timmer 1997). Fertilizer additions follow the exponential growth rate of seedlings, but at each fertilizer addition, slightly more N than is needed is added to the system (Timmer 1997). The luxury uptake of nutrients is characterized by increasing internal nutrient concentrations without significantly increased biomass (Timmer 1997). Greater internal nutrient reserves may improve nutrient retranslocation and nutrient uptake under competitive conditions (Timmer 1997). Exponential nutrient loading has been explored in recent years and has been used with much success to improve seedling growth in eastern Canada in both greenhouse and field experiments (Timmer and Munson 1991, Timmer 1997, Salifu and Timmer 2003).

Several studies have found exponential nutrient loading results in improved seedling growth following out-planting (Malik and Timmer 1995, Miller and Timmer 1997, Xu and Timmer 1998, Salifu and Timmer 2001, 2003). Studies in eastern Canada found exponential nutrient loading increased height and dry matter production in black spruce (*Picea mariana* (Mill.) BSP) (Timmer and Munson 1991, Malik and Timmer 1995), and increased internal plant concentrations of N, P, and K (Malik and Timmer 1995) when compared to conventional fertilizer regimes. Seedlings grown under exponential regimes had superior competitive ability compared to conventional seedlings. This could be due to the increased retranslocation of nutrients from reserves built up during the nursery fertilization phase to actively growing plant tissues (Malik and Timmer 1995).

The greater nutrient reserves of exponentially nutrient loaded seedlings have been shown to enhance the ability of seedlings to retranslocate nutrients, potentially improving growth after planting (Malik and Timmer 1998, Imo and Timmer 2001, Salifu and Timmer 2001, 2003). The retranslocation of nutrients is important for the production of new tissues at all stages of development (Nambiar and Fife 1991), as up to 66% of nutrients required for new growth in young conifers can be acquired through the retranslocation of internal nutrients (Miller 1995). Retranslocation is especially critical to meet nutrient demands of expanding shoots in newly planted seedlings because initial root growth and nutrient uptake is slow (van den Driessche 1985, Burdett 1990). Net retranslocation of N, P and K has been shown to increase by more that 569, 185, and 102%, respectively, in nutrient loaded seedlings compared to seedlings raised under conventional fertilization regimes (Salifu and Timmer 2001).

Although studies of exponential nutrient loading of coniferous species in eastern Canada have shown benefits from this technique, some studies of British Columbia conifer species have been less positive. Exponential fertilization in black spruce was found to produce seedlings with faster growth, increased nutrient uptake efficiency and increased root development, but in the same study, white spruce (*Picea glauca* (Moench)) grew more rapidly with conventional fertilization (Burgess 1990). Burgess (1991) found western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) to have greater growth with exponential fertilization, than with constant rate fertilization. However, the same study found Douglas-fir performed better with conventional fertilization than with 4% or 6% exponential fertilization rates (Burgess 1991). In contrast, Hawkins et al. (2005) found exponentially nutrient loaded western hemlock seedlings performed no better than conventionally fertilized seedlings and exhibited no major increases in net retranslocation in the field. Brown et al. (1996) cautioned that nutrient loading might not always have positive effects, as higher nutrient concentrations in plants may increase their susceptibility to herbivory, moisture stress and frost damage.

Individual seedling fertilization at the time of planting is another method that has been explored as a means to improve regeneration success. Seedlings may be planted with slow release fertilizer packets placed approximately 5 to 10 cm upslope from the seedling. Studies utilizing slow release fertilizer packets have found significant positive growth responses in the first three years, with seedlings displaying greater height and root collar diameter (RCD) (van den Driessche 1988, Bowden 1995). These effects typically diminish after 5 years (Bowden 1995). Brockley (1988) found fertilization at the time of planting to be effective only when competing vegetation was controlled. Much controversy surrounds the benefits of fertilization at the time of planting versus the associated higher planting costs and potential future complications with competing vegetation (Brockley 1988).

Few field studies have been conducted in British Columbia analyzing the effects of nutrient loading on conifer seedling growth, and no field studies of this nature are known to have been conducted on interior Douglas-fir. There have also been no known studies comparing the performance of nutrient loaded seedlings with those receiving extra nutrients at the time of planting. Identifying the benefits of nutrient loading to the growth of conifer seedlings may lead to improved growth in the first years after planting, thereby improving establishment. Improvements in seedling stock may enhance regeneration and may mitigate the need for activities of questionable environmental impact including on-site fertilization and vegetation control with herbicides.

The objective of this study was to compare the growth and nitrogen dynamics of Douglas-fir seedlings raised with exponential nutrient loading, with conventional fertilization in the nursery, or with conventional fertilization plus fertilizer applied at planting over two field growing seasons. I hypothesized that exponential nutrient loading would produce seedlings with greater nutrient reserves for retranslocation and improved competitive ability, thus resulting in greater growth following planting compared to seedlings grown with conventional fertilization. Furthermore, I hypothesized that seedlings planted with additional fertilizer would outperform conventionally fertilized seedlings.

METHODS AND MATERIALS

Barriere field trial

Study Area

The study area was located approximately 10 km northeast of Barriere, British Columbia in the dry, cool subzone of the Interior Douglas-fir biogeoclimatic zone (IDF) (latitude 51° 16' 41" N, longitude 120° 06' 31" W, 1230 m). The continental climate of the IDF is characterized by warm, dry summers, relatively long growing seasons, and cool winters with low to moderate snowfall (Lloyd et al. 1990). Substantial moisture deficits may occur late in the growing season, and frost may occur in any month except July and early August (Lloyd et al. 1990). The mean annual precipitation is 568 mm, and mean growing season precipitation is 221 mm. Mean annual temperature is 4.1 °C, with a mean growing season temperature of 11.1 °C (Lloyd et al. 1990).

The study area was characterized by rolling, short and broken slopes averaging 25-30%, with variable aspects. Prior to harvest, the stand consisted of 60% lodgepole pine (*Pinus contorta* (Dougl. Ex Loud.)), 30% Douglas fir, 5% spruce (*Picea glauca* (Moench) Voss), 2% western red cedar (*Thuja plicata* (Donn ex D.Don)), and 2% subalpine fir (*Abies lasiocarpa* (Hook.) Nutt.), and was classed as moderately productive. Site 1 was located in a lower to toe slope position, with a mesic to sub-hygric moisture regime, and a soil texture ranging from loam to silt loam. This site was located adjacent to the forest edge and approximately 50 m from a riparian area. Site 2 was located in a mid to upper slope position with sub-mesic to mesic moisture regimes, and a soil texture ranging from loam to silt loam.

Experimental Procedure

In 2004, two research sites were located within a 56.7 hectare area that was harvested two years earlier. In 2003, stumps were upturned over the entire area in an effort to prevent the spread of *Armillaria* and *Phellinus* root disease. Planting of the block occurred in the spring of 2004.

Experimental seedlings were grown at the PRT Northland Nursery in Kirkland Lake, Ontario (latitude 48° 09' N, longitude 80° 03' W, 318 m elevation). Douglas-fir seeds (seedlot 39494, latitude 51° 10' N, longitude 120° 03' W, 1200 m elevation) were germinated in Styroblock (PSB) 412A containers in mid-April 2003. Seedlings were grown under one of two nutritional regimes, conventional fertilization or exponential fertilization, both with an N-P-K ratio of 19-9-18 (Figure 3.1). Under the conventional regime, fertilizer was added first at a concentration of 100 ppm. This was raised to 150 ppm after eight weeks. Fertilization of exponentially fertilized seedlings commenced with lower nutrient concentrations that increased at a rate of approximately 2% per day, similar to the seedling growth rate, to a maximum concentration of 403 ppm. Due to slow root expansion, steady state is sometimes difficult to achieve at the early stages of growth in container grown conifers, therefore a modified exponential regime was used at the start of the fertilizer period (Imo and Timmer 1992, Timmer 1997). This modified nutrient regime increased the nutrient concentrations in fertilizer solutions in the first three weeks to allow greater nutrient uptake. This additional quantity was subtracted from the final nutrient application to avoid possible bud damage from excessive fertilization (Imo and Timmer 1992, Timmer 1997). In both the conventional and exponential regimes, nutrient concentrations were reduced in the final four weeks to initiate bud set (Figure 3.1). In both treatments, all nutrients were added in irrigation water. Seedlings were lifted from the Styroblocks in November 2003, and stored at -2 °C.

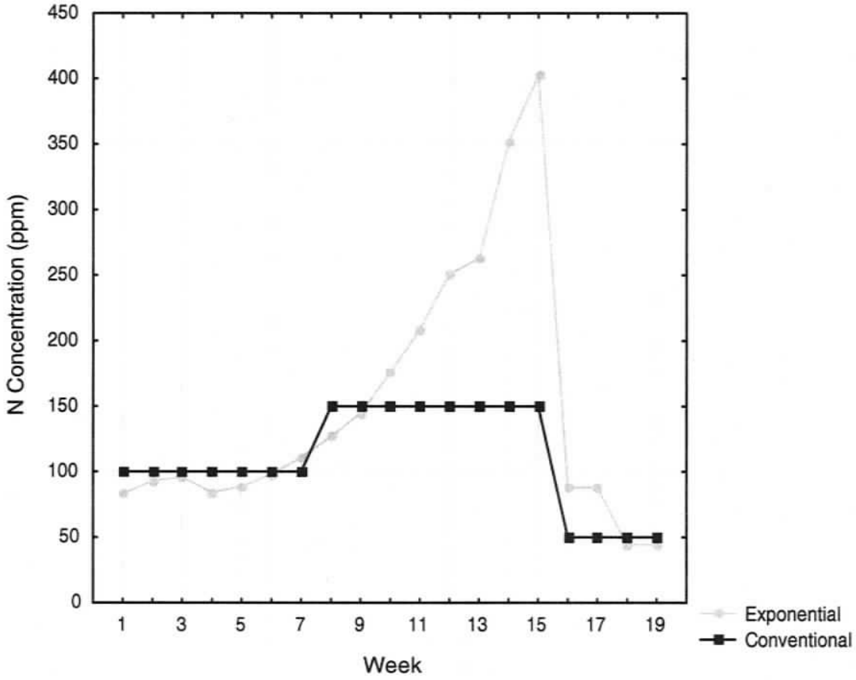

Figure 3.1. Nitrogen (N) concentrations applied to seedlings while in the greenhouse. N concentrations in solution applied to seedlings grown under the exponential regime increased to a peak application of 403 ppm, while the conventional regime had a maximum N concentration in solution of 150 ppm.

In May 2004, seedlings were removed from cold storage and planted at the study site. Six hundred conventionally fertilized seedlings and 300 exponentially loaded seedlings were planted in a complete randomized block design on two sites, 375 m apart located in the cutblock. Half of the conventionally fertilized seedlings were planted with additional fertilizer, in the form of slow release fertilizer packets (RTI Planter's Pak – 10 g biodegradable planting packet containing 16.0% total N, 8.0% available phosphoric acid and 8.0% soluble potash, plus 7.6% S, 0.53% Fe, 0.51% Zn, 0.55% Mn, 0.53% Mg, 0.25% Cu, and 0.05% B). The remainder of the conventionally fertilized seedlings were planted without additional fertilizer.

At each site, 450 seedlings (150 of each treatment) were planted on 3 blocks with 50 seedlings per treatment per block (replicate). Treatments were randomly located within blocks. Although site variations occurred among blocks, each individual block was relatively uniform in slope, aspect, and soil conditions. Seedlings were planted to operational specifications with an approximate spacing of 2.5 m. Immediately following planting, each seedling was measured for RCD and height.

A sub-sample of 30 seedlings (15 conventionally fertilized and 15 exponentially fertilized) was brought back to the University of Victoria (UVic) for further analysis. RCD and height were recorded, and seedlings were divided into needles, stems and roots. All tissues were dried at 60 ^{o}C for 72 hr, weighed, then ground coarsely through a 1 mm sieve using a Wiley Mill (Thomas Scientific, Swedesboro, NJ). Samples were then fine-ground using a Wig-L-Bug Amalgamator (model 3110-3A, Cresent Dental MFG Co., Illinois, USA). Nitrogen analysis of these seedlings was conducted to determine baseline N status for the needle, stem and root sections. Samples were packaged into 6 – 8 mg samples in 10.0 mm diameter tin capsules and analyzed for N content using a FlashEA 1112 Elemental Analyser (ThermoQuest Corp., Italy).

The efficiency at which the N added in the nursery translated to growth was estimated for seedlings from both fertilizer regimes. By estimating the pore space in the

Styroblocks, and assuming seedlings were irrigated to saturation, total N supplied to each seedling was approximated. The seedling biomass divided by N supplied to each seedling was used to determine the efficiency at which the supplied N translated to growth.

At the end of the first growing season in October 2004, 25 core seedlings within each replicate were selected and RCD and height were re-measured. In addition, 8 randomly selected seedlings per replicate per block per site were harvested. These seedlings were measured for RCD and height, then excavated, brought back to UVic and divided into first and second year needles, roots and stems. Each part was dried, weighed, and then bulked in groups of four seedlings. Bulked portions were ground to pass through a 1 mm sieve using a Wiley Mill (Thomas Scientific, Swedesboro, NJ). Ground samples were then analyzed for N concentration using combustion technique carried out on a LECO CNS 2000 Elemental Analyzer (St. Joseph, MI).

In July 2005, the 25 core seedlings within each replicate were re-measured for RCD and height. Six seedlings were harvested per replicate per block per site and analyzed for biomass and N concentration of the first three years growth. Each seedling was divided into first, second, and third year needles and stems, and first and subsequent year's roots. Each section was dried, weighed and bulked in groups of three, then analyzed for N concentration as described above.

University of Victoria - Forest Biology Compound

To allow more intensive sampling of the growth patterns of the three treatments, 150 seedlings were planted in the University of Victoria Forest Biology Compound, Victoria, BC (latitude 48° 27' 60", longitude 123° 18' 50", 60 m elevation). The site is flat and soils are moderately coarse textured, ranging from loamy sand to sandy loam. Fifty seedlings from each fertilizer treatment (conventional, fertilized at planting and exponentially loaded) were planted in May 2004 in a completely randomized design with 1 x 1 m spacing. Immediately following planting, all seedlings were measured for RCD and height. The seedlings were irrigated twice weekly to prevent drought.

Thirty seedlings were excavated in July 2004 (10 of each treatment) and measured for RCD and height. Seedlings were then divided into first and second year roots, needles, and stems. Each plant section was dried at 60 °C for 72 hr, weighed, and bulked in groups of 3 trees. Bulked samples were ground through a 1mm sieve using a Wiley Mill (Thomas Scientific, Swedesboro, NJ) and packaged into 6 – 8 mg samples in 10.0 mm diameter tin capsules. Capsules were analyzed for N concentration using a FlashEA 1112 Elemental Analyser (ThermoQuest Corp., Italy). A similar harvest of thirty seedlings was conducted in October 2004 to provide information on nutrient retranslocation throughout the first growing season. In April 2005, a harvest of 30 seedlings was conducted to determine changes in nutrient status over the winter. A final harvest of 30 seedlings was conducted in July 2005 to determine the growth and nutrient retranslocation patterns through the second season of growth. For all harvests, seedlings were divided into needles and stems by age class, and first and subsequent year's growth of roots. All pieces were dried, ground, bulked in groups of three trees and analyzed for N concentration as described above.

Nutrient retranslocation between the varying age-classes was quantified by:

$$Re = A - B - (D + Le) \tag{3.1}$$

where Re is the amount of nutrient retranslocated, A is the N content of a specific tissue at the previous harvest, B is the N content of tissue of the same age class at the subsequent harvest, D is the nutrient content of dead tissues lost between harvests, and Le is the amount of N lost to leaching (Salifu and Timmer 2001). D and Le were assumed to be negligible (Nambiar and Fife 1991, Helmisaari 1992), therefore percentage net retranslocation was calculated by:

$$\% RE = [(A - B) / A] \times 100 \tag{3.2}$$

Statistical analysis

Barriere Field Site

Some seedlings located at the field site near Barriere were subject to browse damage; these trees were not included in the analysis. Furthermore, trees located in Site 1 - Block 3, were growing on a much colder, wetter site than the rest of the experiment in conditions unfavourable to Douglas-fir, thus this block was also removed from analysis (Table 3.1).

Table 3.1. Sample sizes used in height and root collar diameter analyses following the removal of browsed and off-site seedlings at the Barriere field site.

	Conventional	Exponentially loaded	Fertilizer at planting
May 2004	184	190	185
October 2004	102	104	100
July 2005	90	94	87

Seedling whole plant nitrogen (wpN) content was analyzed with Chi-square analysis. Dry mass was log transformed to meet the assumptions of the ANOVA. All values in the figures and tables are based on non-transformed data. A general linear model - partly nested analysis of variance (ANOVA) was used to test the effect of fertilizer regime on height, RCD, and total dry mass using STATISTICA Version 6 (2003). Analysis for each season was conducted independently. Nutrient treatment was considered to be a fixed factor, site and block were considered to be random factors. The following general linear model was used (Quinn and Keough 2002):

$$Y_{(i)jkl} = \mu + \alpha_i + \beta_{j(i)} + \gamma_k + \alpha\gamma_{ik} + \beta\gamma_{j(i)k} + \varepsilon_{ijkl} \quad (3.3)$$

where;

$Y_{(i)jkl}$ = is the overall value for the *l*th tree in the *k*th treatment for the *j*th block in the *i*th site,

μ = is the overall mean,

α_i = is the variance in mean values of the response variable across all sites,

$\beta_{j(i)}$ = is the random effect of the *l*th tree across all levels of the *j*th block, nested within the *i*th site,

γ_k = is the effect of the *k*th treatment,

$\alpha\gamma_{ik}$ = the variance across all the possible interaction terms between all sites and all treatments,

$\beta\gamma j(i)k$ = the variance across all the possible interaction terms between all the possible blocks within any site and all possible combinations of treatment,

ε_{ijkl} = the random or unexplained error associated with the *l*th tree in the *k*th treatment for the *j*th block in the *i*th site

Means were tested using Tukey's Honestly Significantly Different (HSD) test of hypothesis and Tukey's HSD test for unequal –N when required.

UVic Forest Biology Compound

A one-way ANOVA was used to test for significant differences ($P \leq 0.05$) in height, RCD and total dry biomass among treatments. Dry mass was log transformed to meet the assumptions of the ANOVA. All values in the figures and tables are based on non-transformed data. Nutrient data were analyzed using a Chi-squared test. Means were tested using Tukey's HSD test of hypothesis.

RESULTS

Barriere field trial

Seedling growth parameters

After the first growing season, a large number of the trees had browse damage. In total, 47% of all trees were browsed by the second growing season at the Barriere field site, with 48%, 43% and 50% of the seedlings browsed within the conventional, exponentially loaded, and fertilized at the time of planting treatments, respectively. Browsed trees were excluded from analysis to allow a clearer view of treatment effects.

Seedling mortality among treatments was not significantly different (χ^2, $P > 0 .05$) after two growing seasons. Survival was 93.0%, 93.5% and 95.2% for the conventional, exponentially loaded, and fertilized at the time of planting treatments, respectively.

Two growing seasons after planting, exponentially loaded seedlings did not differ significantly in height or RCD from seedlings grown with the conventional regime. Although there were no significant growth benefits from exponential nutrient loading in the two seasons after planting, there were significant increases in both the height ($P = 0.001$) and RCD ($P < 0.0001$) of seedlings fertilized at planting. Seedlings fertilized at the time of planting had 11% greater height than the exponential treatment and 7% greater height than the conventional treatment (Figure 3.2).

Seedling RCD was significantly ($P < 0.0001$) affected by treatment after both the first and the second growing seasons in the field (Figure 3.3). During the second growing season, the RCD of the seedlings fertilized at the time of planting was 17.1% greater than the exponentially loaded treatment and 9.1% greater than the conventional treatment. There were significant site by treatment interactions ($P = 0.016$) after the second growing season (Table 3.2).

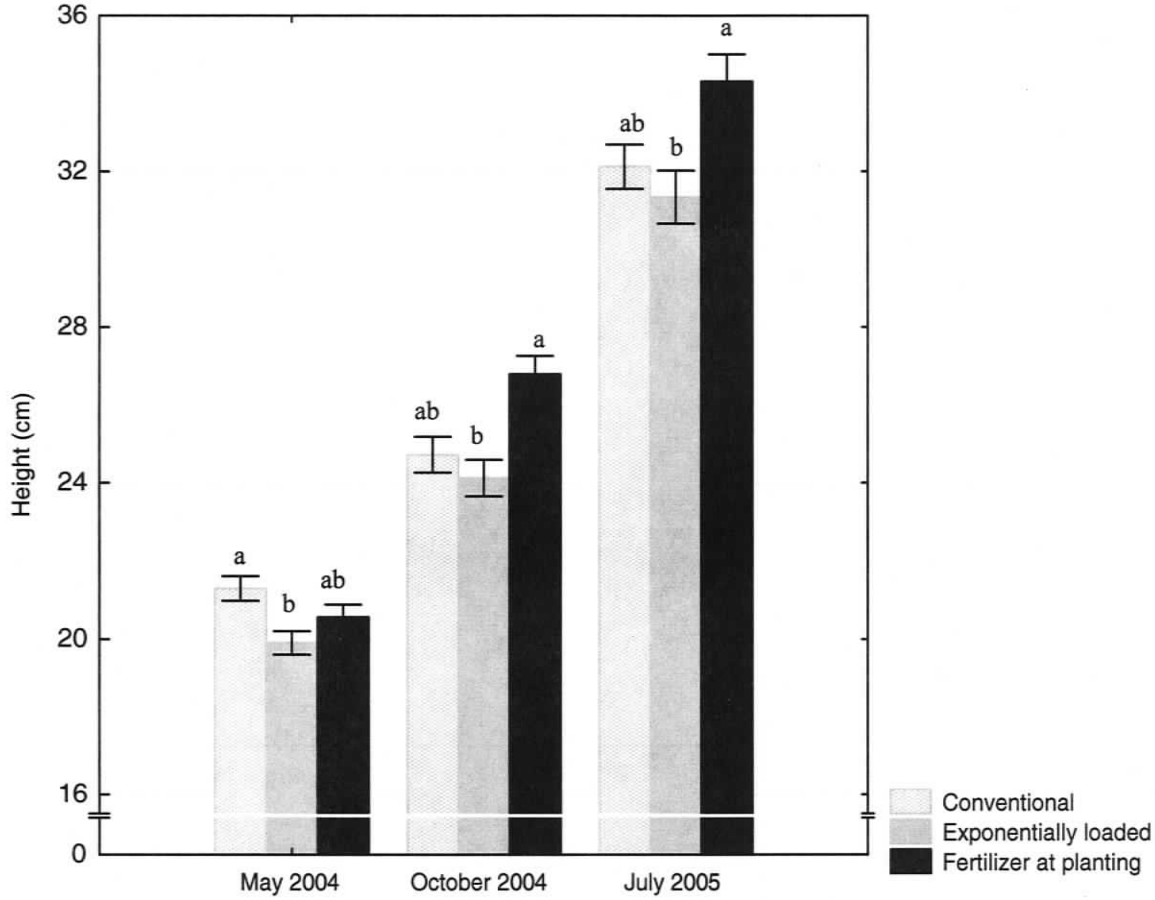

Figure 3.2. Height of Douglas-fir seedlings in three fertilization regimes over two growing seasons in the field. Lines indicate standard error of the mean of all seedlings. Bars marked with different letters denote significant differences within harvests ($P \leq 0.05$).

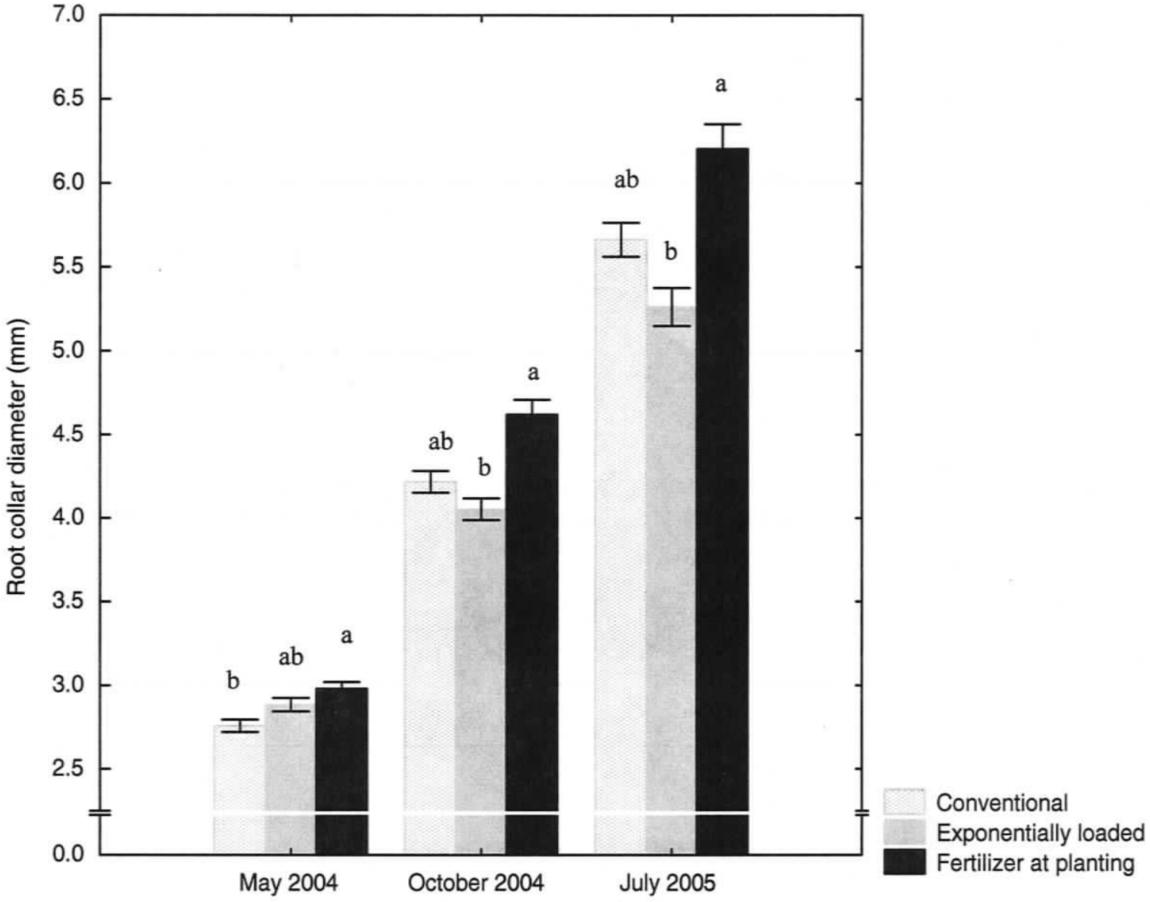

Figure 3.3. Root collar diameter (RCD) of Douglas-fir seedlings in three fertilization regimes over two growing seasons in the field. Lines indicate standard error of the mean of all seedlings. Bars marked with different letters denote significant differences within harvests ($P \leq 0.05$).

Table 3.2. Mean root collar diameter of Douglas-fir two years after planting on the two sites over two growing seasons in the field. Number in parenthesis is *n*. Data are shown as mean ± S.E of all seedlings.

Site 1	Conventional	Exponentially loaded	Fertilizer at planting
May 2004	2.78 ± 0.05 (76)	2.81 ± 0.07 (69)	2.99 ± 0.06 (80)
October 2004	4.09 ± 0.08 (44)	3.90 ± 0.10 (35)	4.59 ± 0.13 (47)
July 2005	5.75 ± 0.15 (43)	4.85 ± 0.13 (33)	6.42 ± 0.27 (37)

Site 2	Conventional	Exponentially loaded	Fertilizer at planting
May 2004	2.74 ± 0.06 (108)	2.93 ± 0.06 (121)	2.98 ± 0.06 (105)
October 2004	4.33 ± 0.07 (58)	4.13 ± 0.09 (69)	4.65 ± 0.13 (53)
July 2005	5.58 ± 0.15 (47)	5.48 ± 0.16 (61)	6.04 ± 0.18 (50)

Seedling biomass allocation

The log of total dry mass differed significantly between nursery treatments at the time of planting, with the conventional seedlings having greater mass than the exponentially loaded seedlings. By the end of the first growing season this trend no longer existed. At that time, seedlings fertilized at planting had greater total dry mass than the conventional or exponentially loaded treatments ($P = 0.042$) (Table 3.3). Although there were significant differences, the means could not be differentiated using Tukey's HSD. Seedlings fertilized at the time of planting had greater average dry mass than both the conventional and exponentially loaded seedlings throughout the second growing season, however means were not significantly different.

Root:shoot ratio was significantly affected by treatment on all three measurement dates (Table 3.4). After the second growing season, the seedlings fertilized at the time of planting had the lowest root:shoot ratio, indicating a greater proportion of biomass was allocated to the above ground systems, in comparison to the below ground systems. The exponentially nutrient loaded seedlings had the highest root:shoot ratio.

Table 3.3. Mean dry mass (g) of Douglas-fir seedlings after two growing seasons in the field. Bold denotes significant P values (≤ 0.05). Data are shown as mean ± S.E of all seedlings. Values marked with different letters denote significant differences within harvests.

	n	P value	Conventional	Exponentially loaded	Fertilizer at planting
May 2004	15	**< 0.0001**	1.71 ± 0.24 a	0.90 ± 0.04 b	---
October 2004	40	**0.042**	3.99 ± 0.21 a	4.13 ± 0.23 a	5.00 ± 0.35 a
July 2005	30	0.078	8.37 ± 0.60 a	8.63 ± 0.94 a	10.42 ± 0.93 a

Table 3.4. Root to shoot ratios of Douglas-fir seedlings after two growing seasons in the field. Data are shown as mean ± S.E of all seedlings. Bold denotes significant P values (≤ 0.05). Values marked with different letters denote significant differences within harvests.

	n	P value	Conventional	Exponentially loaded	Fertilizer at planting
May 2004	15	**0.011**	0.50 ± 0.04 b	0.66 ± 0.04 a	---
October 2004	40	**0.042**	0.84 ± 0.03 a	0.84 ± 0.03 a	0.77 ± 0.03 b
July 2005	30	**0.041**	0.34 ± 0.02 b	0.39 ± 0.03 a	0.33 ± 0.02 b

Nitrogen concentration in plant tissues

The efficiency at which N added in the nursery translated to growth varied greatly between treatments. Seedlings in the exponential regime produced 16.6 mg dry mass per mg N, while seedlings in the conventional regime produced 43.0 mg dry mass per mg N added in the nursery.

There were no significant differences (χ^2, $P > 0.05$) in whole plant N (wpN) concentrations among fertilizer treatment on any harvest date (Table 3.5). Although the exponentially loaded treatment displayed greater average wpN concentrations when compared to the conventional treatment, the difference was not significant. Seedlings planted with fertilizer displayed greater wpN concentrations after the first season, which then decreased during shoot expansion in the subsequent growing season.

Table 3.5. Average whole plant nitrogen concentrations (%) in Douglas-fir seedlings over two growing seasons in the field. Data are shown as mean ± S.E. of all seedlings.

	n	P value	Conventional	Exponentially loaded	Fertilizer at planting
May 2004	15	0.273	1.70 ± 0.06	1.85 ± 0.03	---
October 2004	10	0.301	1.06 ± 0.05	1.09 ± 0.04	1.15 ± 0.03
July 2005	10	0.670	0.94 ± 0.05	1.03 ± 0.06	0.96 ± 0.04

There were no significant differences (χ^2, $P = 0.088$) in foliar N concentration at the time of planting, however, on average needles of exponentially fertilized trees had a greater N concentration than those of conventionally fertilized seedlings, with N concentrations averaging 2.07 ± 0.08% and 1.84 ± 0.09%, respectively. At the end of the first growing season there were no significant differences (χ^2, $P = 0.670$) in new foliage N concentration among treatments. Seedlings in the fertilizer at the time of planting, conventional and exponentially loaded treatments had 1.90 ± 0.08, 1.86 ± 0.09% and 1.81 ± 0.10 % foliar N, respectively. Likewise, there were no significant differences (χ^2, P = 0.301) in new foliage N concentrations following the second growing season with seedlings fertilized at planting, conventional and exponentially loaded treatments containing 0.57 ± 0.02, 0.53 ± 0.02 and 0.60 ± 0.03 % foliar N, respectively.

Nutrient retranslocation

Over the first growing season (May 2004 to October 2004) retranslocation of N from first year foliage varied widely, with 49.1%, 40.5% and 8.0% for the conventional, fertilizer at the time of planting and exponentially loaded treatments, respectively. In absolute terms, 5.7 mg, 4.7 mg and 0.5 mg of N were retranslocated by the conventional, fertilized at planting, and the exponentially loaded treatments, respectively (Figure 3.4). From the end of the first growing season to the end of shoot extension the following year, (October 2004 to July 2005), first-year foliage retranslocated additional N to actively growing tissues. Conventional treatment seedlings retranslocated an additional 3.6 mg N (61%), seedlings fertilized at the time of planting retranslocated 4.6 mg N (67%) and the exponentially loaded seedlings retranslocated 3.8 mg N (60.3%).

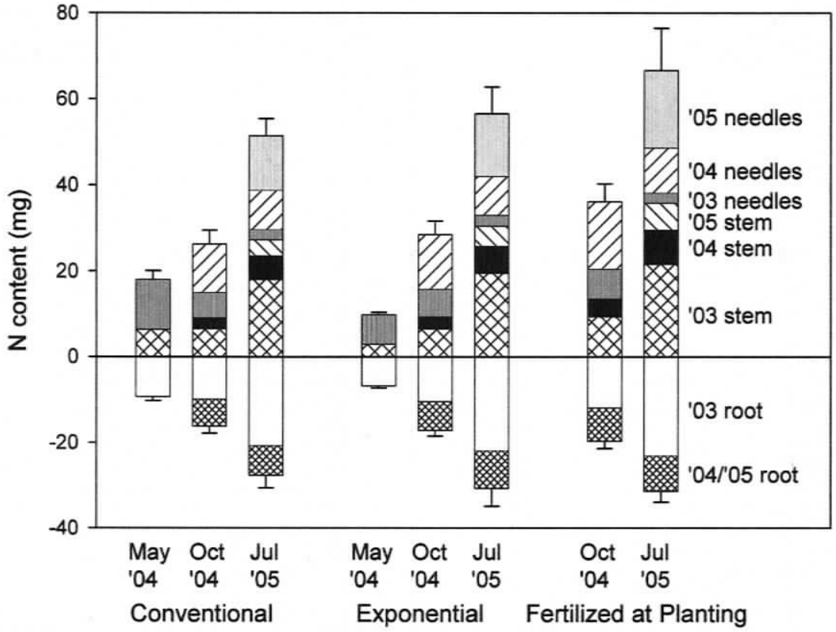

Figure 3.4. Nitrogen (N) content of Douglas-fir seedling components averaged for three fertilization regimes in May 2004, October 2004 and July 2005. Values below the zero line indicate root N content. Values above the zero line indicated shoot N content. N contents for seedlings fertilized at planting in May 2004 are the same seedlings as in the conventional treatment.

University of Victoria – Forest Biology Compound

Seedling growth parameters

At the time of planting, there were significant differences (P = 0.007) in height among the treatments (Figure 3.5). In July 2004, the ranking of treatments remained the same, however the differences were no longer significant (P = 0.386). There were no significant differences in height until July 2005 (P = 0.019), when seedlings in the conventional treatment were largest on average. Differences in RCD were non-significant on all measurement dates, however, during the second growing season, seedlings in both the fertilized at planting and the conventional treatments had larger RCD, on average (Table 3.6).

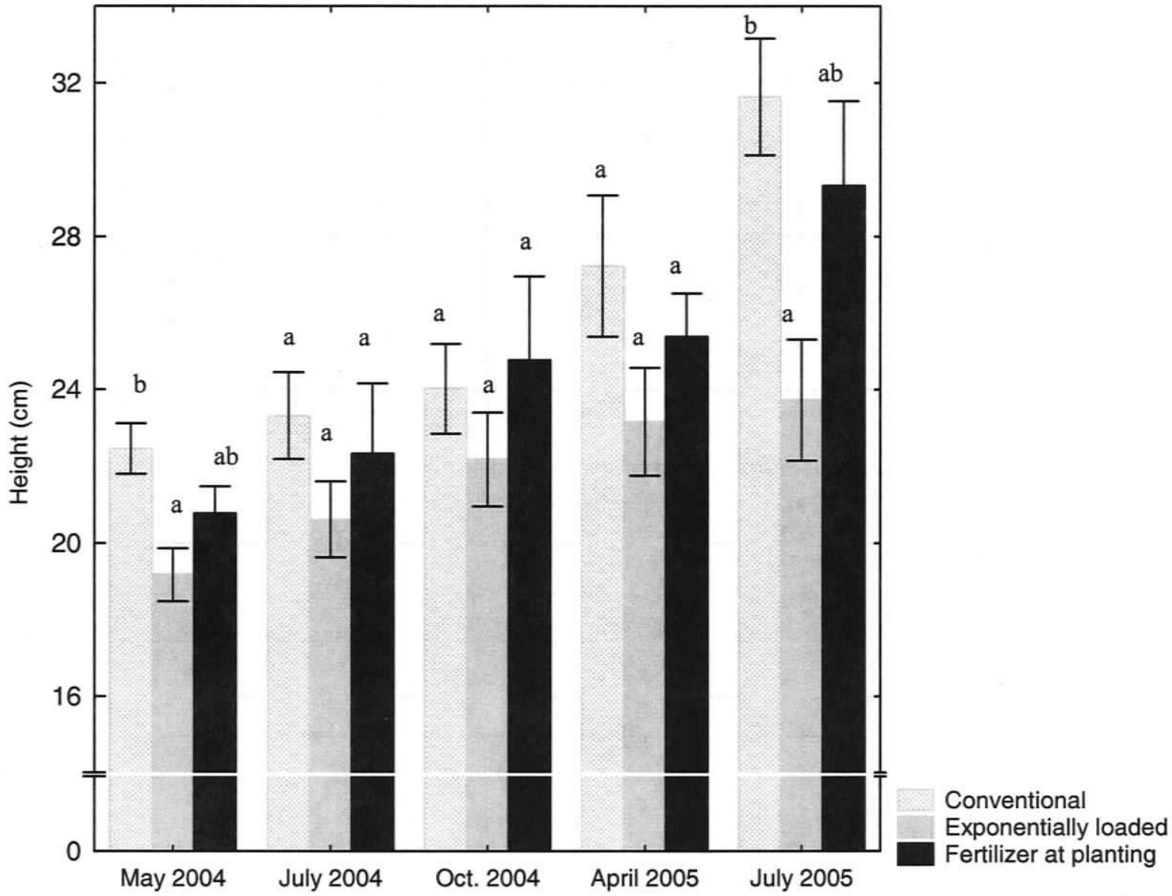

Figure 3.5. Height of Douglas-fir seedlings in three fertilization regimes over two growing seasons following planting. Lines indicate standard error of the mean, *n* = 10. Bars marked with different letters denote significant differences within harvests ($P \leq 0.05$).

Table 3.6. Root collar diameter (mm) of Douglas-fir seedlings in three fertilizer regimes over two growing seasons.

UVic compound	*n*	P value	Conventional	Exponentially loaded	Fertilizer at planting
May 2004	50	0.123	3.48 ± 0.10	3.20 ± 0.10	3.27 ± 0.10
July 2004	10	0.791	4.01 ± 0.23	3.84 ± 0.12	3.89 ± 0.21
October 2004	9	0.077	4.02 ± 0.26	4.26 ± 0.20	4.88 ± 0.31
April 2005	10	0.186	4.26 ± 0.20	4.29 ± 0.21	5.14 ± 0.55
July 2005	10	0.058	8.08 ± 0.52	5.87 ± 0.62	7.24 ± 0.72

Significant differences in log dry mass between nursery treatments existed at the time of planting ($P = 0.001$) (Figure 3.6). In all subsequent measurements over the next two growing seasons, there were no significant differences in log dry mass (July 2004 $P = 0.210$, October 2004 $P = 0.739$, April 2005 $P = 0.157$, and July 2005 $P = 0.061$). Although there were no significant differences, on average, seedlings grown under the conventional regime, both fertilized and not fertilized at planting, exhibited greater dry mass than the exponentially loaded seedlings.

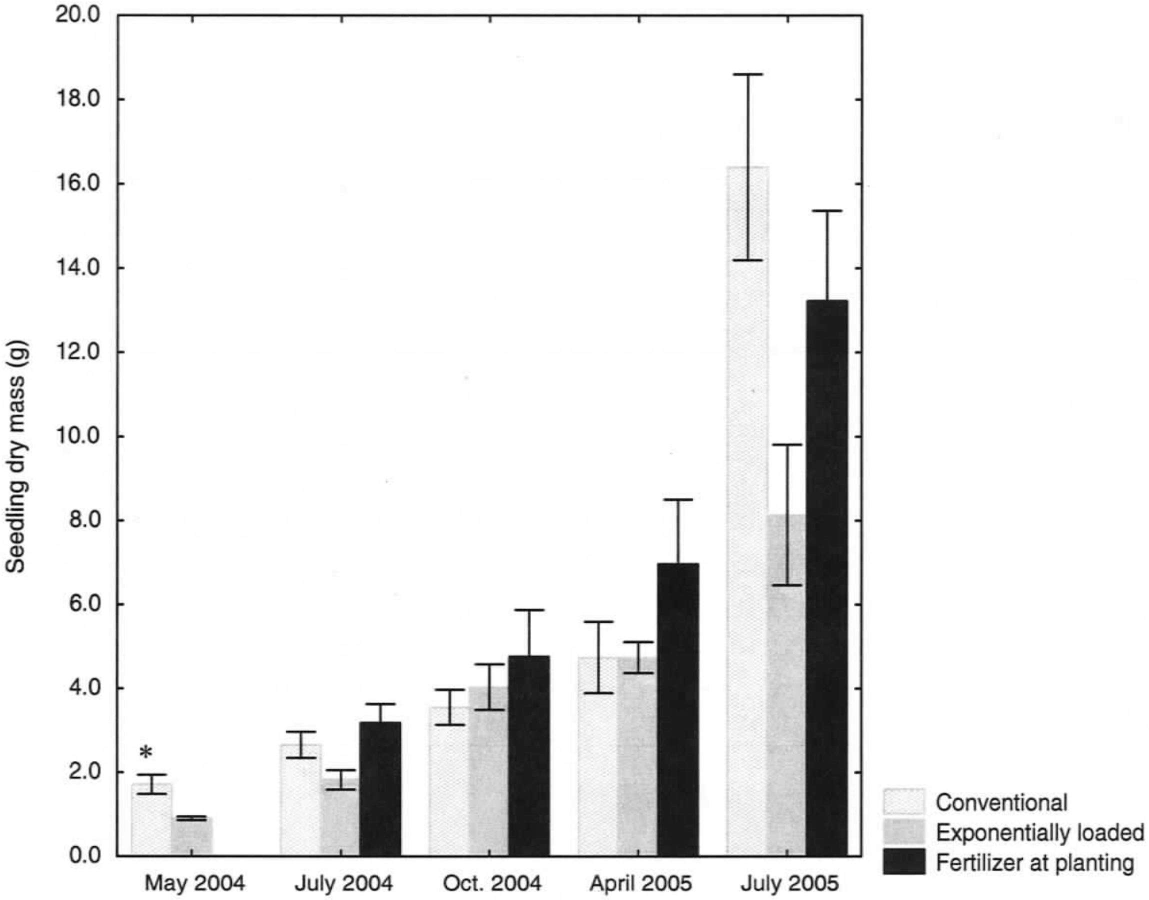

Figure 3.6. Dry mass of Douglas-fir in three fertilizer regimes over the first two years growth following planting. Asterisks denote significant differences ($P \leq 0.05$). Lines indicate standard error of the mean.

No significant differences existed between nursery treatments in wpN concentration at the time of planting (Table 3.7). Significant differences in wpN concentration developed over the first growing season, with seedlings fertilized at the time of planting having greater N concentrations than both the conventional and exponentially loaded seedlings in July and October 2004. Differences in N concentrations diminished over the second growing season, with no significant differences detected among treatments at that time.

Table 3.7. Whole plant nitrogen concentrations (%) of Douglas-fir seedlings in three fertilizer regimes over two growing seasons. Bold denotes significant P (≤ 0.05) values determined by Chi-squared analysis. Letters denote significant differences with harvests.

UVic compound	*n*	P value	Conventional	Exponentially loaded	Fertilizer at planting
May 2004	15	0.273	1.70 ± 0.06	1.85 ± 0.03	---
July 2004	10	**0.042**	0.89 ± 0.06	0.93 ± 0.04	1.13 ± 0.06
October 2004	3	**0.042**	0.99 ± 0.21	0.77 ± 0.03	1.09 ± 0.15
April 2005	3	0.165	1.37 ± 0.13	0.92 ± 0.12	1.08 ± 0.15
July 2005	3	0.165	0.96 ± 0.06	1.11 ± 0.04	1.14 ± 0.04

Immediately following planting there were no significant differences in foliar N concentration (χ^2, P = 0.088), however the exponentially loaded seedlings contained greater average leaf N concentrations than the conventionally grown seedlings (2.07 ± 0.08% and 1.84 ± 0.09%, respectively). In July 2004, there were no significant differences among treatments in foliar N concentrations of newly expanding shoots (χ^2, P = 0.367), however the seedlings fertilized at planting contained greater average foliar N concentrations than the conventional and the exponentially loaded treatments with 1.12 ± 0.07, 0.96 ± 0.07, and 0.95 ± 0.06 % N, respectively. By the fourth and final harvest in July 2005, there was still no significant difference in new foliar N concentration among treatments. At that time the conventional treatment contained 1.40 ± 0.11 % N, the exponentially loaded 1.79 ± 0.08 % N, and the seedlings fertilized at the time of planting, 1.76 ± 0.10 % N.

Nutrient retranslocation

Nutrient retranslocation from older foliage to newly expanding shoots varied in magnitude depending on N content and season (Figure 3.7). By the time of bud set at the end of the first season (October 2004), 49%, 42% and 37% of N had been retranslocated from first-year needles, which corresponded to 5.7 mg, 4.9 mg and 2.5 mg for the conventional, fertilized at planting and exponentially loaded treatments, respectively. Over winter, as growth slowed, nutrients accumulated in the first year foliage, increasing on average by 85%, 5% and 21% for the conventional, fertilized at planting and exponentially loaded treatments, respectively. However, it must be noted that only the increase in the conventional treatment led to N concentrations that were significantly greater than the previous fall. Following the subsequent season's shoot expansion, in the spring of 2005, further N movement within the conventional, fertilized at planting and exponentially loaded treatments was observed with 46%, 30% and 40% retranslocation, respectively.

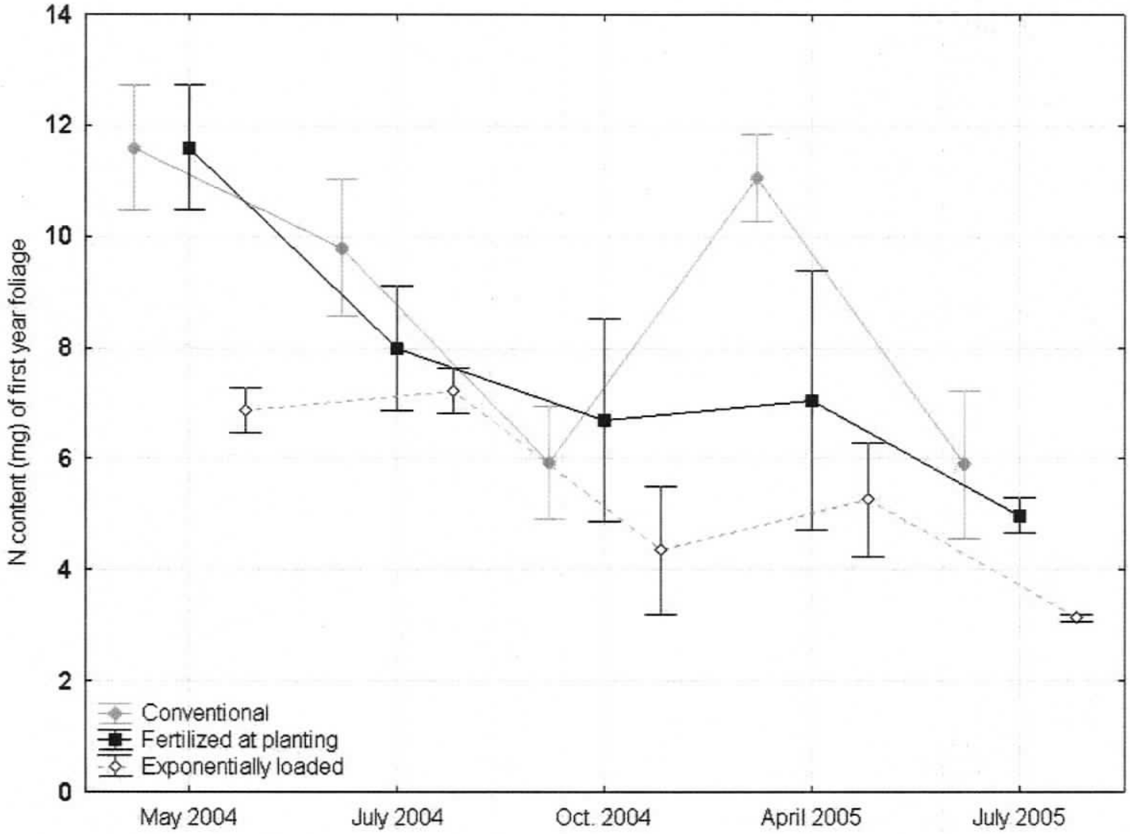

Figure 3.7. Change in nitrogen (N) content in first year foliage in three fertilizer regimes over two growing seasons. Lines indicate standard error of the mean. May 2004 $n = 15$, July 2004 $n = 10$, all subsequent measurements $n = 3$.

Nutrients in second year foliage (2004) followed similar seasonal patterns of N accumulation in the autumn and winter when growth was slow, and rapid loss (assumed retranslocation) in spring when growth rate was greater (Figure 3.8). From July 2004, when shoot expansion was complete, until just prior to shoot expansion in the spring of 2005 (April 2005), there were 181%, 61% and 260% increases in N content of second year foliage for the conventional, exponentially loaded and fertilized at planting treatments, respectively. Nitrogen content of second year foliage rapidly decreased in the conventional and fertilized at planting treatments with the subsequent expansion of shoots in the spring of 2005 (22% and 28% retranslocation, respectively). In contrast, the exponentially loaded treatment accumulated an additional 10% N content between April and July 2005, and did not appear to retranslocate a significant amount of N to the new shoot.

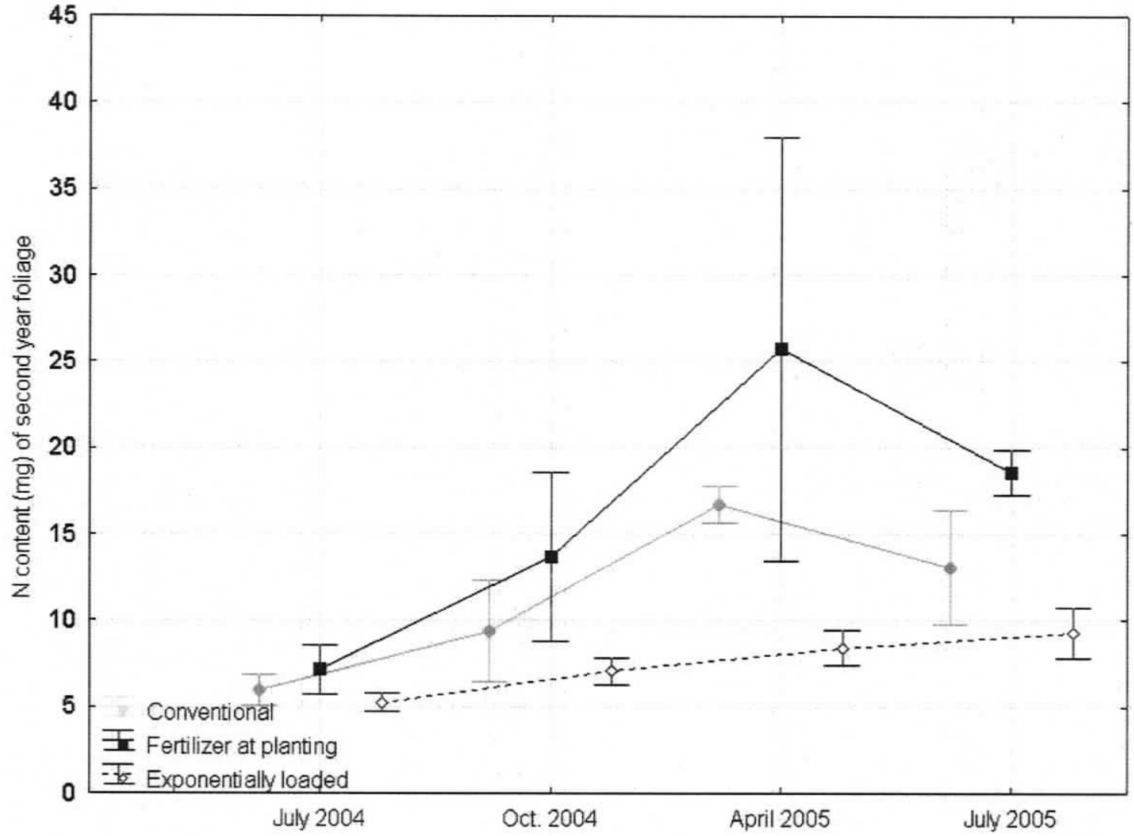

Figure 3.8. Change in nitrogen (N) content in second year needles in three fertilizer regimes over two growing seasons. Lines indicate standard error of the mean. July 2004 $n = 10$, all subsequent measurements $n = 3$.

DISCUSSION

Benefits of nutrient loading

Exponential nutrient loading did not result in any significant benefits to seedling growth in two subsequent seasons following planting. Additional N applied to seedlings in the exponential regime did not appear to be taken up, and did not translate into improved growth, contrary to previous findings in eastern Canada (e.g Miller and Timmer 1997, Timmer 1997, Malik and Timmer 1995, Salifu and Timmer 2001). Although numerous studies with other coniferous species have found beneficial effects of exponential fertilization, this study and the one other (Burgess 1991) conducted on Douglas-fir have found no significant positive growth response. Burgess (1991) found that Douglas-fir grown with conventional fertilizer regimes produced seedlings that had greater biomass than seedlings grown with exponential regimes at 2 and 3% relative addition rate. Only when nutrients were added at 6% did exponential fertilization produce seedlings of greater size.

Burgess (1991) found nutrient additions at a greater exponential rate produced larger seedlings, however nutrient use efficiency in these seedlings was significantly lower. In our study, conventionally fertilized seedlings produced greater biomass per unit N added in the nursery compared to exponentially fertilized seedlings. The exponential treatment received 25% more N, compared to the conventional regime, yet produced less biomass and only 8.8% greater wpN at the time of planting.

Greater average wpN concentrations in the exponentially loaded seedlings at the time of planting resulted in no improvement in height growth or RCD when compared to the conventional treatment over two years in the field, at both the Barriere site and the University of Victoria (Figure 3.2 and 3.3). Hawkins et al. (2005) had similar results with western hemlock, with no significant height differences found between conventional and exponentially loaded treatments after two years in the field. This contrasts with numerous studies of other conifer species that have found positive height and RCD growth responses stemming from nutrient loading (Malik and Timmer 1998, Xu and Timmer 1998, Imo and Timmer 2001, Salifu and Timmer 2003).

Discrepancies in growth response may result from the different amounts of N added in the various experiments. Nitrogen treatments in the studies conducted on black spruce applied 10 mg N per tree to conventionally fertilized seedlings and, on average, 64 mg N per tree to exponential seedlings (Malik and Timmer 1998, Imo and Timmer 2001, Salifu and Timmer 2003). These applications resulted in seedlings containing 1.33% – 1.50% N in the conventional treatment compared to 2.24% - 3.32% N in the exponential treatments (Malik and Timmer 1998, Imo and Timmer 2001, Salifu and Timmer 2003). Seedlings grown with the conventional regime in this experiment received approximately 40 mg N per tree, and the exponential seedlings received approximately 54 mg N per tree. These applications resulted in wpN concentrations of 1.70% and 1.85% for the conventional and exponentially loaded treatments, respectively. Seedlings grown with the conventional regime received substantially more N in my study compared to similar studies on black spruce; likewise, my exponential treatment received slightly less N. This resulted in conventionally grown seedlings with greater wpN concentrations at the time of planting and exponentially loaded seedlings with lower wpN concentrations than was found in the studies with black spruce. Studies that have exhibited significant positive growth response to exponential nutrient loading have been characterized by significantly greater wpN concentrations in exponentially loaded seedlings at the time of planting, unlike my study. Had my study either applied less N in the conventional regime, or applied N at a greater rate in the exponential regime, perhaps we would have achieved greater difference between these treatments. Other nutrient loading trials may have induced nutrient deficiency in the conventional treatments that resulted in poor performance relative to the nutrient sufficient exponential seedlings. This implies it is not the method of fertilizer application in the nursery which has the greatest influence on out-planting performance, but the quantity of nutrients added.

Although our study added relatively high amounts of N to the conventional treatment, a similar study on Douglas-fir found conventional fertilization resulted in improved growth in the nursery compared to exponential fertilization while adding much less N to the system (Burgess 1991). Burgess (1991) found Douglas-fir grown with a conventional regime produced greater growth with 37% less N when compared to seedlings grown with exponential fertilizer regimes. Burgess (1991) postulated that results of exponential nutrient loading may be species specific. As a fast growing conifer species, Douglas-fir may have high nutrient demands. Douglas-fir appears to require nutrients to be added at an exponential rate of at least 6% to show a significant positive growth benefit at the end of the nursery period (Burgess 1991). This high nutrient demand may be the underlying reason for the reduced growth rate in most exponential fertilization treatments. Burgess (1991) postulated that the exponential treatments set the initial level of N supplied to seedlings at too low a level, thereby inhibiting early growth to such a degree that the seedlings never recover.

Seedlings fertilized at planting exhibited greater height and RCD growth than seedlings raised with either the conventional or the exponentially loaded regimes. Increased height and RCD growth of seedlings fertilized at planting have been documented in previous studies (van den Driessche 1988, Brockley 1988, Bowden 1995). Growth immediately following planting may improve with fertilization at planting, however increased competition with surrounding vegetation has been documented (Brockley 1988, Imo and Timmer 1999 and 2001), and was observed in this study. At the time of harvest, the root systems excavated from the seedlings fertilized at planting were often entangled in the roots of surrounding vegetation; a phenomenon that was not observed with the conventional or exponentially loaded treatments. Brockley (1988) found similar results when seedlings were planted with increasing amounts of slow release fertilizer. Overall height growth of the seedlings fertilized at planting was less at the UVic Forest Biology Compound compared to the Barriere field site most likely because of greater competition with adjacent vegetation.

Differences among mean treatment effects were much larger at the UVic Forest Biology Compound when compared to the field site in Barriere. These differences could partly be the result of below-ground competition with adjacent vegetation, the coarse-textured soil conditions, the smaller sample size, or the transfer of seed out of the intended seed zone, all of which occurred at the University of Victoria site. Movement of this seedlot from interior British Columbia to a more southern, low elevation site may have played a role in the growth patterns in our study. Conventional seedlings fertilized at planting and grown for two years at the University of Victoria were shorter in height than those grown in Barriere, but were larger in average RCD. Seedlings in Victoria generally experienced slightly warmer temperatures and a longer growing season, however because seedlings were irrigated regularly, drought should not have been a major factor.

Biomass allocation

Although exponential nutrient loading did not result in increased height growth in the nursery, mean seedling dry mass at the time of planting was greater than in the conventional treatment (Table 3.3). The elevated dry mass of the exponential regime, compared to the conventional regime, was a direct result of greater biomass allocation to the root systems, indicating greater investment in acquiring below-ground resources, such as N in comparison to the above-ground, carbon (C) acquiring components (Malik and Timmer 1998). Root:shoot ratio for exponentially loaded seedlings remained significantly greater (Table 3.4) after two growing seasons at the Barriere field site.

The ability of many plants to switch the allocation of resources between the root and the shoot is one of the most fundamental responses to changes in mineral nutrition (Forde and Lorenzo 2001). The difference in biomass allocation between nursery fertilization treatments is interesting, as optimal partitioning theory states that plants tend to allocate more N to roots to support nutrient uptake under conditions of limited nutrient availability (Reynolds and D'Antonio 1996). Total nutrient availability between the conventional and exponentially grown seedlings was similar, however biomass allocation varied. A greater allocation to roots in exponential nutrient treatments has been found in other studies (Burgess 1991, Timmer 1997), and may reflect a pattern of allocation determined early in seedling growth when nutrients are in low supply. Greater biomass allocation to roots at the time of planting may improve long-term growth and survival by allowing greater nutrient uptake on nutrient deficient sites and increasing the ability of seedlings to withstand drought (Burgess 1991). The shift towards a higher root:shoot ratio in exponentially loaded seedlings may be beneficial over the long term.

N retranslocation

Previous studies have found exponential nutrient loading enhances nutrient retranslocation in seedlings (Salifu and Timmer 2003), however we did not observe similar trends in our study. Two contrasting theories explain the forces driving retranslocation efficiency. The first theory postulates that retranslocation is more efficient in trees growing on infertile soils (Stachurski and Zimka 1975, Miller et al. 1979). The second theory postulates that the efficiency of retranslocation is not determined by soil nutrient availability (Fife and Nambiar 1982, 1984, Chapin and Kedrowki 1983, Birk and Vitousek 1986), but by tree growth rate (Nambiar and Fife 1987). There was a substantially lower amount and proportion of N retranslocated in the exponentially loaded treatment compared to both the conventional and fertilized at planting treatments at both the Barriere and UVic sites. The reduced retranslocation in the exponentially loaded treatment is likely a result of reduced growth sinks of expanding shoots compared to the conventional treatments. This supports the hypothesis of Nambiar and Fife (1987, 1991) that retranslocation is driven by sink strength, or the demand for nutrients from expanding shoots, rather than by soil fertility. Retranslocation may also be driven by the nutrient pool available in the old shoot. No consistent trends occurred in our study to support this, as the exponentially loaded seedlings had the greatest shoot N at planting, however this did not translate to greater N retranslocation.

Frequent measurements at the UVic Forest Biology Compound provided us with the ability to monitor changes in foliar N content over time, thus inferring the retranslocation of nutrients from first year foliage to subsequent year's foliage (Figure 3.6 & 3.7). Magnitude of N movement varied among treatments, however the general pattern of N retranslocation, replenishment and accumulation was consistent. This pattern of N movement between foliage age classes has been documented in the past (Fife and Nambiar 1982, 1984, Nambiar and Fife 1991). By October 2004, the end of the first field season, N content of the first year foliage decreased by 49%, 42%, and 37%, for the conventional, fertilized at planting and exponentially loaded seedlings, respectively. This decrease in N was assumed to be retranslocation. Nitrogen accumulated in this foliage cohort throughout the autumn and winter months, followed by rapid retranslocation in the spring of the second growing season, coinciding with that year's shoot extension. Phases of retranslocation coincide with greater sink strength in the spring and summer when shoot growth is at a maximum, and N accumulation occurs in autumn and winter when growth rates, and sink strength, are very small (Nambiar and Fife 1991, Helmisaari 1992). Chapin and Kedrowski (1983) found that needles were the major sites of nutrient storage over winter, which is supported by the substantial increase in N content from October to April (Figure 3.6 and 3.7).

Fertilization at the time of planting

Fertilization at the time of planting resulted in improved height, RCD, and dry mass in two years of growth following planting at the Barriere field site. After one growing season at the Barriere site, seedlings fertilized at planting had 20.7% and 25.3% more dry mass than the exponentially loaded and the conventionally fertilized seedlings, respectively. Similar results have been found in previous studies (van den Driessche 1988, Brockley 1988, Bowden 1995), yet scepticism still exists surrounding the benefits of this treatment. Increased planting costs, complications with competing vegetation and magnification of drought effects (Brockley 1988) have made fertilization at the time of planting a controversial option in forestry operations. Fertilization at the time of planting requires careful consideration of site conditions before this method should be used. Sites characterized by dry climates or sites that are prone to problems with competing vegetation should not be fertilized at the time of planting (Brockley 1988). Fertilization at the time of planting requires sufficient soil moisture to release and distribute nutrients (Brockley 1988). When warm, dry weather follows planting, there may be a negative impact as the osmotic effect of the high salt concentrations may aggravate already dry soil conditions, and magnify effects of drought for seedlings (Brockley 1988). In addition, competing vegetation has been found to benefit from the added nutrients, thus increasing both above-ground and below-ground competition, as observed in my study (Brockley 1988, Imo and Timmer 1999, 2001). The gains from fertilizing at the time of planting must be weighed against the increased costs of planting and subsequent implications of competing vegetation.

Conclusions

In theory, seedlings grown with exponential nutrient loading are grown at a steady state, achieving constant internal nutrient concentrations. From a physiological perspective, this enhanced nutrient status would be expected to result in seedlings with a more balanced root:shoot ratio, greater growth following out-planting, and increased nutrients for retranslocation. This was not, in fact, the case with Douglas-fir, and our findings are supported by another similar study (Burgess 1991). Explanations for the lack of growth benefits from the exponential treatment may include the following:

(I) Seedlings that are pre-adapted to their environment are known to perform best in the field (Burdett 1983). Exponentially grown seedlings were exposed to high N concentrations at the end of their period in the nursery and then transferred to the field, where lower N concentrations likely existed. Hawkins and Henry (1999) found that seedlings transferred from high N concentrations to lower N concentrations grew less than seedlings transferred between conditions of similar N. Transferring seedlings from conditions of high nutrients to potentially lower nutrients may induce nutrient stress, thereby reducing growth following planting.

(II) At the beginning of the nursery period, exponentially grown seedlings may have been inhibited by low levels of N. Nitrogen concentrations applied to seedlings in the exponential regime were low at the beginning of the nursery phase compared to seedlings raised in the conventional regime. This lower level of N may have negatively impacted the subsequent growth potential of the seedlings, and seedlings may have been unable to recover by the completion of the experiment.

(III) To induce greater levels of luxury consumption in Douglas-fir, exponential nutrient loading may require higher N concentrations than we used in this study. Burgess (1991) found exponential nutrient loading only improved growth in Douglas-fir when applied at a rate of 6% (83 mg N applied per tree), which was substantially more than the 54 mg N per tree added in my study.

(IV) A portion of the nutrients applied during the peak application rates to the exponential treatment may have been lost from the growing medium in the nursery through leaching and rendered unavailable for uptake by the seedlings.

(V) The biomass produced per unit N applied in the nursery was 61% greater in the conventional treatment than in the exponential treatment. Perhaps this greater efficiency of N use resulted in seedlings that were more efficient after planting when nutrients were likely in limited supply.

It may be beneficial to determine the response of Douglas-fir after planting when grown with higher rates of exponential fertilizer application. Comparing the growth response with the cost-effectiveness of nursery practices may provide a clearer picture of the benefits of alternative nursery fertilization regimes. Likewise, it may be useful to determine the benefits of conducting conventional fertilization at higher rates of N addition to determine if greater seedling growth could be achieved in the field. Further studies should also be conducted on the cost effectiveness of fertilization at the time of planting, considering the impacts of competing vegetation on a variety of site conditions.

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CHAPTER 4. SUMMARY AND GENERAL CONCLUSIONS

AMMONIUM AND NITRATE CONTROLLED ENVIRONMENT STUDY

Summary of results

The objective of this study was to identify the optimal N source ratio for Douglas-fir by examining the growth effects of culture in varying ratios of NH_4^+ and NO_3^-. Seedlings were grown in one of six NH_4^+:NO_3^- treatments, and growth and internal N concentrations were compared. The greatest relative growth rate (RGR) and total dry biomass occurred in the 20:80, 40:60 and 60:40 NH_4^+:NO_3^- treatments. On average, the greatest RGR occurred within the 60:40 NH_4^+:NO_3^- treatment, however this treatment was characterized by decreasing internal N concentrations, indicating internal nutrient stress. In all six treatments, a greater proportion of NH_4^+ was taken up compared to NO_3^-, when both ions were available in solution. Seedlings grown with high NO_3^- concentrations had greater overall N uptake, as inferred from the frequency of nutrient additions, compared to seedlings grown in high NH_4^+ concentrations, although this uptake was less efficiently translated to increases in internal N content. High NH_4^+ concentrations (80:20 and 100:0 NH_4^+:NO_3^-) produced seedlings that had reduced growth, reduced biomass, lower internal N concentrations and lower rates of net photosynthesis and root respiration compared to treatments containing relatively more NO_3^-. Results indicate that, for the environmental conditions under which this experiment was performed (pH 4.0 and 20 °C), optimal growth occurs when both NH_4^+:NO_3^- are available in relatively equal proportions in solution.

Ammonium and NO_3^- nutrition influenced plant morphology. Seedlings grown at high NH_4^+ concentrations had longer thinner root systems, with fewer or smaller laterals than seedlings grown at high NO_3^- concentrations which had shorter, but more robust root systems and long lateral root development. Seedlings grown at high NO_3^- concentrations had the greatest root:shoot ratio and the greatest specific leaf area (SLA), while seedlings grown in the 40:60 and 60:40 NH_4^+:NO_3^- treatments had the lowest root:shoot ratio and SLA, respectively.

Conclusions

The reduced growth and decreasing internal N concentrations in the high NH_4^+ treatments (60:40, 80:20 and 100:0) indicates NH_4^+ toxicity was occurring in Douglas-fir at concentrations greater than 214 μM. Maintaining NH_4^+ concentrations below 214 μM in solution under constant-feed conditions should prevent NH_4^+ toxicity from occurring while allowing maximum growth to occur. There did not appear to be effects of NO_3^- toxicity at the maximum concentrations of 363 μM. There appeared to be a synergistic effect when both NH_4^+ and NO_3^- ions were readily available in solution as the greatest wpN concentrations, RGR and biomass occurred in the 20:80, 40:60 and 60:40 treatments. Nutrient solutions containing NO_3^- as the sole N source resulted in sub-optimal growth, indicating a minimal level of NH_4^+ (as low as 71 μM) was beneficial to seedling growth.

The results of this study support the hypothesis of Kronzucker et al. (1997, 2003) that Douglas-fir, as an early successional species, may be poorly adapted to conditions of high NH_4^+ concentrations. Ammonium uptake by Douglas-fir, under laboratory conditions, has been described as excessive and insufficiently regulated, with high levels of uptake and efflux being recorded with corresponding low levels of growth (Kronzucker et al. 2003). My study demonstrated preferential uptake of NH_4^+ when both ions were available in solution, however more rapid short term uptake of NH_4^+ never translated to improved growth or elevated internal N concentrations. Furthermore, symptoms of toxicity such as root damage and chlorosis developed in seedlings raised with high NH_4^+ concentrations. All treatments with high NH_4^+:NO_3^- ratios were marked by internal nutrient stress which diminished when NH_4^+ concentrations decreased.

Recommendations

Recommendations from the NH_4^+ and NO_3^- controlled environment study are as follows:

(I) When raising seedlings with root temperatures of 20 °C and a pH near 4.0, optimal NH_4^+:NO_3^- ratios should be in the range of 50:50. The 60:40 NH_4^+:NO_3^- ratio revealed decreasing internal wpN concentrations, indicating internal nutrient stress. This increase in internal nutrient stress may lead to reduced growth or survival. Reducing the NH_4^+:NO_3^- ratio slightly may produce seedlings with the greatest RGR that maintains constant internal wpN concentrations.

(II) Consideration should be given to temperature and pH when determining optimum N source ratios. When raising seedlings with root temperatures below 20 °C, NO_3^- uptake may become inhibited (Gessler et al. 1998). When raising seedlings at a pH greater than 4.0 (up to pH 5.5) expect NH_4^+ uptake to increase, which may be reflected in elevated susceptibility to NH_4^+ toxicity.

(III) Nursery managers should consider effects on seedling morphology when selecting a nutrient formulation for container grown seedlings. Root morphology was strongly linked to N source ratio. Container grown seedling may grow best with relatively equal proportions of NH_4^+ and NO_3^- as seedlings grown in these ratios typically suffered neither from the more shallow root system characterized by seedlings raised in high NO_3^- concentrations, nor did they suffer from the thin and linear root system of seedlings raised in high NH_4^+ concentrations.

Future research

Conducting a large, all encompassing study examining the growth response of Douglas-fir to varying ratios of NH_4^+ and NO_3^- at a range of pH levels and a range of temperatures could provide valuable information on morphological and physiological response of Douglas-fir to these N sources. Conducting the experiment at a single pH or a single temperature limits the operational use of the results. In addition to studies incorporating pH and temperature, it would be useful to conduct similar studies with mycorrhizae to determine the influence fungal associations have on N uptake. Conducting studies utilizing isotope labelled NH_4^+ and NO_3^- may allow researchers to see, in more detail, which ion is being taken up, and the role each N source plays in growth. Conducting studies for periods longer than 45 days may provide more definitive results for treatments showing marginal signs of nutrient stress, as in the situation of the 60:40 NH_4^+:NO_3^- treatment. Bedell et al. (1999) found that when Douglas-fir was grown in soil conditions, implications of NH_4^+ and NO_3^- nutrition only became obvious after 40 days of treatment.

NUTRIENT LOADING FIELD TRIAL

Summary of results

The objective of this study was to compare the field performance of Douglas-fir seedlings grown with exponential nutrient loading or conventional fertilization in the nursery; and to compare the growth of these seedlings with that of conventionally fertilized seedlings given additional fertilizer at the time of planting. After two years of growth in the field, no significant benefits of exponential nutrient loading were found in terms of growth, biomass or internal N concentrations. Conventionally grown seedlings planted with additional fertilizer at the Barriere field site exhibited the greatest height and RCD growth and the greatest biomass. Seedlings from all treatments contained similar wpN concentrations after two seasons in the field. At the UVic Forest Biology Compound, conventionally grown seedlings, planted either with or without additional fertilizer, performed the best. Nitrogen retranslocation, in terms of N content, was greatest in seedlings raised with the conventional fertilizer regime, both with or without additional fertilizer, compared to seedlings raised with the exponentially loaded regime.

Conclusions

When nutrients were applied at an exponentially increasing rate of 2%, nutrient loading of Douglas-fir did not produce seedlings with greater growth potential, contrary to studies with other species (e.g. Malik and Timmer 1995, Salifu and Timmer 2001, 2003). Alternatively, conventional fertilization produced seedlings with the greatest growth. These differences may be attributed to the nutrient demands of Douglas-fir. Studies that have shown positive benefits of nutrient loading mainly have been conducted on relatively slow growing coniferous species such as black spruce (*Picea mariana*

(Mill.) BSP) that are naturally found on nutrient poor sites (Timmer and Munson 1991, Malik and Timmer 1995, Salifu and Timmer 2001). Douglas-fir is a relatively fast growing species and may have greater nutrient demands. Applying nutrients at a greater exponential rate (e.g. 6%) may result in positive growth benefits as seen in earlier studies (Burgess 1991). To induce significant luxury consumption in Douglas-fir it may be necessary to apply nutrients at a higher rate than we did in this study. The economic, morphological and physiological benefits of applying greater N to seedlings in the nursery would require further analysis.

An alternative explanation of the poor growth response to exponential nutrient loading may lie in the degree of change in nutrient supply between the nursery and the field. Seedlings pre-adapted to the future field environment are thought to perform best in the field (Burdett 1983). When transferred to the field from the nursery, nutrient loaded seedlings that had being receiving elevated levels of N, were subject to conditions where N supply was probably much lower. This transfer from high nutrient conditions to lower nutrient conditions may have induced nutrient stress. Alternatively, conventionally grown seedlings received comparatively lower levels of N in the nursery, and when transferred to the field, may not have suffered from such severe nutrient stress as the exponentially loaded seedlings.

Nursery fertilization regimes altered biomass allocation patterns within the seedlings. Exponential nutrient loading and conventional fertilization altered root:shoot ratios, with nutrient loaded seedlings allocating a greater proportion of biomass to root systems, while conventionally fertilized seedlings, both with or without fertilizer at planting, allocated greater biomass to shoot systems. Greater allocation to root systems may favour the long-term survival of the nutrient loaded seedlings if water deficits occurred in the years immediately following planting (Burgess 1991). Balancing adequate internal nutrient concentrations, as well as root:shoot ratios in the seedlings may be key to developing high quality planting stock.

Conventional fertilization, with the application of additional fertilizer at planting, resulted in significant benefits for growth and biomass at the Barriere field site. Fewer benefits were seen at the UVic Forest Biology Compound. Variations in the benefits of fertilization at the time of planting appear to be linked to site characteristics. Relatively dry sites and sites with a high level of competing vegetation may inhibit seedling growth. The high salt concentrations of the fertilizer packets may accentuate these limitations drawing water away from the seedlings when water is limited, therefore use on dry sites is not recommended (Brockley 1988). Additionally, the below-ground competition from roots of competing vegetation that can arise from the placement of fertilizer packets in close proximity to the root systems of the seedlings appeared to negatively impact growth at the UVic Forest Biology Compound.

Recommendations

Recommendations from the nutrient loading field trial are as follows:

(I) Growers of Douglas-fir seedlings should continue using conventional fertilization regimes in the nursery as exponential nutrient loading in Douglas-fir does not appear to have significant benefits.

(II) The decision to apply fertilizer at the time of planting, in the form of slow release fertilizer packets, should be made on a site-by-site basis. Fertilizer packets at the time of planting should not be used on drought-prone sites or sites that are at a high risk for developing problems with competing vegetation.

(III) Nursery fertilizer regimes influenced seedling morphology. Seedlings raised in the conventional regime had lower root:shoot ratios. Fertilizer regimes that promote an elevated root:shoot ratio to reduce the risk of drought-induced mortality should be used on sites prone to water deficiencies (coarse soils, warm climate, etc).

Future research

Experiments should be conducted to examine the field performance of Douglas-fir seedlings supplied with higher rates of exponential nutrient loading (e.g. 6%). Previous studies have indicated greater growth in the nursery can be achieved (Burgess 1991); however, an understanding of how this growth translates to the field is essential. Similarly, higher rates of conventional fertilization may enhance growth of Douglas-fir and should be examined as another alternative to improving regeneration. Further studies should be conducted to more clearly identify sites in which fertilization at the time of planting is appropriate and to determine the cost-effectiveness of this technique.

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