Seedling Mineral Nutrition, the Root of the Matter

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Abstract: Plants have the marvelous ability to take up inorganic mineral nutrients as atoms or simple molecules and process them into proteins, enzymes, and other organic forms. This paper reviews the 14 essential mineral nutrients, their roles within the plant, their target concentrations in tree seedling nursery culture, and their effects on seedling growth and performance after planting. Three areas of active research in tree nutrition over the past 20 years with relevance to the Pacific Northwest are discussed: steady state nutrition, exponential fertilization and nutrient loading; adaptation to nitrogen form in forest soils and organic nutrient uptake; and variation in nitrogen uptake within species.

Keywords: nutrient targets, nutrient translocation, steady state nutrition, nutrient loading

Essential Mineral Nutrients

Plants are the basis for life on earth, not only because they use energy from the sun to capture carbon dioxide (CO_2) and convert the carbon into carbohydrates, but also because they capture nitrogen (N), phosphorus (P), potassium (K), and other minerals from the soil and convert them into forms that heterotrophs (like us) can use. More than 60 mineral elements have been identified in plants, including gold, silver, lead, mercury, and arsenic, but only 14 elements are considered "essential" in most plants (Raven and others 2005). A formal definition of an essential element was published by Arnon and Stout (1939), and this definition is widely cited to the present day, including in the 1990 Target Seedling Symposium proceedings (Bigg and Schalau 1990). Epstein and Bloom (2005) modified the definition, stating that an element is essential if it fulfills either one or both of two criteria: 1) it is part of a molecule that is an intrinsic component of the structure or metabolism of a plant; or 2) plants deprived of this element exhibit abnormalities in growth, development, or reproduction.

The 14 essential elements make up a small fraction of plant dry mass, but they are truly essential. On average, a tree is 60% to 90% water, depending on the tissue; thus 10% to 40% is dry matter. Of this dry matter, 90% to 96% is carbon from CO_2, oxygen from O_2 and CO_2, and hydrogen from water. These elements are also essential, but they do not come from minerals and so are not considered to be mineral nutrients. The remaining 4% to 10% of plant dry weight is made up of mineral elements. The 14 essential mineral elements are divided into macro- and micronutrients depending on whether their average concentration is greater than or less than 100 ppm (mg/kg) in dry matter, respectively.

The essential mineral elements are presented in Table 1 in descending order by average concentration and range of concentration in crop plant dry matter. Nickel (Ni) is the most recent addition to the list. Its essentiality was not proven until the 1980s (Epstein and Bloom 2005). Ni is required to activate urease, an enzyme found in higher plants, including conifers (Chalot and others 1990; Todd and Gifford 2002). Urease is associated with the conversion of urea to ammonium. It is very difficult to eliminate Ni from experimental nutrient solutions and induce deficiency (Epstein and Bloom 2005). Ni deficiency in the field has been shown only in pecan trees (*Carya illinoensis*) on the Gulf Coast Plain of the US (Wood and others 2003). Although only needed by some plant species, four other mineral elements have been shown to be essential. Silica (Si) is required at a mean tissue concentration of 0.1% to strengthen and protect the epidermis in some algae, horsetails, and grasses.

Table 1. The 14 essential mineral elements for plants, presented in descending order by average concentration and range of concentration in crop plant dry matter (from Epstein and Bloom 2005).

Element	Symbol	Average Concentration	Range of Concentration
Macronutrients (%)			
Nitrogen	N	1.5	0.5-6
Potassium	K	1.0	0.8 -8
Calcium	Ca	0.5	0.51-6
Magnesium	Mg	0.2	0.05-1
Phosphorus	P	0.2	0.15-0.5
Sulphur	S	0.1	0.1-1.5
Micronutrients (ppm)			
Chlorine	Cl	100	10-80,000
Iron	Fe	100	20-600
Manganese	Mn	50	10-600
Boron	Bo	20	0.2-800
Zinc	Zn	20	10-250
Copper	Cu	6	2-50
Molybdenum	Mo	0.1	0.1-10
Nickel	Ni	0.05	0.05-5

Sodium (Na) is required at a mean tissue concentration of 10 ppm in some halophytes (for example, saltlover [*Halogeton glomeratus*]) and in plants with C4 and CAM photosynthesis to regenerate phosphoenolpyruvate. Cobalt (Co) is required (0.1 ppm) for an enzyme complex in N-fixing bacteria associated with some plants, including leguminous trees and alder (*Alnus* spp.). Selenium (Se) may be essential for some plants from Se-rich soils (for example, *Astragalus* spp.).

Mineral nutrients in plants are usually measured as a concentration (percentage or parts per million [ppm] in dry matter). A number of methods exist to measure these elements, but generally the plant material to be analyzed is dried and then combusted or digested to remove the C, H, and O. The ash or digest is then analyzed for the elements of interest by various methods. Results may also be presented as nutrient content. Nutrient content is the total amount (weight in g or mg) of an element in a given amount of plant tissue (for example, 1 g dry matter or 100 needles). Percentage concentration can be calculated from content by dividing the element weight by the sample dry weight and multiplying by 100. It is important to distinguish between content and concentration, as fast-growing seedlings may have low nutrient concentrations due to dilution but may have high nutrient contents due to their large size.

Some of the essential mineral nutrients play many roles in plants, but others play only one role. An exhaustive description of the functions of each element can be found in most plant physiology texts; therefore, only brief descriptions of the major functions of each element are presented in Table 2.

What Are the Targets for Nutrients?

We know that seedlings require essential nutrients, but how do we know they contain sufficient quantities of nutrients? Severe deficiency of a given element results in characteristic deficiency symptoms detailed in most plant physiology texts. Moderate deficiency, however, can limit growth but otherwise not be obvious. Moderate nutrient deficiencies are

Table 2. Major functions of essential mineral elements (adapted from Epstein and Bloom 2005).

Integral in carbon compounds	
N	a constituent of all amino acids and proteins, amides, nucleic acids, nucleotides, and polyamines.
S	a constituent of several amino acids and, thus, proteins, and coenzymes.
Integral in energy acquisition and utilization and in the genome	
P	plays a key role in nucleotides and nucleic acids, and in all metabolites dealing with energy acquisition, storage and utilization—sugar phosphates, adenosine phosphates (AMP, ADP, ATP).
Associated with the cell wall	
Ca	contributes to cell wall stability, but is also important in signaling and regulating some enzymes.
B	contributes to cell wall stability
Integral constituents of enzymes	
Cu	in metalloenzymes
Mn	in superoxide dismutase enzymes, part of the water-splitting complex of photosystem II; also activates some enzymes
Mo	in nitrogenase and nitrate reductase
Ni	in urease
Zn	in metalloenzymes and activates some enzymes
Activate or control enzyme activity	
Cl	part of the water-splitting complex of photosystem II
Fe	activates several enzymes and is also a part of heme proteins, ferredoxin, and Fe-S proteins
K	activates many enzymes and is a major cellular osmoticum
Mg	activates more enzymes than any other other nutrient; part of chlorophyll

thus difficult to diagnose. The classic plant stress response curve can be modified to show response of any growth parameter (for example, height or biomass) to any nutrient (Figure 1). At low internal nutrient concentrations, growth is severely limited; as concentrations rise, growth increases to the critical or optimum point where maximum growth is achieved with minimum nutrient concentration (Figure 1). As nutrient concentrations in the growing medium rise further, the plant has reached its genetically programmed maximum for growth, and so accumulates nutrients in a phase called "luxury consumption". Eventually, nutrient supply and internal nutrient concentrations may become so high as to be toxic, and growth declines (Figure 1).

Depending on the mobility of the element, nutrient deficiency symptoms will manifest differently among plant tissues. Some elements like N, P, and K are easily recycled and retranslocated within the plant. Deficiencies of these mobile nutrients become evident in older foliage, but not until the deficiency is well developed. Immobile elements like Ca and some micronutrients exhibit deficiency symptoms in new tissues, and these symptoms are observed at an earlier stage.

Landis (1985) defined a standard range of target values for mineral nutrient concentrations in needle tissue of conifer container and bareroot nursery stock that are still in use (Table 3). The critical concentration will vary by species, age of tissue, stocktype, and growing medium. Critical concentrations have been defined more narrowly for some species and age classes.

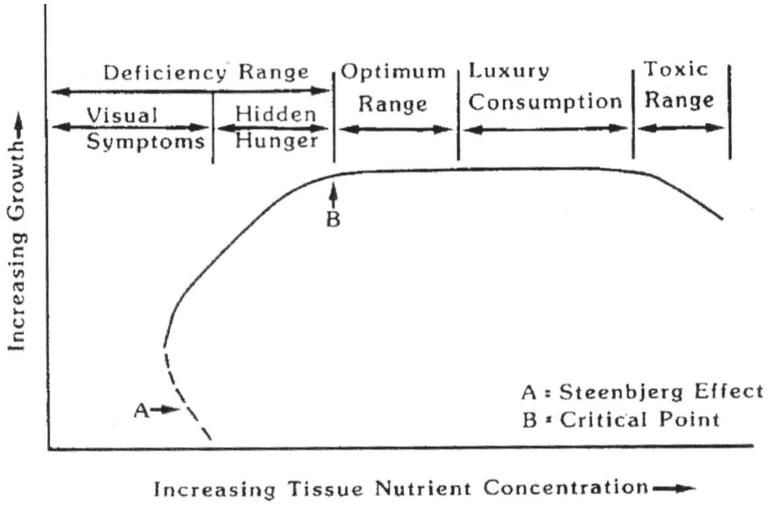

Figure 1. Plant growth curve in response to tissue nutrient concentration (from Landis and others 2005; adapted from Chapman 1967).

Table 3. The standard range of values for mineral nutrient concentrations in conifer needle tissue of container and bareroot nursery stock (Landis 1985), the adequate range for white spruce container stock (van Steenis 2002), and the minimal nutrient concentration in whole seedlings for optimum growth of 10- to 16-week-old Douglas-fir and white spruce (van den Driessche 1989).

	Standard Range		Adequate Range	Minimal Concentrations	
Element	**Container**	**Bareroot**	**White spruce**	**Douglas-fir**	**White spruce**
Macronutrients (%)					
N	1.3-3.5	1.2-2	2.04	2.2	2.46
P	0.2-0.6	0.1-0.2	0.33	0.24	0.39
K	0.7 -2.5	0.3-0.8	1.12	0.89	1.46
Ca	0.3-1.0	0.2-0.5	0.51	0.12	0.2
Mg	0.1-0.3	0.1-0.15	0.15	0.12	0.1
S	0.1-0.2	0.1-0.2	0.14	0.2	0.13
Micronutrients (ppm)					
Fe	40-200	50-100	98	39	50
Mn	100-250	100-5,000	326	35	100
Zn	30-150	10-125	63	30	33
Cu	4-20	4-12	7	11	15
B	20-100	10-100	26	52	46
Mo	0.25-5.0	0.05-0.25	1.4	4	5
Cl	10-3000	10-3000			

Critical concentrations are typically defined for single elements with all other nutrients at optimum concentration. This situation is rarely found in nature, and a limitation in one nutrient may negate the response to increasing concentrations of another element. Thus nutrients should not only be supplied in adequate concentrations, but also in balanced proportions. This concept underlies the diagnosis and recommendation integrated system (DRIS) developed by Beaufils (1957) and reviewed in the 1990 Target Seedling Symposium proceedings (Bigg and Schalau 1990). Ingestad (1970) defined optimum nutrient ratios for birch as N 100: K 50: P 8.4: S 9: Ca 6: Mg 6: Fe 0.7: Mn 0.4: B 0.2: Cu, Zn, Cl 0.03: Mo 0.007. In young Douglas-fir seedlings provided with free access to nutrients in aeroponic culture, Everett (2004) defined similar optimum macronutrient ratios of N 100: K 45: P 15: S 9: Mg 2: Ca 1. The Ca proportion was surprisingly low, but was likely due to the small proportion of cell wall in the young (8-week old) seedlings used in that study.

In British Columbia, most stock is grown in containers, and liquid fertigation is the norm. Target tissue nutrient levels in nurseries are based on experience, and the targets stated by Landis (1985) are well known. Regular monitoring maintains targets (for example, foliar N during active growth is 2% to 3% of dry weight) (van Steenis 2002). In Washington and Oregon, more seedlings are outplanted as transplants or bareroot stock. Pre-plant fertilizers are incorporated prior to sowing or transplanting, followed by liquid fertilization. Target nutrient concentrations are often those defined by Landis (1985). Some growers have a target growth curve for each stocktype and species, and nutrients are adjusted to keep the seedlings on the target growth trajectory. All growers surveyed use different fertilizer regimes for different species and stocktypes. Most start the season with high levels of N, decrease N supply over the growing season, and monitor foliar nutrient concentrations frequently (biweekly to monthly). More complex methods of analysis, including vector analysis (Timmer and Armstrong 1987), and DRIS as described by Bigg and Schalau (1990), are not widely used.

Why Do Nutrient Targets Matter? Effects of Nutrients After Outplanting

Shoot-to-root Ratio—Optimum nutrient content and balance will result in optimal physiological functioning and growth in the nursery, and will have a major influence on survival and growth of planting stock once it leaves the nursery. Shoot-to-root ratio is determined, to a large degree, by fertilization in the nursery. High N fertilization, especially with ammonium, can result in excess shoot growth relative to root growth. A greater proportion of nitrate encourages lateral branching of roots (Everett and others 2010). The shoot-to-root ratio can affect seedling survival after outplanting, particularly on dry sites. A large, transpiring shoot may create an unsustainable demand for water on a small root system.

Nutrient Retranslocation—Since N, P, and K are mobile nutrients within the plant, and root establishment may be slow after outplanting, high nutrient content in seedling shoots will allow retranslocation of mobile nutrients to support new growth before uptake begins. In young conifers, 32% to 40% of N used in new leaf growth is remobilized N from older tissues, mostly older needles (Millard 1996), but seedlings need to accumulate nutrient reserves before retranslocation can support new growth. Nutrient loading (see below) promotes accumulation of nutrients for retranslocation, and remobilization of N in black spruce (*Picea mariana*) has been increased 569% by nutrient loading (Salifu and Timmer 2001). Retranslocation rates have been correlated with shoot growth rate in radiata pine (*Pinus radiata*), and in this fast-growing species, retranslocation has been observed from needles as young as 2 months old (Nambiar and Fife 1991). Slow-growing, shade-tolerant species may conserve nutrients in older needles for a longer time to maintain functioning of these leaves (Hawkins and others 1998). In young seedlings of Douglas-fir (*Pseudotsuga menziesii*) and Pacific silver fir (*Abies amabilis*), retranslocation was not observed from older needles until the third growing season (Hawkins and others 1998). These results support the suggestion of Munson and others (1995) that retranslocation efficiency is species-specific and is related to both plant growth potential and short-term imbalances in nutrient supply. Under conditions of low nutrient availability, retranslocated N from stems and roots of deciduous species can make up almost 100% of the N found in new leaves (Salifu and others 2008).

Mycorrhizae—High levels of fertilizer in the nursery can reduce root colonization by mycorrhizae or alter the species of fungal partner. In a meta-analysis of 31 studies involving N fertilization and 20 studies of P fertilization, mycorrhizal abundance decreased 15% under N fertilization and 32% under P fertilization, on average (Treseder 2004). Decreased mycorrhizal colonization may have negative effects on performance after outplanting, depending on the tree species and planting environment (for example, Teste and others 2004; Ósakarsson 2010).

Storage Molds—When seedlings are in cold storage over winter, the environment is moist and encourages the growth of *Botrytis* spp. and storage molds. If foliar N is high, seedling susceptibility to storage mold is increased. van Steenis (2002) suggests that foliar N concentration should be less than 2% in stored seedlings to reduce the development of storage molds.

Cold Hardiness—Many studies have investigated the influence of various mineral elements on cold hardiness of tree seedlings. In general, nutrient deficient plants are less cold hardy than nutrient sufficient plants (Bigras and others 2001); fertilization, however, has also been associated with fall frost damage or early release of dormancy, flushing, and increased risk of frost damage the following spring (Colombo and others 2001). Individual mineral nutrients can have different effects on cold hardiness. K fertilization has been shown to increase frost hardiness in a number of conifer species (Bigras and others 2001; Colombo and others 2001). The effects of N fertilizer on cold hardiness, however, may be positive, negative, or none because results depend on the timing and amount of N application. Fertilizing with high levels of N in the mid to late growing season may delay bud induction, favor continued growth in indeterminate species (for example, western hemlock [*Tsuga heterophylla*] and western redcedar [*Thuja plicata*]), or lead to lammas growth in determinate species (for example, Douglas-fir).

Prolonged growth can have a negative effect on seedling cold hardiness in the fall and winter (Hawkins and others 1995). High N applied in early fall during the hardening phase, however, can increase winter hardiness. In field-grown trees, foliar N has been shown to be positively correlated with frost hardiness in September (Hawkins and Stoehr 2009).

Herbivory—Nursery fertilization can influence the susceptibility of seedlings to herbivory by mammals or insects after outplanting. Plants with high levels of N (which often correlates with lower levels of tannins or phenolics) have been shown to be more damaged by browsing in some studies (for example, Close and others 2004).

Developments Since the 1990 Target Seedling Symposium

The heyday of applied tree seedling nutrition research in the Pacific Northwest could be considered to be the 1970s to 1990s. The successful extension of research results over this period, and the facilitated communication between nursery managers and scientists, led to improved nursery practices, seedling health, and, ultimately, seedling survival. In British Columbia, seedling survival rates in plantations increased from 54% in 1982 to 87% in 1990 (Brown 1993). During the past 20 years, there has been increasing interest in ecophysiological questions in tree nutrition and the interactions between soil processes and tree nutrient uptake. In the following section, three active areas of research in tree nutrition will be discussed.

Steady State Nutrition and Nutrient Loading: Growing Regimes to Mimic Natural Growth and Optimize Nutrient Uptake

The techniques of steady state nutrition, exponential nutrition, and nutrient loading originated in the seedling nutrition work of Torsten Ingestad at the Swedish University of Agricultural Sciences in the 1970s and 1980s. Ingestad aimed to develop growing regimes that would induce seedlings to grow in natural developmental patterns and optimize nutrient uptake (Ingestad and Lund 1986; Ingestad 1987). Theoretically, unshaded tree seedlings grow at an exponential rate. The more branches or roots there are, the more lateral branches and roots will be formed. Exponential root growth allows seedlings to exploit an ever-increasing volume of soil, thus an ever-increasing pool of nutrients. As the plant increases in size, the root area over which nutrient uptake occurs increases accordingly, and nutrient concentrations remain at a steady state within the plant.

Seedlings in containers or nursery beds are not able to develop a naturally spreading root system. According to Ingestad, the optimum fertilization method should mimic natural exponential growth by adding nutrients at an exponentially increasing rate, matching plant relative growth rate. Thus low amounts of fertilizer are added when seedling root systems are small and unable to take up large quantities of nutrients, then fertilizer application is increased exponentially as seedlings increase in size. The proof of optimum nutrition is constant internal plant nutrient concentrations over time, called steady state nutrition. This contrasts with the declining internal concentrations observed when plants increase in size but nutrient application remains constant. Steady state nutrition is very difficult to achieve when seedlings are grown in pots in nutritional studies, and results of these studies have been criticized based on the episodic nature of nutrient application.

The concept of exponential nutrition can be extended to exponential nutrient loading, where nutrients are supplied at an exponentially increasing rate exceeding seedling growth rate. Extra nutrients are stored in the seedling for retranslocation after outplanting. There is evidence that seedlings can accumulate greater stores of nutrients with exponential compared to constant-rate nutrient loading (Timmer 1997).

The concepts of exponential fertilization and nutrient loading were tested in Ontario in the mid-late 1990s and early 2000s by Dr Vic Timmer at the University of Toronto and his students and colleagues. Results of many experiments with black spruce, white spruce (*Picea glauca*), red pine (*Pinus resinosa*), larch (*Larix occidentalis*), *Eucalyptus* spp., and China fir (*Cunninghamia lanceolata*) were published, showing greater growth, nutrient uptake, and mycorrhizal colonization after outplanting, particularly on nutrient-deficient sites (reviewed in Hawkins and others 2005). Timmer (1997) has also shown improved nutrient retranslocation and reduced planting shock in exponentially fertilized trees.

Two experiments testing the exponential nutrition and nutrient loading concepts have been conducted with species from the Pacific Northwest, western hemlock and Douglas-fir. Hawkins and others (2005) compared conventional versus exponential nutrition in western hemlock. Seedlings were grown in Styroblock™ 410A containers (80 cm^3 [4.9 in^3]) and were fertilized twice per week over the growing season with 20N:20P_2O_5:20K_2O all-purpose fertilizer applied in three treatments: constant rate (100 mg N/L, total 83 mg N/seedling), 2% per day exponential to a maximum of 250 mg N/L (total 134 mg N/seedling), and 3% per day exponential to a maximum of 559 mg N/L (total 236 mg N/seedling). Seedlings from each nursery treatment were outplanted the following spring after cold storage with or without a 9-g (0.3-oz) 26N:12P_2O_5:6K_2O Silva Pak slow release fertilizer package (Reforestation Technologies International, Salinas, CA).

At the end of the summer season in the greenhouse (1 September), the 3% exponential treatment increased N concentration in all plant parts by approximately 10% compared to the constant rate treatment. By December, there were no significant differences in N concentration among treatments due to continued growth after fertilization ended in early September. One year after outplanting, N concentrations were highest in seedlings fertilized at outplanting followed by 3% exponential seedlings; after that time, foliar N concentrations did not differ significantly among nursery or fertilized-at-outplanting treatments (Hawkins and others 2005).

At the time of lifting in December, there was no significant effect of exponential versus conventional fertilization treatment on height, biomass, or root-to-shoot ratio. As well, there was no significant effect of treatment on duration of shoot growth. Three years after outplanting, greenhouse treatment had no significant effect on height, but root-collar diameter and height increments were 10% greater in the

3% seedlings compared to conventional seedlings. This was supported by results of a pot experiment, where seedlings from the three nursery treatments were planted in pots with 10 or 100 mg/L N supply and grown for a second summer. In the pot experiment, biomass of new roots was increased by exponential fertilization. Seedlings fertilized at the time of outplanting had greater height, height increment, and root-collar diameter (RCD) 3 years after outplanting than those not fertilized at outplanting. The effects of post-outplanting fertilization outweighed any effects of nursery fertilization (Hawkins and others 2005) (Figure 2).

The study with relatively fast-growing, indeterminate western hemlock did not show the same degree of positive response to exponential fertilization seen in studies with other species. Any gains in N concentration and RCD due to exponential fertilization were in the order of 10% (reviewed in Hawkins and others 2005), whereas gains in height or RCD from fertilization at outplanting were in the range of 15% to 20%. A likely reason for the limited relative response to exponential fertilization treatments in western hemlock is the high rates of fertilizer applied in all treatments. Large seedlings with high growth rates call for high rates of fertilization, so even constant-rate seedlings received high levels of N. In many studies with slower growing trees, the maximum N application rate in constant-rate trees is 40 mg N, and may be as low as 10 mg N (reviewed in Hawkins and others 2005). Seedlings from British Columbia nurseries typically get 80 to 125 mg N per seedling and have 2% to 2.6% foliar N, so conventionally fertilized stock performs well.

The study with western hemlock showed constant-rate fertilization can produce seedlings that are equal to exponentially fertilized seedlings if the rate of N application is high. The next question is then, "Can exponential fertilization produce seedlings of similar quality to conventionally fertilized seedlings using similar quantities of N?" To address this question, an experiment was conducted with interior Douglas-fir, a species that does not have the complication of semi-indeterminate growth seen in hemlock.

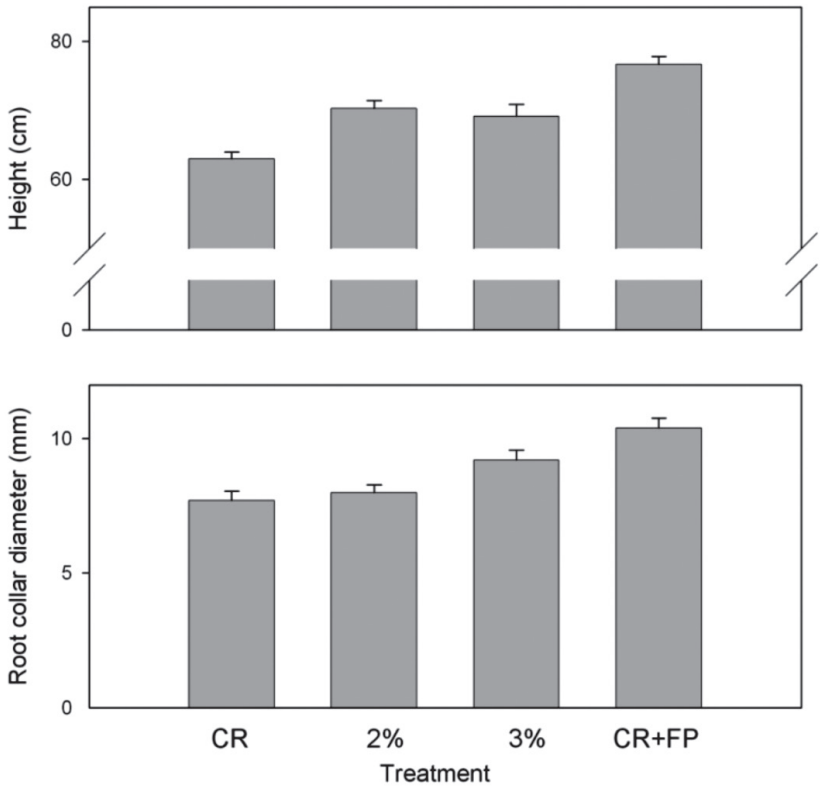

Figure 2. Mean (± S.E.) height and root-collar diameter of western hemlock seedlings three growing seasons after outplanting in the field. Seedlings were grown with one of three nursery fertilization treatments (constant rate, 2% exponential, 3% exponential) and outplanted with (+FP) or without slow-release fertilizer. Treatments indicated in the figure are: constant rate seedlings outplanted without fertilizer (CR); 2% exponential seedlings outplanted without fertilizer (2%); 3% exponential seedlings outplanted without fertilizer (3%); and constant rate seedlings outplanted with fertilizer (CR+FP) (adapted from Hawkins and others 2005).

Everett and others (2007) compared conventional versus exponential fertilization in interior Douglas-fir grown in Styroblock™ 412 A containers (125 cm^3 [7.6 in^3]) in an operational nursery. Two fertilization treatments were applied. Fertilizer was applied once per week in a 19N:4P_2O_5:15K_2O formulation at a constant rate (100 or 150 mg N/L, total 40 mg N/seedling) or 2% per day exponential to a maximum of 403 mg N/L (total 54 mg N/seedling). Seedlings were lifted and cold stored and outplanted the following spring. Seedlings from the conventional treatment, only, were outplanted with or without a 10-g (0.4-oz) 16N:8P_2O_5:8K_2O Planter's Pak of slow-release fertilizer.

Twenty-five percent more N was applied to exponentially fertilized seedlings; at outplanting, however, the exponential seedlings had only slightly greater mean foliar N concentration than conventionally fertilized seedlings (1.85% versus 1.7% N; Figure 3). Exponentially fertilized seedlings had smaller dry mass but greater root-to-shoot ratio than constant-rate treatment seedlings at outplanting. After 2 years in the field, exponential fertilization did not confer any significant benefits to Douglas-fir seedlings; these seedlings, however, still had the greatest root-to-shoot ratio. Seedlings fertilized at outplanting had greater dry mass and height than those not fertilized at outplanting (Everett and others 2007) (Figure 3).

Both studies with relatively fast-growing conifer species from the Pacific Northwest reached the conclusion that exponential fertilization did not confer any dramatic benefits over adequate constant-rate fertilization, and that fertilizer at outplanting outweighed any differences in nursery fertilization treatment. Everett and others (2007) suggested that fast-growing species may be unable to take advantage of exponential fertilization over a whole growing season because they are self-shading and accumulate more non-photosynthetic biomass. There may also be a danger of setting the initial level of N supply at too low a level, inhibiting early growth to such an extent that the seedlings never catch up. The combined results suggest that it is not the method of fertilizer application in the nursery that has the greatest influence on outplanting success, but rather the quantity of nutrients in the seedlings.

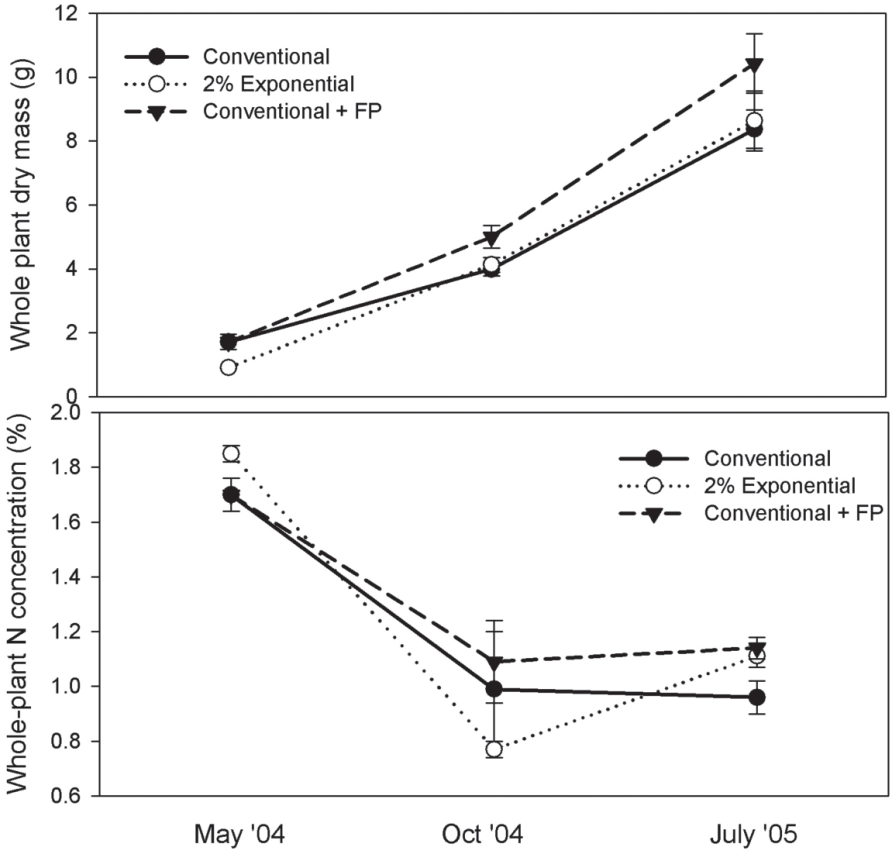

Figure 3. Mean (± S.E) whole plant dry mass and N concentration of Douglas-fir seedlings at the time of outplanting (May 2004), and 5 and 14 months after outplanting in the field. Seedlings were grown with one of two nursery fertilization treatments (conventional; 2% exponential) and outplanted with (+FP) or without slow-release fertilizer. Treatments indicated in the figure are: conventional rate seedlings outplanted without fertilizer (Conventional); 2% exponential seedlings outplanted without fertilizer (2% Exponential); and conventional seedlings outplanted with fertilizer (Conventional+FP) (adapted from Everett and others 2007).

Adaptation to N Form

Plants take up N as ammonium, nitrate, and in organic form; in forest soils, however, these N sources are generally found in low concentrations. In a study on soils near Jordan River, British Columbia, a maximum of 4.5 µmol nitrate, 5 µmol ammonium, and 8 µmol amino acid-N per g soil was measured in mid-summer (Metcalfe 2008). The fact that plants can take up organic forms of nitrogen, primarily amino acids or small peptides, has been known for over 60 years. It was not appreciated until the last 20 years, however, that amino acids can comprise a substantial proportion of the N absorbed by forest plants, particularly in cold soils. This work has been mainly led by scientists working in the boreal environments of Sweden and Alaska (reviewed in Näsholm and others 2009). Plants that take up organic N can bypass the N mineralization step in decomposition and compete with soil microbes for access to soil N. Advantages of organic N uptake for plants include a competitive advantage over other plants through earlier access to N released by decomposition. Theoretically, organic N uptake may also be metabolically "cheaper," as the first step in assimilation of inorganic N into organic compounds is bypassed.

Based on the evidence of organic N uptake in natural environments, researchers in Sweden have developed an organic N fertilizer called arGrow based on the amino acid arginine. Arginine is positively charged, and therefore binds to soil cation exchange sites, reducing N runoff compared to negatively charged nitrate. Results of trials comparing arGrow to ammonium nitrate fertilizer indicate that seedlings grown with arGrow have better root systems with more fine roots, larger stem diameter, and higher foliar N levels (Ohlund and Näsholm 2002). These improvements have resulted in 40% greater volume after 7 years in the field in trees grown with arGrow compared to trees grown with ammonium nitrate in the nursery. Holmen Skog (Gideå and Friggesund, Sweden) is fertilizing more than half their seedlings (>15 million) with arGrow, and other Swedish forest companies are evaluating its use (Confederation of Swedish Enterprise 2010).

Soil temperature and pH influence the form of N in the soil. Cold, acid soils have a greater proportion of N as ammonia, whereas warm, neutral soils have more N as nitrate. In warmer soils where the N mineralization cycle is more rapid, plants take up more N in inorganic form. Disturbed soils have a relatively high proportion of nitrate due to higher rates of decomposition. Agricultural soils have much higher proportions of nitrate than forest soils. There is growing evidence during the past 15 years that some plants have adapted to preferentially take up the N form most common in their environment.

Kronzucker and others (1997) measured uptake of radiotracer ^{13}N-labelled ammonium and nitrate by white spruce. They found that uptake of ammonium was up to 20 times greater than uptake of nitrate, and that assimilation of ammonium was more efficient than that of nitrate. Preference for ammonium had been observed before in conifers, but Kronzucker and others (1997) went on to attribute the failure of conifer plantations on disturbed sites, in part, to the lower ammonium-to-nitrate ratio on these sites relative to undisturbed forest soils. This interpretation of the results was hotly debated, but the Glass and Kronzucker labs continued to publish a volume of excellent work on ammonium and nitrate uptake in trees. They have provided substantial evidence that late-successional species, such as white spruce, have a "preference" for ammonium, while faster-growing early successional species, such as Douglas-fir and trembling aspen (*Populus tremuloides*), have higher rates of nitrate uptake and may exhibit futile cycling of ammonium from root cells (for example, Min and others 2000; Kronzucker and others 2003; Britto and Kronzucker 2006). Work with microelectrodes has also shown greater nitrate than ammonium net uptake along the roots of Douglas-fir and lodgepole pine (*Pinus contorta*) (Hawkins and others 2008) (Figure 4); most whole seedling studies, however, show best growth with a mixture of ammonium and nitrate. Everett and others (2010) showed that at pH 4, Douglas-fir seedlings grew best and had stable internal N concentrations with an NH_4:NO_3 ratio of 40:60 or 20:80.

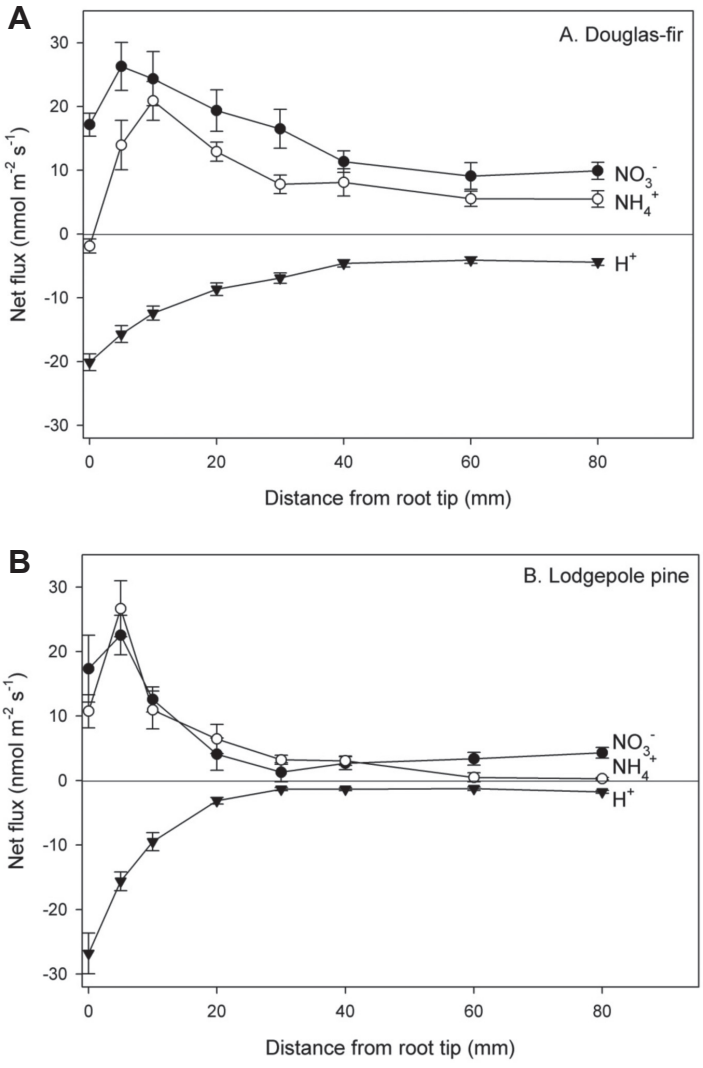

Figure 4. Mean net fluxes of NO_3^-, NH_4^+, and H^+ (nmol m^{-2} s^{-1}) (± S.E.) at various distances from the primary root tip of Douglas-fir (A) and lodgepole pine seedlings (B). Negative flux values indicate efflux (adapted from Hawkins and others 2008).

Variation in N Uptake within Species

Genetic variation exists among species in nutritional characteristics such as nutrient uptake and utilization, but variation also exists within species in N-uptake efficiency and N-use efficiency. A number of studies published in the past 20 years have looked for genetic variation in the efficiency of nutrient uptake and have asked the question, do fast-growing families have higher rates of nutrient uptake or greater nutrient uptake efficiency than slow-growing families?

In studies with Douglas-fir and interior spruce (*Picea glauca* (Moench) Voss X *Picea engelmannii* Parry ex. Engelm.) grown in containers or in aeroponic culture, fast-growing families were shown to exhibit greater plasticity in biomass allocation to roots versus shoots in response to N availability than slow-growing families. Most plants respond to a high N supply by increasing biomass allocation to shoots, whether this is due to an accelerated growth trajectory (for example, Coleman and others 2004) or a true allometric shift. Fast-growing families of Douglas-fir (Hawkins 2007) and interior spruce (Miller and Hawkins 2003) grown with a high N supply allocate proportionally more biomass to shoots, but more to roots at low N supply than slow-growing families. Fast-growing seedlings also have greater rates of N uptake, greater N productivity (more biomass produced per unit of N supply), and higher N utilization indices (more biomass produced per unit of plant N concentration) than slow-growing seedlings (Figure 5).

An examination of N uptake rates by roots of fast- and slow-growing interior spruce families using N depletion measured with macroelectrodes showed that fast-growing families had greater rates of NH_4 uptake, particularly at high NH_4 concentration, and greater rates of NO_3 uptake, on average (Figure 6) (Miller and Hawkins 2007). In Douglas-fir roots, mean family net influx of ammonium (NH_4^+), measured with microelectrodes in high- and low-nutrient treatments, was significantly correlated with measures of mean family biomass (Hawkins 2007). These results indicate that efficient nutrient uptake and utilization contributes to higher growth rates of trees.

Further afield, a study examining variation in nutrient concentration and growth in six clones of radiata pine (*Pinus radiata*) planted across New Zealand on a range of site qualities showed some clones differed significantly in their nutritional characteristics (Hawkins and others 2010). Clones with consistently high N or P uptake across a range of sites were identified. These results suggest that selection of families or clones with efficient nutrient uptake or nutrient use should be considered for inclusion in tree breeding programs.

With the rapidly developing genomics resources for some tree species, the ability will come to select or even create genotypes with particular characteristics. Work is under way to identify and characterize nitrate and ammonium transporters, as well as genes for enzymes involved in nitrogen assimilation in trees. Potentially, genes that result in high rates of N uptake or assimilation could be identified. Genotypes with these genes could then be selected or the genes inserted into individuals with desirable growth rates or wood characteristics. Public resistance to genetically modified organisms may be less for non-food crops, particularly if these plants can be made sterile. These technologies are being employed in Sweden, China, and other countries where transgenic trees are being created that grow more quickly or have greater fiber length, more biomass, greater drought or salt tolerance, higher energy content, or improved bioenergy properties.

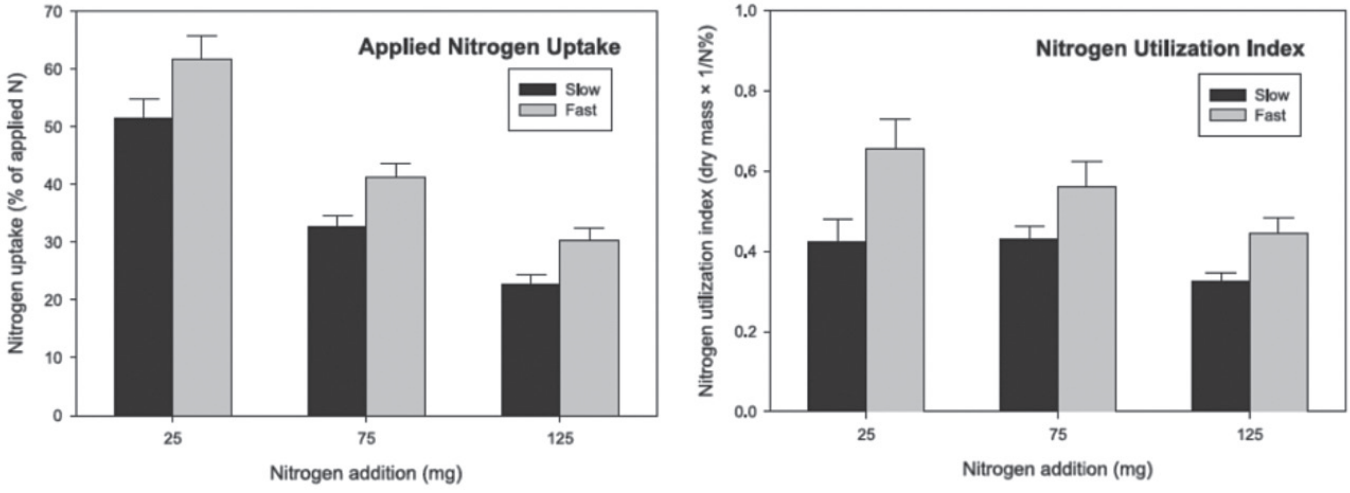

Figure 5. Mean (± S.E.) applied nitrogen uptake (percentage of the applied nitrogen taken up) and nitrogen utilization index (dry mass produced per unit plant N concentration) of slow- and fast-growing families of interior spruce in three fertility treatments after 175 days (adapted from Miller and Hawkins 2003).

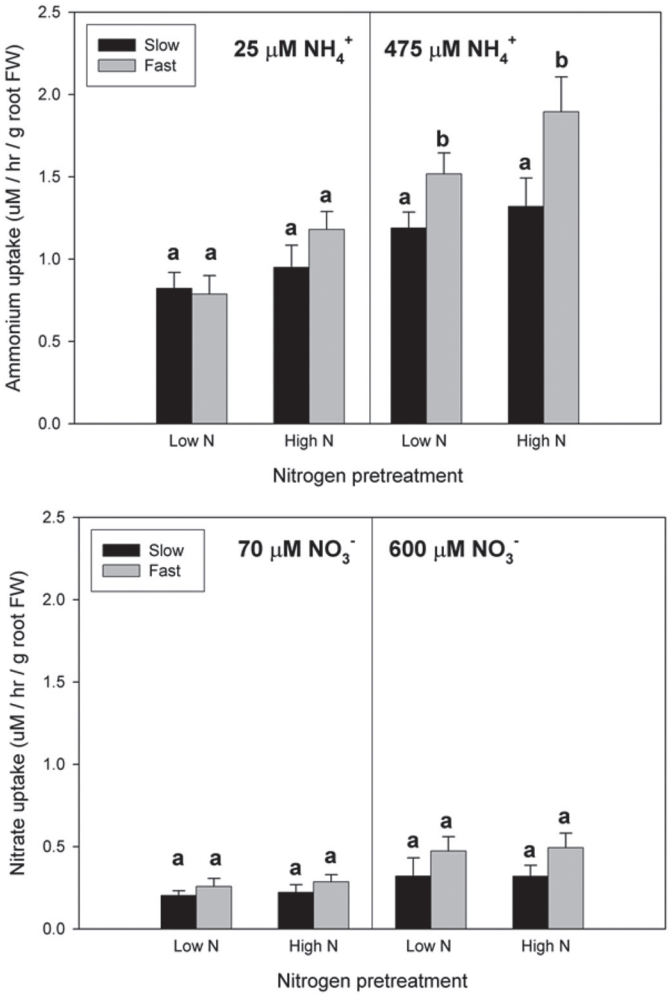

Figure 6. Mean (± S.E.) short-term ammonium and nitrate uptake rates of slow- and fast-growing families of interior spruce measured at two concentrations of each ion. Bars surmounted by the same letter indicate no significant difference in the means within each concentration of each ion (from Miller and Hawkins 2007).

Conclusion

Forest renewal management techniques in the Pacific Northwest have improved dramatically during the past 30 years. In British Columbia, the greatly improved seedling survival rates have been attributed to a greater diversity of stocktypes (including more container stock), increased seedling vigor (based on improved nursery techniques, including nutrition), improved stock handling, and more site preparation and brushing (Brown 1993). A major factor driving these changes was increased funding for research (Brown 1993). When a good measure of success has been achieved, it is easy to become complacent and to forget the effort and investment that enabled this achievement; but there is still much to be done in forest research to continue to build on the successes of the past. In tree nutrition, we need to learn more about growing regimes to optimize the performance of valuable, genetically improved stock. With more focus on restoration, we need to understand the nutrition of a greater diversity of tree species. There is still much work required to understand the relationships between tree species and their mycorrhizal partners, both in the nursery and in the field. The interaction of nutrition and cold hardiness, disease resistance, and browsing are also areas where more research is needed to maximize survival after outplanting. Overarching all of these challenges is the very real possibility of climate change that may have great impact on long-lived forest trees. Now, more than ever, we need research and sound science to make the best decisions for the future.

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