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Production and outplanting success of nutrient-loaded aspen seedlings

by

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Abstract

Quality aspen (*Populus tremuloides* Michx.) nursery seedlings are difficult to produce. In this thesis I explored methods to improve the nutrient status (loading) of aspen seedlings. Tissue nutrient concentrations doubled in seedlings treated with a shoot growth inhibitor (SGI) compared to exponential fertilization which resulted in poor quality (small) seedlings with lower nutrients. Nutrient loaded and standard feed SGI seedlings were outplanted on a reclamation site in two capping materials (peat-mineral-mix (PMM) and salvaged forest floor). Superimposed were also four different fertilizer regimes. Loaded aspen seedlings always outperformed standard seedlings, grew better on PMM capping material, and a controlled release fertilizer had a longer lasting effect on seedling growth than the immediate release fertilizer. These differences continued in the second growing season. SGI treatment allows for the production of nutrient loaded aspen seedlings, produces uniform and quality seedling stock, and can reduce broadcast fertilization needs on reclamation sites.

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Chapter 1: Introduction

1.1 Forest reclamation

Resource extraction plays an integral role in the economy and psyche of Alberta, Canada as evidenced by myriad factors including the name of Edmonton, Alberta's professional hockey team: The Oilers. In the Oil Sands region of northern Alberta, open-pit surface mining results in landscape-scale disturbance of the boreal forest. Approximately 710 square kilometers have been disturbed by oil sands surface mining to date and reclamation efforts have begun on a tenth of that area (Government of Alberta 2013). Up to 4,800 km^2 could eventually undergo surface mining for oil sands extraction. Albertan environmental law requires mined areas to be reclaimed to "self-sustaining ecosystems" of "equivalent land capability." The ultimate goal of forest reclamation is to restore disturbed sites to self-sustaining ecosystems composed of native tree and understory species. The reclamation strategies employed on such sites are of great interest as they influence the trajectory of ecosystem recovery (Rayfield et al 2005; Macdonald et al. 2012).

The Oil Sands region of northern Alberta extends north of the town of Fort McMurray, Alberta (56° 43' N, 111° 23' W) and is located within the Central Mixedwood subregion of the Boreal Forest Natural Region (Natural Regions Committee 2006). As the largest Natural Subregion in Alberta, the Central Mixedwoods are defined by undulating planes of upland forests and wetlands that experience long, cold winters and warm, short summers. Within the Subregion, upland sites are characterized by mosaics of aspen (*Populus tremuloides*), mixedwood, and white spruce (*Picea glauca*) forests on medium to fine textured gray Luvisolic soils while jack pine (*Pinus banksiana*) forests form on coarse textured Brunisols. Low lying areas within the region are dominated by black spruce (*Picea mariana*) fens on Organic soils. Climate averages taken from 1971-2000 show that average daily temperatures range from -18.8 °C in January to 16.8 °C in July with an annual average of 0.7°C (Environment Canada 2013). The growing season in the area is from April to September with an average temperature of 11.7°C and 342.2 mm of rainfall. From October to March the average temperature is -10.2°C and an average of 155.8 mm of precipitation fall as snow (Environment Canada 2013).

Surface mining involves the removal of overburden (including rock, soil, and the vegetation they support) to expose the resource of interest. Depending on the location and character of the material being extracted, surface mining can be undertaken by strip mining, open-pit mining, or mountaintop removal. The nature of the Athabasca Oil Sands deposit requires that open-pit mining methods be used where surface mining is undertaken, resulting in pits several kilometers wide and up to 100 m deep. In preparation for mining, trees are removed from the site, followed by the removal of topsoil (the A and B soil horizons (depth to ~30 cm)), suitable subsoil (depth to 3m), and any remaining material (overburden) overlying the resource of interest. Topsoil is removed and stored separately from subsoil in "stockpiles" before being used as capping material for reclamation sites while overburden is used for construction of structures like dams or reclaimed as hill structures (overburden dumps). While draglines and bucket wheels were once used for overburden removal and oil sands extraction in northern Alberta, trucks and shovels, which allow for more selective mining, are now utilized for these tasks (George 1998). Currently, open-pit surface mines recover oil sands deposits up to 75 m below the undisturbed ground level (Government of Alberta 2013).

However, topsoil contains a valuable seedbank, mycorrhizal propagules, and microbes that can play an invaluable role in reclamation and many of the benefits topsoil can supply to a reclaimed site decrease over time when it is stored in stockpiles (Iverson and Wali 1982; Visser et al 1984; Miller et al. 1985; McKenzie and Naeth 2007). In order to take advantage of these soil services, the direct transfer of topsoil without stockpiling is increasingly being used in reclamation. Direct transfer involves removing topsoil from a site where mining is beginning and moving to a reclamation site where overburden has already been constructed into a landform awaiting topsoil without storing the topsoil.

Promising results of the effects of direct transfer on ecosystem recovery after surface mining suggest that this method will become more common in the future (Iverson and Wali 1981; Koch et al. 1996; Bakker et al. 1996; Ross et al 2000; Holmes 2001).

When reclaiming former surface mining sites, the first step involves the placement of the overburden material. This material forms the landform to be reclaimed. "Landforming" is a critical step in reclamation that affects the aspect, elevation and hydrology of the reclaimed area (Schor and Gray 2006). When shale overburden and compressed sand are removed from oil sands mine sites they become fragmented and increase in volume (Stolte et al. 2000). Saline shale overburden is also prone to weathering, slumping and erosion. Accordingly, the overburden materials are capped with at least 80 cm of subsoil suitable for plant roots (e.g. nonsaline, and appropriate pH) (Rowland et al. 2009). The last soil component to be added to a reclamation area is the capping material which is composed of salvaged topsoils. Depending on the original forest type of the donor site the soil was salvaged from, topsoil can be dominated by peat materials (from lowland forests and peatlands) or by upland forest surface soils. These materials are used to increase soil water-holding capacity and nutrient availability. Depending on their origin the capping materials are called peat-mineral mix (PMM) and forest floor material (FFM). Peat-mineral mix refers to the peat, which is readily available in the footprint of oil sands mines, that is often mixed with underlying mineral subsoil during salvage. FFM includes a mixture of organic forest floor material and underlying mineral soil (mostly A and B horizons). Reclamation prescriptions vary in the type and depth of capping material application with some reclamation sites in the area containing no organic capping material, whatsoever. Capping material is often applied at depths of 10 to 50 cm (Lanoue 2003).

Revegetation efforts follow soil reconstruction. Non-native, agricultural cover crops, such as barley (*Hordeum vulgare* L.), were once planted widely within the oil sands region to provide erosion control and quick vegetation cover

(Rowland et al 2009). However, an increasing emphasis on the use of native species has been placed on restoration efforts in Alberta. Revegetation efforts now largely include planting native tree seedlings (mostly white spruce and aspen) and encouraging forest floor understory development (increasingly by planting shrub seedlings.)

Generations of scientists have dedicated their efforts to the production of tree seedlings with optimum outplanting performance. Seedling quality is uniquely tested in the context of reclamation where harsh conditions such as soil fertility, pH, salinity, contamination and compaction; competing vegetation; and lack of protection from animal browse and harsh weather events can limit tree seedling outplanting success and growth (Bussler et al 1984; Andersen et al. 1989; Cassleman et al 2006). Natural resource managers seek to convert highly disturbed lands (such as former mining sites) to self-sustaining forests through multi-step processes that often include planting nursery-grown seedlings. These seedlings must survive on harsh sites where the entire soil profile has been reconstructed. Thus, quality standards for seedlings to be planted in reclamation sites are exceptionally rigorous and sometimes require unique plant attributes (such as bioremediation capacity).

Current reclamation practices also often include the broadcast fertilization of reclaimed sites to decrease nutrient limitations. This practice, in addition to being costly, can lead to increased competition between herbaceous species and tree seedlings and negative environmental effects of fertilizer leaching and runoff. Nitrogen leaching is of particular concern for reclamation soils in the northern Alberta oil sands region as they contain elevated nitrogen levels as compared to natural soils (Rowland et al. 2009). Certain Albertan ecosites can also have high B horizon soil phosphorous (P) levels. This is of particular importance on reclamation sites where soil profiles have been disturbed, potentially increasing B horizon soil within the rooting zone (Lanoue 2003). Alternative fertilization methods are under investigation (including slow release fertilizers and localized application of fertilizers with respect to tree seedlings). However, more attention has been placed on creating seedlings that can successfully establish with lower levels or in the absence of field fertilization application. One method of growing tree seedlings more highly suited to nutrient-poor environments characteristic of reclamation areas is through the process of "nutrient loading" seedlings.

1.2 Trembling aspen

Trembling aspen (*Populus tremuloides* Michx.) of the genus Salicaceae, is a broadleaved, deciduous tree native to North America (Burns and Honkala 1990). Aspen is a fast growing, early successional, shade-intolerant species and exhibits an indeterminate growth pattern. Trembling aspen is the most widely distributed tree species in North America, and grows in mid- to upper riparian zones across a broad range of climates (Howard 1996). Trembling aspen can be found in single-tree stands or associated with spruce (*Picea* spp.), fir (Abies spp.), pine (*Pinus* ssp.), or scrub oaks (*Quercus* spp.) (Howard 1996). Aspen stands develop across a broad range of soil textures though growth is usually optimized on nutrient-rich, well-drained loamy upland soils (Krajina et al. 1982; Haeussler and Coates 1986; Burns and Honkala 1990). Reproduction can naturally occur by seed or, more commonly, by vegetative sprouting from the roots. Both aspen seedlings and cuttings can be transplanted onto disturbed sites though seedlings are more economical to grow on a large scale, establish a better rooting system, and afford a greater genetic diversity than cuttings (Howard 1996; Snedden et al. 2010). Thus, in this thesis, only aspen seedling growth and development will be considered.

As aspen's role in commercial forestry and land reclamation increases, so does the interest in the factors that promote aspen growth. Soil parameters have proven useful in predicting aspen performance. Chen et al. (1998b) found total soil nitrogen (N) to be the nutrient most important in determining the height growth of naturally regenerating aspen in northern British Columbia. Forest floor potassium and calcium; mineral soil N, Ca, magnesium (Mg), and sulfur (S); and soil pH have also been positively correlated with aspen growth in the field and greenhouse (Chen et al. 1998; Lu and Sucoff 2001; Paré et al. 2001; Pinno et al.

2009). Soil phosphorous (P), however, has not been significantly correlated with aspen growth (Pinno et al. 2012).

Pinno et al. (2012) offer an interesting insight into aspen seedling growth responses on soils commonly used in Alberta oil sands reclamation and fertilization regimes in their 2012 greenhouse study. This study used eight different soils including organic-mineral soils (PMM and FFM), various B horizon soils, subsoil and tailings sand. When the eight different soils studied were not fertilized, aspen growth was greatest on organic-mineral material soils. NPK fertilization resulted in greater performance than PK or N fertilization across most soil types. However, FFM showed similar height growth under the PK, N, and NPK regimes. With NPK fertilization, seedlings on PMM had the greatest height growth and those on FFM the least, growing less than seedlings on the pure mineral soils. These findings emphasize the importance of the interaction between fertilizer regime and soil type for aspen on reclamation soils.

Foliar nutrients prove less reliable in predicting the growth of aspen and closely related *Populus* species. In one study (Chen et al. 1998b), only total N (r=0.52) and extractable boron (B) (r=0.55) had correlation coefficients greater than |0.5| when correlated to site index. Jug et al. (1999) found foliar nutrient concentration to be a poor predictor of hybrid aspen height growth on soils with nutrient availabilities within the optimal range. On weed-free plots in agricultural soils in central Saskatchewan, Canada hybrid poplar foliar P concentration was strongly correlated to tree diameter growth (r= 0.88) while there was no significant relationship between foliar N and diameter growth.

While general characteristics of aspen growth responses may be used as guidelines, care must be taken in comparing aspen field studies as climate and other local factors have been shown to affect the accuracy of aspen height growth predictions from site index (Chen et al. 1998a).

Perhaps because of aspen's wide distributional range and prevalence in multiple ecosystem types, aspen regeneration is often taken for granted as a natural process that needs no outside help from humans. However, as human activities cause increasingly large areas of land to be disturbed (for example the development of oil sands mines in northern Alberta) the reproduction of aspen from suckers or natural regeneration from seed can no longer be relied on and natural resource managers must carefully consider the future of aspen in our forested landscapes. The use of containerized aspen seedlings for reclamation of such sites is becoming an increasingly common practice. Though decades of rigorous scientific research have been dedicated to the production of high quality conifer seedlings, the same cannot be said of aspen. As a result, aspen seedling stock quality remains poorly understood and outplanting success is unreliable (van den Driessche et al. 2003; Landhäusser et al. 2012; Macdonald et al. 2012). The relative newness of producing aspen seedlings on a large scale means that there is still a great deal to be understood about the greenhouse practices that optimize outplanted aspen seedling success.

1.3 Nutrient loading

Nutrient loading is the process by which nutrients are provided to a seedling in excess of the quantities needed for plant growth but at concentrations that do not decrease biomass production through either toxicity or complex nutrient or soil chemistry interactions such as pH or salinity problems (Timmer 1996). Nutrient loading coincides with the plant nutrient uptake status that is also known as "luxury consumption" (Elrifi 1985). Figure 1-1 demonstrates the "nutrient loading" range of seedling biomass and nutrient concentration. Though seedling biomass does not increase at nutrient levels above those needed for plant growth, nutrient concentration does increase in seedling tissue. Increased seedling performance of nutrient loaded seedlings as compared to seedlings fertilized to sufficiency levels have been demonstrated in numerous outplanting studies (including Imo and Timmer 1999; 2002). These results suggest that nutrient loaded seedlings are able to utilize the nutrients stored in their tissues following outplanting on nutrient poor sites. It should be noted that nutrient loading can only provide these benefits for nutrients that are mobile within plants (N, P, K, Mg, Zn) and not plant-immoblie nutrients (S, Ca, B, Fe, Mn, Cu). Excess consumption of plant-immoblie nutrients by seedlings may predispose them to toxicity of those nutrients if outplanted onto a site high in such nutrients.

The mechanism for achieving luxury consumption of nutrients can vary. Rather than providing nutrients at constant rates over the course of the growing season, many nutrient loading studies have focused on the exponential increase in the availability of nutrients to plant over the growing season. In addition to nutrient loading, another concept pertinent to the fertilization of seedlings is the concept of "steady-state nutrition" which strives to create plants with constant internal nutrient ratios (Ingestad 1982). A steady state of nutrient uptake accompanies optimum nutrition, the point at which internal nutrient ratios match nutrient uptake rates (Ingestad and Lund 1979). Steady-state nutrition and optimum ratios suggest that an exponential fertilization regime is likely to maximize plant growth and nutrient uptake by matching available nutrients to the exponential growth rates of tree seedlings (Munson and Bernier 1993). The application of fertilizer in exponentially increasing concentrations as the growing season progresses will be referred to as "exponential fertilization" and has been successfully used to nutrient load the seedlings of several coniferous species (Ingestad and Lund 1979).

As the use of exponential fertilization regimes increased in popularity among those growing coniferous seedlings, modifications to the traditional natural-log curve have been tested for their efficacy. Modified exponential fertilizer regimes often spread the fertilizer out somewhat more evenly across the growing season to prevent potential negative effects of extremely high rates of fertilizer application at the end of the growing period. These slight modifications prevent salinity build up from high fertilizer application rates which could negatively affect plant root health and water relations. Most recent publications using exponential nutrient loading fertilization regimes on angiosperm tree species have employed a modified exponential design (Birge 2006; Zabek and Prescott 2007).

Nutrient loading studies

When tree seedlings achieve luxury consumption levels of nutrient concentrations after having been fertilized under an exponential regime or they are said to have been exponentially nutrient loaded. A number of examples of coniferous tree species that have been successfully exponentially nutrient loaded exist (black spruce- Quoreshi and Timmer 2009; Japanese and hybrid larch- Qu et al. 2003; Chinese fir-Xu and Timmer 1998).

However, results of exponential nutrient loading experiments performed on angiosperm tree species have shown quite different results. A study done on Holm Oak (Oliet et al. 2009) found no significant differences between exponential and conventional fertilization at equal rates of application per plant per season. The results of a study of both red and white oak yielded mixed results though nitrogen content was not significantly increased with experimental fertilization, as compared to conventional (Birge et al. 2006). The experimental design of a study on eucalyptus doesn't allow for comparison of exponential and conventional fertilization application methods at the same fertilization rate per plant per season (Close et al. 2005). Most relevant to the potential application of such fertilizer treatments to aspen, three clones of hybrid poplar (*Populus trichocarpa* Torr. and Gray x *Populus deltoides* Marsh.) showed no consistent, significant differences between conventional and exponential fertilization application regimes (Zabek and Prescott 2007). Table 1 summarizes the results of this study. Table 1-2 is a brief comparison of the methods and findings of the studies mentioned in this paragraph.

Greenhouse operators often use basic morphological traits of seedlings or foliar nutrient analyses to assess seedling quality. When judged by these standards, the true advantages of nutrient loaded seedlings may not be taken into account. The luxury consumption of nutrients does not increase seedling height and leaf nutrient analyses at one point in time may not provide enough information to measure steady-state nutrient accumulation. Since seedling quality is often determined by dry weight and height, greenhouse operators may be reluctant to incur the risks inherent to adapting a new fertilization technique, the additional operational complications of nutrient loading, and increased fertilizer costs associated with nutrient loading seedlings. Nutrient loading may decrease total seedling height but can cause measures known to be important to seedling outplanting success such as root:shoot ratio (RSR) and root carbohydrate levels to increase (Landhäusser et al. 2012). To overcome this challenge, alternative criteria for assessing seedling quality and predicting field performance may be necessary.

Growth strategies and nutrient loading

Given aspen's deciduous habit and indeterminate growth strategy and the limitations of various nutrient loading and plant tissue nutrient analysis methods, I suggest a new approach to nutrient loading aspen. Rather than applying an exponential or modified exponential fertilization regime to aspen seedlings the use of a shoot growth inhibitor (SGI) has been proposed to facilitate luxury nutrient uptake in aspen seedlings. The SGI paclobutrazol has been shown to uniformly induce shoot growth cessation and mid-season bud set in containerized aspen seedlings (Landhäusser et al. 2012). It is hypothesized that by treating aspen seedlings with a shoot growth inhibitor (thus inducing bud set in the middle of the growing season) and following this treatment with elevated fertilization rates aspen seedlings will be enter a state of luxury consumption of nutrients rather than allocating all available resources to biomass growth. An additional method suggested for growing hardy, nutrient-loaded seedlings is moving the containerized seedlings outside of the greenhouse at six weeks after germination to subject them to ambient wind and temperature fluctuations more similar to those experienced on outplanting sites. The results of this method will be discussed in Chapters 2 and 3 as they provide a unique solution to the challenge of growing high-quality, nutrient-loaded aspen seedlings.

1.4 Objectives

The overall objective of this thesis was to determine if aspen seedlings can be nutrient loaded and, if so, what effect nutrient loading would have on seedling outplanting performance on an oil sands reclamation site.

In Chapter 2, results of a nutrient loading study are presented. The objective of this study was to investigate the efficacy of exponential fertilization and early shoot growth termination and fertilization regime on aspen seedling tissue nutrient concentration and content. This study examined data on the morphology, foliar, and woody concentrations and contents of seedlings grown under exponential fertilization and with SGI application.

Chapter 3 presents the results of a study looking at the outplanting performance of two types of nutrient-loaded aspen seedling stocks on two capping materials treated with each of four different fertilizer regimes. The study includes data taken over two outplanting seasons. Morphological characteristics of seedlings including mortality, height, root collar diameter, root, stem, and leaf dry weight, and root:stem ratio were compared across these treatments. Foliar nutrient concentrations of aspen seedlings and soil available nutrient levels were also evaluated.

Finally, Chapter 4 summarizes the main findings of the two studies, details management implications of the findings, and suggests areas where future research could benefit natural resource managers.

Tables

Table 1-1: Abbreviated results of orthogonal contrasts comparing seedling biomass, root to shoot ratio (RSR) and relative growth rates (Rg) between conventional and modified exponential fertilization applications to the un-rooted cuttings of three hybrid poplar clones. Asterisks represent the only three measures in which there were significant differences between seedlings produced under conventional (C) and modified experimental (ME) methods (P>0.05) (Zabek and Prescott 2007).

Clone	Comparison	Leaf	Stem	Root	Plant	RSR	Rg
49-177	C vs ME			*			
DTAC-7	C vs ME						
15-29	C vs ME	*				*	

Table 1-2: Comparison of methods and findings of selected tree seedling nutrient loading studies. Information is given on the author(s), year of publication, species studied, treatment (exponential:E or conventional:C and fertilization), tissue type analyzed and findings. Numbers in the treatment column represent percentages of standard nursery fertilization rates for the species studied.

Study		Species	Treatment	Tissue	Findings
Author(s)	Year				
Oliet et al.	2009	*Quercus ilex*	0, 25 E, 100E, 100C	Woody	100 treatments successfully loaded, no significant difference between 100E and 100C in seedling N content or post-transplant performance
Birge, Salifu, and Jacobs	2006	*Quercus rubra, Quercus alba*	0, 50E, 100C, 100E, 150E, 200E, 250E, 300E, 350E, 400E	Foliar and woody	Stem dry mass significantly increased with 100E vs. 100C treatment but N content not significantly different (in either leaf or stem for either species)

Close, Bail, Hunter, and Beadle	2005	*Eucalyptus globulus*	100C, 200E	Foliar	Foliar N and SLA at age 16 weeks, were higher in 200E, cannot conclude if due to exponential nutrient-loading or higher fertilization rates
Zabek and Prescott	2007	*Populus trichocarpa* x *Populus deltoides*	0, 50C, 50ME, 100C, 100ME, 200C, 200ME	Total plant	No consistent, significant differences between modified experimental (ME) and conventional fertilization application methods were found across the clones.
Qu, Quoreshi and Koike	2003	Larix kaempferi, L. gmelinii × L. kampferi	100C, 100E, 200E, 400E	Woody	Exponential fertilization increased N concentration and content of the whole plant when compared to conventional fertilization at the same nitrogen application per plant over the growing season.
Quoreshi and Timmer	2009	*Picea mariana*	100C, 100E, 200E, 400E (control & inoculated w/ ectomyco-rhizae)	Woody	"Results indicate that high exponential fertilization combined with fungal inoculation may be effective for producing both nutrient-loaded and ectomycorrhizally infected planting stock."
Xu and Timmer	1998	*Cunninghamia lanceolata*	0, 100C, 100E, 300E, 500E	Total plant	Increased biomass and nutrient uptake in 100E as compared to 100C. Successful loading and steady state consumption at 300E and 500E.

Figures

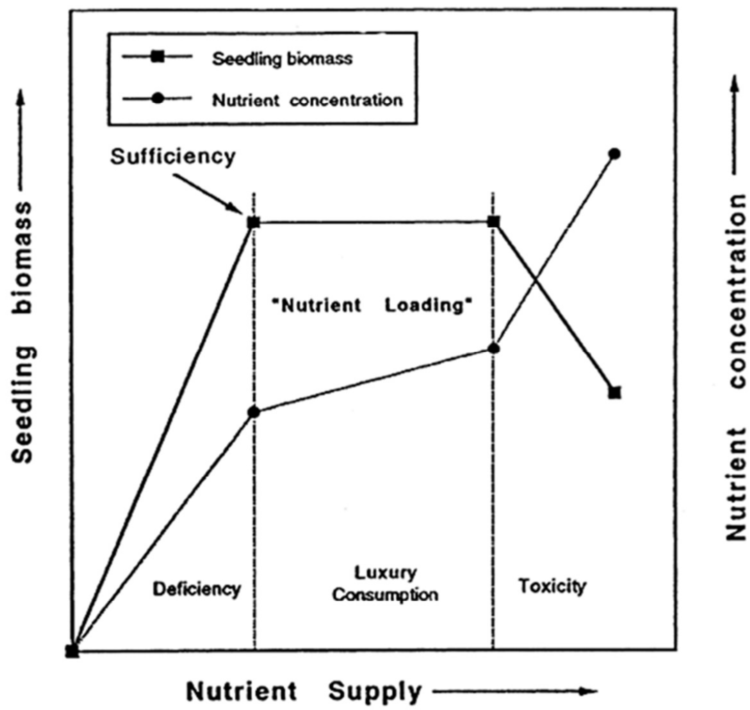

Figure 1-1: Optimum nutrient loading occurs when a seedling contains the highest nutrient concentration associated with maximum seedling biomass (Timmer 1996).

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Chapter 2: Premature shoot growth termination allows nutrient loading of seedlings with an indeterminate growth strategy

2.1 Introduction

Trembling aspen (*Populus tremuloides* Michx.) is a deciduous species in the Salicaceae family that has the distinction of being North America's most widely distributed tree (Little 1971; Burns and Honkala 1990). While both seedlings and cuttings are options for producing aspen planting stock, seedlings are more economical to grow on a large scale, establish a better rooting system, and afford a greater genetic diversity than cuttings (Howard 1996; Snedden et al. 2010). Despite increasing interest in aspen seedlings for afforestation projects, ranging from mine reclamation and restoration to short-rotation plantations, obtaining quality aspen seedling stock that delivers predictable outplanting performance continues to be a challenge, particularly on stressful sites (van den Driessche et al. 2003; Landhäusser et al. 2012a; Macdonald et al. 2012). Aspen's reputation as having poor planting stock quality might be related to its indeterminate growth strategy, which allows aspen seedlings to continuously grow in height during nursery production as long as growing conditions are favorable thereby allocating most resources to current growth and little to the storage of excess nutrients and carbohydrates.

Nutrient loading is the process by which nutrients are provided to the seedling in excess of the quantities needed for plant growth (luxury consumption), but at concentrations that do not decrease biomass production through either toxicity or other complex nutrient or soil chemistry interactions such as pH or salinity (Elrifi 1985;Timmer 1996). Outplanting studies suggest that nutrient loaded seedlings are able to utilize the accumulated plant-mobile nutrients (e.g. nitrogen (N), phosphorous (P), and potassium (K)) stored in their tissues resulting in greater growth and survival, particularly on nutrient poor sites (Salifu et al. 2009; Barker 2010; Louranen and Rikala 2011).

One common procedure for producing nutrient loaded seedlings is exponentially increasing fertilizer nutrient concentrations as the growing season progresses (Ingestad and Lund 1986). Nutrient loading through exponential fertilization has been successfully demonstrated in many evergreen coniferous seedlings with determinate or periodic growth strategies (Borchert 1991) including white spruce (McAlister and Timmer 1998), black spruce (Quoreshi and Timmer 1998), Chinese fir (Xu and Timmer 1998), and white and loblolly pines (Barker 2010). However, information on the nutrient loading of deciduous species using exponential fertilization is lacking, in particular those with an indeterminate growth strategy (Borchert 1991). For example, in a study of three hybrid poplar clones, only one clone showed an increase in woody tissue nutrient concentration between conventional and exponential fertilization (Zabek and Prescott 2007).

Another potential option for producing nutrient loaded aspen seedlings is by inducing premature bud set whereby height and new leaf area growth are terminated while photosynthesis and resource uptake continues (Landhäusser et al. 2012a). This process can be achieved via blackout (artificially shortening day length) or through the application of an artificial shoot growth inhibitor (SGI), with the SGI resulting in a more uniform seedling response (Landhäusser et al. 2012b). According to the growth-limitation hypothesis, restricting height growth while resource uptake continues should result in an imbalance between carbon and nutrient supply and demand which might lead to a diversion of photosynthates and nutrients to reserves and storage rather than growth (Chapin et al. 1990; Körner 1998; 2003). Paclobutrazol, a shoot growth inhibitor (SGI), has been shown to reliably induce the mid-season formation of buds, which are not broken by optimal growing conditions. This artificial termination of shoot growth has shown to improve seedling carbohydrate reserves in aspen seedlings (Landhäusser et al. 2012a). Paclobutrazol is a synthetic plant growth regulator that inhibits gibberellin biosynthesis (Hedden and Graebet 1985) and has also been shown to increase plant tolerance to a number of stresses, such as low-temperature (Upadahyaya and Davis 1989; Zhou et al. 2012), salt stress (Sharma et al. 2011), and water stress (Fletcher and Nath 1984).

When evaluating the transplanting success of nutrient loading deciduous seedlings, it is important to keep in mind that only the nutrients stored in the woody tissues will be available for translocation within the plant upon outplanting. Accordingly, nutrient status analyses should focus on the woody tissues rather than the foliage which is abscised at the end of the growing season. Multiple predictors of seedling outplanting success, including both physiological and morphological characteristics, are needed but in terms of nutrient status, nutrient concentration is appropriate if seedlings are of similar size and age while nutrient content may be more important if seedlings vary widely in size or age (Grossnickle 2012).

The objective of this study was to investigate the efficacy of exponential fertilization and early shoot growth termination on dormant aspen seedling tissue nutrient concentration and content. We hypothesize that exponential fertilization and SGI application will both result in nutrient accumulation in aspen seedlings in relation to conventionally fertilized seedlings. To address these hypotheses, we conducted two related studies on the nutrient status of aspen seedlings compared between: 1) exponential and conventional fertilization, and 2) SGI application and fertilization rate.

2.1 Methods

2.2.1 Seedling production and treatments

A total of 675 aspen seedlings were grown in Styroblock containers (5-12A, Beaver Plastic, Edmonton, Alberta) for one growing season. The seed used was from an open-pollinated seed source collected from several populations of trees in the greater area of Edmonton, AB, Canada (53°34′ N; 113°31′ W; elevation 668 m a.s.l.). Seeds were sown on March 26, 2010 into cavities (5 cm diameter and 12 cm deep, volume 220 ml) filled with peat and perlite (9:1, by volume) at Smoky Lake Forest Nursery (Smoky Lake, AB, Canada 54°6′ N; 112°28′ W; 598 m a.s.l.). All seedlings were germinated and established under standard greenhouse growing conditions used for aspen seedling stock (temperature: 21˚C average, max 28˚C, min 18˚C; relative humidity: >70%) and fertigated daily with a balanced standard nutrient fertilizer (78 ppm N (equal proportion of NO_3^- and NH_4^+), 77 ppm P, 161 ppm K, 46 ppm sulphur (S), and a balanced blend of chelated micronutrients) included in every watering with an automated mist irrigation system until May 12.

On May 12, seedlings were moved outside at which point they were assigned to either the exponential fertilization study or the shoot growth inhibitor study for a total of five different treatment combinations (Table 1-1). Each treatment was replicated nine times in separate blocks that contained 15 seedlings (sub-samples) each. In the exponential fertilization study, the first treatment was conventional fertilization (treatment 100-100), which applied a constant rate of 100% of a standard commercial nursery fertigation solution throughout the early (May 12-July 12) and mid-growing season (July12-September 4). The second treatment was an exponential (EXP) fertilization regime in which nutrient concentrations were calculated for 3-day intervals throughout the growing season and applied to the seedlings. To calculate the concentrations applied during each 3-day interval we used the methodology described in Timmer (1996) using the equivalent of 110 mg N seedling^{-1} (same quantity as the 100% treatment) over a period of 105 days. The starting minimum N concentration was 39 ppm which was 50% of the standard 78 ppm N aspen fertilizer mix used in the 100% treatment. When plotted, an exponential regression through the points created by the concentrations the fertilization regime followed the equation $y = 39 + 6^{-6} * e^{0.6638x}$. To allow for comparisons between exponential and continuous fertilization treatments (Close et al. 2005), we applied the same amount of total fertilizer over the growing season in the exponential treatment as in the continuous treatment (100%).

For the shoot growth inhibitor study, four treatments were used, which combined different fertilizer concentrations (100% or 150% of the standard nutrient concentration (see above)) that were applied at two different times (early- and mid-growing season), and a superimposed shoot growth inhibitor (SGI) treatment (see Table 1-1). All treatments, except the exponentially fertilized treatment, received a 100% fertilizer concentration until July 12. Styroblocks were weighed daily and the seedlings were fertigated when the saturation of the peat-perlite mixture dropped below 80%, either daily or on alternate days.

On June 24, when average seedling height had reached about 30 cm, paclobutrazol (4 g L^{-1} active ingredient; Bonzi®, Syngenta, North Carolina, USA) was applied to the seedling blocks assigned to the SGI treatment by soaking the Styroblocks in water containing 5 mL L^{-1} of Bonzi. Individual styroblocks were submerged into tubs of prepared paclobutrazol solution for 5 minutes each, allowing the solution to be absorbed through the holes in the bottom of the styroblocks to the point of saturation. Rods were positioned on top of the styroblocks to keep them submerged during the treatment. Mid-season fertilization concentrations (100% or 150%) were applied on July 12 when SGI treated seedlings had visibly set bud. Fertilizers were applied until September 4 in all treatments. After September 4, fertilization was discontinued and the seedlings received only water when the peat-perlite mixture was below 80% saturation. The seedlings remained outside and were allowed to naturally go dormant and harden. Seedling height growth was measured on June 3, June 21, August 3, September 3, and in November after the seedlings were dormant.

2.2.2 Plant morphology and tissue nutrient concentration

In late August 2010, before leaf senescence began, two mature and fully expanded leaves were removed from the top 10 cm of each seedling and pooled within each block for nutrient analyses. Leaves were washed with de-ionized water, dried at 70°C for 2 days in an oven and ground to a fine powder in a Wiley Mill (Thomas Scientific, Swedesboro, NJ, USA) passing a 40-mesh (0.4mm) screen.

In November, three representative fully dormant seedlings from each block were selected to determine seedling characteristics through destructive sampling. The resulting measures were averaged by block resulting in nine replications per treatment. Height and root collar diameter of the seedlings were measured and the root systems were washed carefully to remove peat and perlite particles. Root volume was determined by water displacement (Olesen 1971). The woody tissue was separated at the root collar into root and stem tissue, and all tissues were dried to constant weight at 70°C. Dry weights of root and stem tissues were determined, and root:stem ratio (RSR) calculated. Dried stem and root tissues were ground to pass 0.4 mm mesh.

Total N concentration of leaf, stem, and root samples was determined by the Kjeldahl method (Kalra and Maynard 1991). Concentrations of P, K, and S were determined by inductively coupled plasma optical emission spectrometry (ICP-OES) after microwave digestion (EPA Method 3051, U.S. Environmental Protection Agency, Washington, D.C.). Non-structural carbohydrate concentrations were determined for the woody samples. Water-soluble sugars were determined colorimetrically at 490 nm, after water soluble sugars were extracted three times using hot-ethanol. Remaining starch was digested in the residue using enzymatic digestion, and starch concentrations were determined as glucose equivalents by colorimetric measurement of glucose hydrolyzate at 525 nm (Chow and Landhäusser 2004). Total non-structural carbohydrate (TNC) reserves at the tissue level were calculated by adding their respective sugar and starch concentrations.

2.2.3 Statistical analyses

For the first comparison, the effect of exponential (EXP) and the nursery standard constant rate (100-100) fertilization regimes on seedling nutrient status, morphological characteristics and carbohydrate reserves were compared by t-test. A repeated measures ANOVA was used to compare seedling height over the growing period in these two treatments. For the second comparison, the effects of fertilizer concentration (100-100 and 100-150) and SGI (SGI and no SGI) on seedling nutrient status, morphological characteristics and carbohydrate status were tested via two-way ANOVA. The Student-Newman-Keuls test, which controls Type I error at an experimental level, was used to compare significant differences among treatment means. All variables met the assumptions for normality and homogeneity of variance so no data transformations were necessary. All statistical analyses were performed using the GLM and REG procedures in SAS (SAS 9.2, SAS Institute, Cary, NC, USA).

2.3 Results

2.3.1 Exponential vs. constant rate fertilization

Seedlings with constant rate (100-100) or exponential (EXP) fertilization exhibited very different growth patterns (Figure 2-1). Seedlings with the constant rate fertilization continued to grow throughout the growing season, only slowing height growth towards the end of the growing season. Exponentially fertilized seedlings, on the other hand, had very slow initial growth which only increased when fertilizer rates started increasing in the later part of the growing season as indicated by the significant time by treatment interaction. By the end of the growing season EXP seedlings reached a height of only 19.1 cm while the 100-100 seedlings were 36.8 cm tall (Figure 2-1).

At the end of the growing season, EXP seedlings had lower stem and root dry weight than 100-100 seedlings. Root volume and dry weight of EXP seedlings was approximately half that of 100-100 seedlings (Table 2-2). As a result, seedlings had much poorer plug fill and were relatively small for the container size to be securely handled by planters. However, the stem dry weight was proportionally lower than the root dry weight in EXP seedlings resulting in a higher RSR than in the 100-100 seedlings (Table 2-2). Exponentially fertilized seedlings did have higher stem concentrations of N, P, K, S, and TNC (all $P < 0.001$) than 100-100 seedlings (Tables 2-2 & 2-3). However, given the large differences in height and dry weight of seedlings, the 100-100 seedlings had much higher nutrient and TNC content than EXP seedlings (Table 2-3).

2.3.2 Shoot growth inhibitor and fertilization rates

Treating seedlings with a shoot growth inhibitor (SGI) resulted in shorter seedlings ($P < 0.001$), while increased nutrition resulted in taller seedlings ($P < 0.001$) with the tallest seedling in the 100-150 treatment and the shortest in the

100-100 SGI (Table 4). While root collar diameter, stem dry weight, and root volume mirrored the height response described above (Table 2-4), root dry weight and root volume were least in the 100-100 SGI treated seedlings with no differences among the other three treatments (Table 2-4). However, regardless of fertilizer regime, SGI treated seedlings had higher RSRs than non-SGI treated seedlings ($P < 0.001$; Table 2-4).

Increased fertilizer uniformly increased seedlings' N, P, K, and S woody tissue contents, i.e. the total measure of nutrient in both root and stem tissues ($P < 0.001$; Table 2-5). However, despite lower seedling heights, SGI did not affect woody tissue N content at either fertilizer rate ($P = 0.902$) (Table 2-5). For P and S, SGI decreased woody tissue nutrient content at the 100-100 but not the 100-150 fertilizer rate while for K, SGI resulted in lower woody tissue K contents for both fertilization levels (Table 2-5).

Foliar and stem N, P, and S concentrations increased between 25 and 30% when seedlings had their shoot growth prematurely terminated (Figure 2-2). However, increasing the fertilization rate in mid-season did not translate into higher nutrient concentrations in these tissues when combined with the SGI application. This is in contrast to seedlings with no SGI application, where an increase in the fertilization rate (100-150) resulted in greater foliar and stem nutrient concentrations. Interestingly however, this increase in tissue nutrient concentration was less than the increase observed in a 100-100 seedling that had been treated with SGI (Figure 2-2).

Root tissue N, P, and S concentrations responded very differently to the SGI and fertilization treatments than stems. Root N and P concentrations increased with higher fertilization rate and with SGI application, indicating an additive effect. Root S concentration increased with fertilization, but was not impacted by the SGI treatment (Figure 2-2). Overall, root N concentrations were similar to those in stems while P and S concentrations were higher in roots than in stems.

Potassium concentrations in all three tissues responded in very different patterns to the SGI treatment than the concentrations of the other three macro nutrients. While K concentration increased as expected with increased fertilization in all three tissues, K concentrations in SGI treated seedlings were much lower, particularly in the root and leaf tissues. However, in both these tissues K concentrations increased when SGI treated seedling received the higher fertilizer treatment. Stem K concentration did not follow this pattern as the SGI treated seedlings at the lower fertilization rate had somewhat higher stem K concentration than seedling treated with the higher fertilization rate (Figure 2-2).

Total non-structural carbohydrate reserve concentrations in roots were generally twice the concentrations measured in stems, while TNC contents were 4 to 7 times higher in roots than in stems (Table 2-6). SGI treatment and lower fertilization level resulted in lower stem TNC contents ($P < 0.001$), though 100-150 SGI seedlings had contents equal to 100-100 seedlings (Table 2-6). Root TNC contents showed a significant interaction between SGI and fertilization ($P < 0.001$) and were highest in 100-150 SGI seedlings and lowest in 100-100 SGI seedlings, with no difference between 100-100 and 100-150 treatments (Table 2-6). Seedlings treated with SGI had significantly higher TNC concentrations in both the root and stem tissues (both $P < 0.001$) than untreated seedlings, while increasing the rate of fertilization did not affect TNC concentrations in roots and stems ($P = 0.857$ and $P = 0.455$, respectively) (Table 2-6).

2.4 Discussion

As suggested by the growth-limitation hypothesis, the artificial restriction of height growth in aspen seedlings while maintaining physiological activity of leaves and roots resulted in a diversion of photosynthates and nutrients to reserve storage rather than growth. Although only tested here in aspen, cessation of height growth might be a necessity to significantly increase nutrient and carbon woody tissue concentrations in species with indeterminate growth. In the EXP fertilization regime, aspen seedlings ceased to grow in height (with terminal budset) during the initial period of fertilization where nutrient additions were very low resulting in higher woody tissue nutrient concentrations than continuously fertilized seedlings. Regardless, due to their small size EXP seedlings had much lower overall nutrient content along with lower root dry weight and very poor plug-fill making them undesirable for outplanting. Therefore, the exponential fertilization regime tested here does not appear to be the optimal approach for producing high-quality aspen seedling stock. Higher initial fertilization and slower supply rates might be an option to modify the exponential fertilization regime; however, higher initial rates will likely lead to continuous growth of aspen seedlings and not to early bud set, which appears to be a requirement for this species to accumulate nutrients in tissues. This response to EXP fertilization in aspen is in stark contrast to species with determinate and periodic growth strategies where budset is driven by endogenous factors and exponential fertilization has shown promise and been successful at nutrient loading seedlings (McAlister and Timmer 1998; Quoreshi and Timmer 1998; Xu and Timmer 1998; Birge et al. 2006; Salifu et al. 2009). These internally scheduled "resting" periods likely allow for seedlings to accumulate resources prior to the next flush.

In aspen, terminating shoot growth prematurely is very successful and uniform with the use of a SGI (Paclobutrazol) (Landhäusser et al. 2012a). In our study, treatment with SGI produced shorter seedlings with higher RSRs and increased woody tissue nutrient and carbohydrate concentrations compared to conventionally grown aspen seedlings with no SGI application. Seedlings without induced budset (treatments 100-100 and 100-150) continued to grow in height throughout the growing season, resulting in the allocation of available nutrient and carbon resources to growth rather than reserves. This is highlighted by the fact that non-SGI treated seedlings with higher fertilizer rate (100-150) did not have higher tissue nutrient concentrations than SGI-treated seedlings treated with a lower fertilizer rate (100-100 SGI). Overall, the application of SGI generally increased woody tissue nutrient reserves more than an increased application of fertilizer indicating that costly fertilizations do not need to be increased in order to create nutrient loaded seedlings.

Both the stem and root tissues in aspen seedlings seem to accumulate nutrients at similar rates although there appears to be a somewhat preferential storage of P in the roots. Root P concentration plays an important role in promoting root growth (Fernandez et al. 2007) and has also been shown to be a source of P for shoot growth in *Eucalyptus grandis* (Reis and Kimmins 1986). Rapid new root growth is seen as the key seedling developmental trait to ensure successful seedling establishment (Grossnickle 2012) so preferentially storing P in root tissues may be a mechanism for promoting root growth. Similarly, carbohydrate reserves are also stored preferentially in root tissue of aspen seedlings where they are thought to promote root growth and root water uptake (Landhäusser et al. 2012a).

Though not different in N content, seedling N concentrations were higher in SGI-treated seedling than in non-SGI seedlings. Nitrogen status in aspen seedlings has been linked to seedling growth, net assimilation, and water use efficiency (DesRochers et al. 2003), all of which should offer a benefit to seedlings upon outplanting. In our study, foliar N concentrations of the SGI-treated seedlings were only 1.9%, which is still well below the optimal foliar N concentrations of 3-4% for young aspen cuttings (Coleman et al. 1998). We feel that nursery practices and fertilizer blends (e.g. the proportion of NO_3^- to NH_4^+ (Landhäusser et al. 2010) or micronutrients influencing uptake) can still be adjusted to further increase N loading of seedlings.

Contents and concentrations of K in seedling tissues did not follow the same pattern of response to the treatments as tissue concentrations of N, P, and S. Paclobutrazol is known to negatively affect K concentrations in treated trees (Blanco et al. 2002; Iuchi et al. 2008). The foliar K concentrations of the SGI-treated seedlings, at approximately 6.5 mg g^{-1}, are well below the optimal foliar K concentrations of 15 mg g^{-1} for hybrid poplars (Hansen 1994). Because of this, caution should be used when applying paclobutrazol to seedlings to avoid internal nutrient imbalances which could negatively impact outplanting success. However, Landhäusser et al. (2012b) found that lower foliar K concentrations in SGI-treated seedlings did not impede outplanting performance of aspen seedlings. In addition, aspen seedling performance did not benefit from K field fertilization (van den Dreissche et al. 2003) indicating the K may not limit aspen field performance in many instances.

Root total non-structural carbohydrate (TNC) concentration is positively related to aspen growth upon outplanting (Landhäusser et al. 2012a). Though large differences in seedling height resulted in SGI-treated seedlings having lower TNC contents, both stem and root TNC concentrations were higher in SGI-treated seedlings than in non-SGI seedlings. Increased TNC concentrations resulting from SGI treatment is another indication that treatment with SGI may be beneficial to aspen seedling outplanting performance.

Nutrient loaded aspen seedlings are likely to outperform conventionally fertilized seedlings on both nutrient poor and highly competitive sites (Salifu et al. 2009; Louranen and Rikala 2011); On poor sites, improved performance of nutrient loaded seedlings has been related to increased root growth (Timmer 1996) which is critical for increasing outplanting survival on resource limited sites (Grossnickle 2012). On highly competitive sites, loaded seedlings offer the potential of fast aboveground growth and leaf area development thereby suppressing weeds and reducing the need for herbicide application (Timmer 1996). In the context of mine reclamation and restoration where nutrient cycling between the plants and soil is poorly developed, sites are often repeatedly broadcast fertilized which accelerates the growth of undesirable weed species and potential environmental contamination via runoff and leaching of unabsorbed fertilizer (Macdonald et al. 2012). The use of nutrient loaded aspen seedlings in such circumstances could potentially provide a mechanism for trees to access nutrients in the short-term and reduce or eliminate the need for early stand fertilization.

In summary, the EXP fertilization produced poor aspen planting stock and cannot be recommended as a treatment for nutrient loading aspen seedling stock.

SGI application, on the other hand, resulted in complete height growth cessation and a subsequent switch from allocating nutrients and carbohydrates from growth to reserves which resulted in nutrient and carbohydrate loaded aspen seedlings. It also appears that with the application of SGI lower fertilization rates can be applied during seedling production to achieve the same woody tissue nutrient concentrations observed in seedlings not treated with SGI. Therefore, we recommend initiating premature budset in aspen in order to produce nutrient loaded nursery planting stock and suggest that this may also be an appropriate technique for producing nutrient loaded seedlings of other species with indeterminate growth strategies.

Tables

Table 2-1: Description of the five fertilization treatments used in this study. Fertilizer concentration represents a percentage of a standard nursery fertilizer regime (100% or 150%) used for commercial aspen planting stock in early (May 12 – July 12) and mid (July 12 – September 4) season. SGI is the application of a shoot growth inhibitor. The exponential fertilization regime represents the equivalent nutrient amount supplied to seedlings in the 100 -100 constant rate fertilizer regime.

Treatment	Fertilization		SGI
	Early-Season	Mid-Season	
100-150 SGI	100	150	Yes
100-150	100	150	No
100-100 SGI	100	100	Yes
100-100	100	100	No
EXP	Exponential	Exponential	No

Table 2-2: Average, plus standard error in parentheses, of morphological characteristics and tissue total non-structural carbohydrate (TNC) concentration and content of aspen seedlings treated with an exponential (EXP) and constant rate (100-100) fertilization regime (n = 9).

Treatment		100-100	EXP	*P*
Root Collar Diameter	(mm)	4.7 (0.12)	3.4 (0.12)	< 0.001
Stem Dry Weight	(g)	1.32 (0.06)	0.42 (0.02)	< 0.001
Root Dry Weight	(g)	2.91 (0.07)	1.56 (0.11)	< 0.001
Root Volume	(ml)	12.60 (0.65)	6.29 (0.46)	< 0.001
Root:Stem Ratio		2.24 (0.23)	3.77 (0.13)	< 0.001
Stem TNC Concentration	(%)	13.50 (0.32)	14.77 (0.28)	0.009
Stem TNC Content	(g)	0.178 (0.01)	0.061 (0.01)	< 0.001
Root TNC Concentration	(%)	31.40 (0.75)	29.99 (0.80)	0.219
Root TNC Content	(g)	0.917 (0.04)	0.472 (0.05)	< 0.001

Table 2-3: Nutrient concentration and content of stem, root, and foliar tissues of seedlings treated with exponential (EXP) and constant rate (100-100) fertilization regime. Woody tissues (stem and root) values are from dormant seedlings harvested in November and foliar tissue values are from samples harvested in late-August. Values are averages with standard error of the mean in parentheses (n = 9). ND = Not Determined.

Nutrient		Nutrient Concentration			Nutrient Content		
	Tissue Type	Exponential	100-100	*P*	Exponential	100-100	*P*
N	Stem	13.83 (0.43)	8.92 (0.23)	< 0.001	5.70 (0.32)	11.71 (1.29)	< 0.001
	Root	12.60 (0.77)	8.95 (0.19)	< 0.001	19.63 (1.47)	26.04 (1.05)	0.002
	Foliar	13.57 (0.26)	12.60 (0.23)	0.012	ND	ND	ND
P	Stem	1.76 (0.03)	1.30 (0.04)	< 0.001	0.74 (0.04)	1.71 (0.10)	< 0.001
	Root	2.31 (0.09)	2.33 (0.06)	0.909	3.63 (0.29)	6.77 (0.21)	< 0.001
	Foliar	1.82 (0.03)	1.74 (0.02)	0.037	ND	ND	ND
K	Stem	6.73 (0.11)	6.06 (0.13)	< 0.001	2.79 (0.17)	7.95 (0.29)	< 0.001
	Root	6.07 (0.41)	7.39 (0.15)	0.008	9.55 (0.95)	21.51 (0.59)	< 0.001
	Foliar	6.96 (0.13)	9.58 (0.44)	< 0.001	ND	ND	ND
S	Stem	1.00 (0.03)	0.70 (0.02)	< 0.001	0.41 (0.02)	0.91 (0.04)	< 0.001
	Root	1.08 (0.05)	1.01 (0.03)	0.225	1.69 (0.13)	2.93 (0.12)	< 0.001
	Foliar	2.11 (0.01)	1.80 (0.01)	< 0.001	ND	ND	ND

Table 2-4: Average, plus standard error in parentheses, of morphological seedlings characteristics at the end of the growing season of seedlings that were treated with a different combination of two fertilizer rates and a shoot growth inhibitor (SGI) treatments. Different letters indicate a difference between means ($P < 0.05$) (n = 9).

Treatment	Height (cm)	Root Collar Diameter (mm)	Root Dry Weight (g)	Stem Dry Weight (g)	Root Volume (ml)	Root:Stem Ratio
100-150 SGI	30.8 (0.75) c	4.0 (0.06) c	3.17 (0.18) a	0.94 (0.05) c	11.72 (0.99) a	3.36 (0.08) a
100-150	40.2 (1.08) a	5.4 (0.13) a	3.05 (0.10) a	1.62 (0.10) a	14.49 (1.16) a	1.92 (0.15) c
100-100 SGI	26.2 (0.61) d	3.7 (0.67) d	2.23 (0.09) b	0.69 (0.03) d	8.23 (0.68) b	3.23 (0.17) a
100-100	36.8 (1.00) b	4.7 (0.12) b	2.91 (0.07) a	1.32 (0.06) b	12.60 (0.65) a	2.24 (0.23) b

Table 2-5: Average, plus standard error in parentheses, of nutrient content of woody tissue (combined stem and root) from dormant aspen seedlings harvested in November that had been treated with different combinations of two fertilizer rates and a shoot growth inhibitor (SGI). Different letters indicate a difference between means (*P* < 0.05) (n = 9).

Treatment	N (mg)	P (mg)	K (mg)	S (mg)
100-150 SGI	53.08 (2.21) a	10.48 (0.51) a	28.11 (1.71) b	4.38 (0.20) a
100-150	50.67 (2.34) a	10.56 (0.45) a	33.74 (0.47) a	4.80 (0.25) a
100-100 SGI	35.58 (1.54) b	6.50 (0.25) c	18.45 (0.67) c	2.78 (0.08) c
100-100	37.74 (0.97) b	8.48 (0.25) b	29.46 (0.70) b	3.84 (0.14) b

Table 2-6: Average, plus standard error in parentheses, of total non-structural carbohydrate (TNC) concentration and content at the end of the growing season in stem and root tissues of aspen seedlings that had been treated with different combinations of two fertilizer rates and a shoot growth inhibitor (SGI). Different letters indicate a difference between means (*P* < 0.05) (n = 9).

Treatment	Stem TNC Concentration (%)	Root TNC Concentration (%)	Stem TNC Content (g)	Root TNC Content (g)
100-150 SGI	17.20 (0.29) a	35.30 (0.67) a	0.163 (0.01) b	1.12 (0.08) a
100-150	13.40 (0.17) b	30.16 (0.66) b	0.217 (0.12) a	0.918 (0.04) b
100-100 SGI	17.54 (0.34) a	34.32 (0.72) a	0.122 (0.01) c	0.765 (0.04) c
100-100	13.50 (0.32) b	31.40 (0.75) b	0.178 (0.01) b	0.917 (0.04) b

Figures

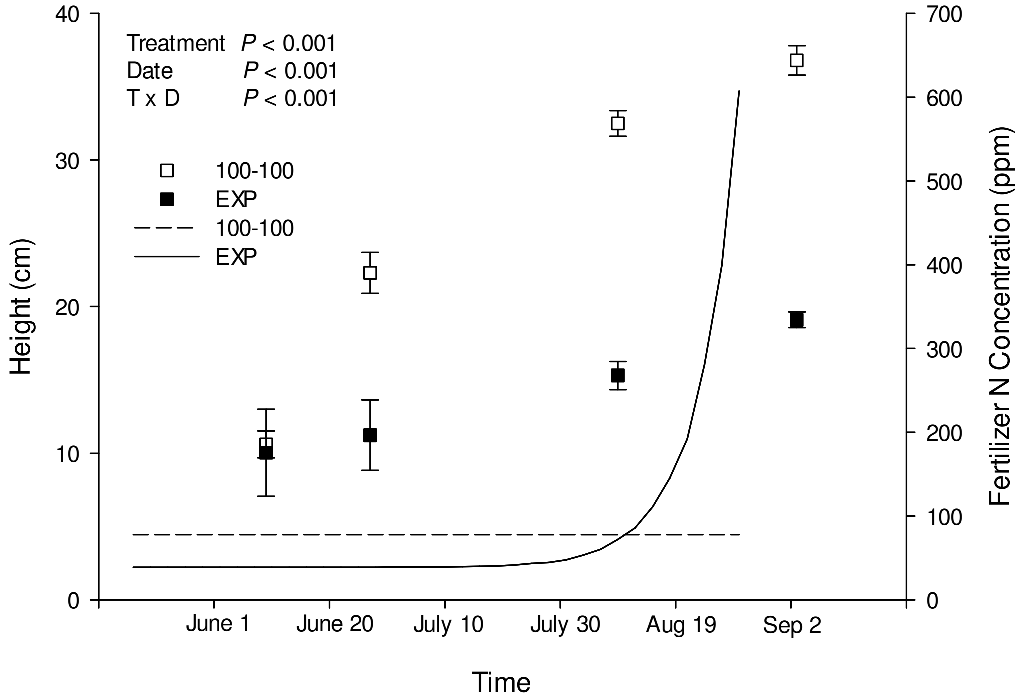

Figure 2-1: Average height of seedlings treated with an exponential (EXP) and constant rate (100-100) fertilization regime over the 2010 growing season. Error bars indicate one standard error of the mean (n = 9). Lines indicate the N concentration for both treatments over time as a surrogate for the fertilizer regime. The *P*-values are the results of a repeated measures ANOVA performed to compare seedling height by fertilization regimes across the growing season.

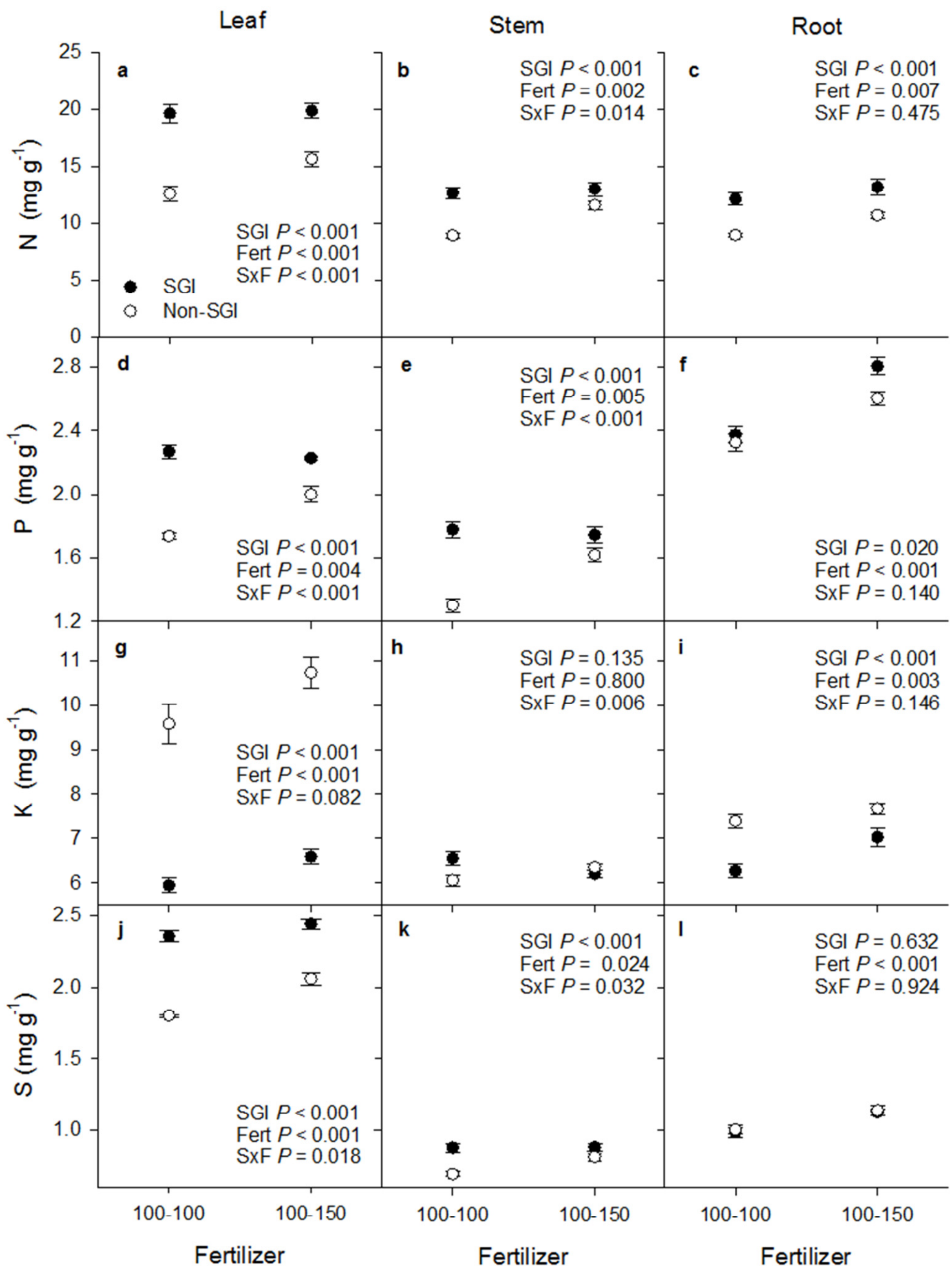

Figure 2-2: Average total N, P, K, and S concentrations in stem, root, and foliar tissues of aspen seedlings that had been treated with different combinations of two fertilizer rates and a shoot growth inhibitor (SGI). Leaves were sampled in late August while stems and roots were sampled on dormant seedlings in November. Error bars indicate one standard error of the mean (n = 9).

Endnotes
A version of this chapter has been published. Schott et al. 2013. New Forests. 1-13.

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Chapter 3: Outplanting performance of nutrient loaded aspen (*Populus tremuloides*) seedlings on an oil sands reclamation site

3.1 Introduction

The successful reclamation of mined areas to forested lands is a challenge that must be faced in an integrative, multi-disciplinary manner. In reclaimed mine areas, resource managers have the unique ability to manipulate factors as variable as capping material, fertilization regime, and seedling stock in order to facilitate tree seedling establishment and influence the trajectory of a reclaimed ecosystem (MacDonald et al. 2012). However, the optimum combination of these factors for facilitating seedling performance remains poorly understood.

Surface mine reclamation projects across the globe must tackle obstacles such as such as low soil fertility, competition from other vegetation, and soil compaction in order to establish tree seedlings on former mining sites. Successfully reclaiming mined areas remains a priority in the Oil Sands Region of northern Alberta, Canada where environmental law requires the reclamation of land to “equivalent land capability” after mining operations cease (Alberta Land Conservation and Reclamation Council 1991). Oil sands mining disturbed approximately 71,500 ha of land between 1967 and 2010, much of which was subject to surface mining characterized by the removal of up to 60 m of overburden (Government of Alberta, 2010). The disturbed organic matter and surface soils are combined to form capping material and stored separately from subsoils. Site reclamation involves forming landforms by contouring subsoils and the subsequent placement of a capping material (mixture of organic matter and surface soils). Due to variants in vegetation and topography, a variety of capping materials may be available in the region including forest floor material (FFM) and peat-mineral mix (PMM). Once soil placement has been completed, native species are used for revegetation. Oil sands reclamation areas are commonly fertilized with IAFs for up to five years after revegetation efforts begin (Pinno et al. 2012).

Despite being the most widely distributed tree in North America, the understanding of the requirements for trembling aspen (*Populus tremuloides* Michx.) seedlings to successfully establish canopy closure on mine reclamation sites remains limited (Burns and Honkala 1990; Macdonald et al. 2012). Producing and obtaining aspen seedling stock that reliably shows outplanting success on stressful sites is a continuing challenge (van den Driessche et al. 2003; Landhäusser et al. 2012; Macdonald et al. 2012). High quality seedling stock is critical for reforestation and reclamation efforts.

However, a recent study demonstrated the use of shoot-growth inhibitors (SGIs) such as paclobutrazol, to be a precursor for producing dormant aspen seedling stock with high woody nutrient concentrations (Schott et al. 2013). Additionally, seedlings treated with SGI exhibit desirable root carbohydrate reserves and root:stem ratios (RSRs), seedling stock measures known to be important for aspen outplanting success (Grossnickle, 2012; Landhäusser et al. 2012). The use of paclobutrazol has also been shown to increase plant tolerance to low temperatures and salt and water stresses (Fletcher and Nath 1984; Upadahyaya and Davis 1989; Sharma et al. 2011; Zhou et al. 2012). However, the outplanting success of aspen seedlings treated with SGI, or nutrient-loaded aspen seedlings, had not previously been studied.

The use of broadcast field fertilization is a practice commonly used in an attempt to improve tree seedling establishment on reclamation areas (Andersen et al. 1989; Casselman et al. 2006; Pinno et al. 2012). To date, the immediately available fertilizers (IAFs) commonly used in agriculture are also most prevalent in reclamation. These fertilizers release large amounts of nutrients upon application, often resulting in nutrients being lost from the rooting zone and contaminating the environment through leaching. Additionally, the growth of competing understory vegetation is stimulated through the application of such fertilizers (Chang and Preston 2000).

Controlled-release fertilizers (CRFs) have gained attention for their potential to minimize the negative effects of IAFs while still mediating nutrient limitations on harsh sites. Unlike IAFs, CRFs slowly release their nutrients, minimizing leaching and continually providing seedlings with nutrients throughout the growing season in a manner than parallels seedling nutrient demands (Goertz, 1993). Though CRFs have been shown to have positive effects on tree seedling outplanting performance in other forestry settings (Arnott and Burdett, 1988; Fan et al. 2002; Jacobs et al. 2005), their effectiveness in stimulating aspen seedling growth and performance in a mine reclamation setting remains unclear. In previous studies, aspen seedlings have shown varied responses to IAF fertilization trials with nitrogen, phosphorous, soil pH caused nutrient deficiency or excess, and water all limiting growth depending on site characteristics (DesRochers 2003; Van Cleve 1973; van den Driessche, 2003). To date, however, no studies have assessed aspen seedling growth response to both IAF and CRF on contrasting capping materials.

In this study, I compare the potentially interacting effects of nutrient status of aspen seedling stock, capping material, and field fertilization regime on aspen seedling outplanting performance over two growing seasons. The field study was conducted on a mine reclamation site in the oil sands region of northern Alberta, Canada.

3.2 Methods

3.2.1Research area

This study was carried out on Mud Dump 8 (MD8) (56° 57' 41" N, 111° 18' 49" W), an overburden dump located within Suncor Energy Inc.'s Millennium Mine oil sands lease.

This study was conducted within the Central Mixedwood subregion of the Boreal Forest Natural Region of Alberta (Natural Regions Committee, 2006) which is characterized by an undulating landscape that includes upland aspen, white spruce (*Picea glauca*), jack pine (*Pinus banksiana*) or mixed forests and wetlands dominated by black spruce (*Picea mariana*) fens. Climate at the Fort McMurray weather station (56°39'0" N, 111°13'0" W, Environment Canada climate normals 1971-2000) has average daily temperatures ranging from -18.8 °C in January to 16.8 °C in July with an annual average of 0.7°C. The average temperature during the April-September growing season is 11.7°C with the rest of the year averaging -10.2°C. Average annual precipitation totals 455.5 mm including 342.2 mm of rainfall (all of which falls during the growing season) and 155.8 mm of snow. Precipitation in Fort McMurray during the 2011 growing season totaled 237.9 mm and the average temperature during this period was 12.2°C. From October 2011 to March 2012, 50.8 mm of precipitation fell as snow, and the average temperature was -6.7°C. The 2012 growing season had 349.8 mm of precipitation and an average temperature of 12.7°C (Environment Canada, 2013).

3.2.2 Site setup and capping material

Soils utilized as capping materials for reclamation were originally located on an upland area within the footprint of the overburden dump. The area occupied a "d" ecosite (Beckingham et al. 1996), a classification characterized by an early-successional overstory of aspen, balsam poplar (*Populus balsamifera*) and/or white birch (*Betula papyrifera*), submesic to mesic moisture regime and medium nutrient regime. From August 2008 to January 2009 the site was stripped and soils were stockpiled according to soil type. The forest floor material was composed of the top 30 cm of natural upland soil and included L, F, H, and A and part of the B horizons of a gray luvisol. Once the FFM was stripped, another lift of the remaining B and upper part of the C horizon to a soil depth of 60 cm was stripped separately. Suitable C-horizon material (low sodic till) was then salvaged to a maximum depth of 300 cm. Peat-mineral mix was derived from peatland sites, where the organic soil layer including some of underlying mineral soil was salvaged to a depth of 30 cm. All salvaged materials were stockpiled for 2 years before being used as capping materials for the overburden dump.

In 2009, the construction of the sodic overburden dump, Mud Dump 8, was completed and the landform surface was covered with 1 m of the low sodic till C-horizon material (salvaged between 60 and 300 cm). For the study, a 1.3 ha area was selected and covered with either FFM or PMM materials. For the FFM treatment, the low sodic till was covered with 30 cm of subsoil followed by 20 cm of FFM and for the PMM treatment the low sodic till was covered with a 50 cm layer of PMM. Surface soil placement began in August 2010 and was completed by early June 2011. The FFM and PMM capping materials were placed in alternating strips across the research area; each strip was 20 × 65 m, arranged as five paired blocks (40 m × 65 m). A fertilizer regime treatment was superimposed on the material treatments by dividing each strip into four 15 m by 15 m fertilizer treatment plots for a total of 40 plots. The plot size allowed for a 5 m buffer where capping materials changed and fertilizer treatments were spatially separated. Fertilizer regimes (see below) were randomly assigned to these plots.

3.2.3 Fertilizer treatments

Four fertilizer treatments were applied: (1) high application rate of an immediately available fertilizer (high IAF); (2) low application rate of and IAF (low IAF); (3) high application rate of a controlled-release fertilizer (CRF); and (4) an unfertilized control. The IAF was a 20-20-20 N-P-K water soluble Peters General Purpose fertilizer while the CRF was a 15-9-12 N-P-K granular Osmocote Plus fertilizer with an 8-9 month release period. Both products contain a mixture of chelated micronutrients and are produced by The Scotts Company LLC (Marysville, OH, USA).

Fertilizer was hand spread with and EarthWay Ev-N-Spread hand held seeder (EarthWay; Bristol, IN, USA) from June 28-30th 2011. Fertilizer rates were chosen to allow for comparison between treatments. Plots (n=5) assigned to the high IAF treatment were fertilized at a rate of 500 kg fertilizer ha^{-1} or 15 kg fertilizer per 15 m × 15 m plot. Low IAF plots received half of the previous fertilization rate (i.e. 7.5 kg per plot). CRF treated plots received 20 kg fertilizer per plot equivalent of 670 kg of fertilizer ha^{-1}) which resulted in a nitrogen application rate that was equivalent to the high IAF treatment.

3.2.4 Aspen seedling stock type production and outplanting performance

For this study, aspen seedlings were grown under two different sets of conditions resulting in: high and low feed seedlings. All seedlings were sown and grown in Styroblock containers (5-12A (220 ml), Beaver Plastic, Edmonton, Alberta) for one growing season at Smoky Lake Nursery (Smoky Lake, Alberta, Canada 54°6′ N; 112°28′ W; 598 m a.s.l.). Seedling production followed the protocols developed for nutrient loading of aspen described and tested in Chapter 2. Seedlings were grown from open-pollinated, Fort McMurray sourced seed sown on March 26, 2010 into cavities filled with peat and perlite (9:1, by volume). Standard greenhouse growing conditions for aspen (temperature: 21˚C average, max 28°C, min 18°C; relative humidity: >70%) were used to germinate and establish the aspen seedlings. Seedlings were fertigated with a balanced nutrient fertilizer (78 ppm N, 77 ppm P, 161 ppm K, 46 ppm S and a balanced blend of chelated micronutrients) which were included in every watering with an automated mist irrigation system until May 12, 2010, at which point seedlings were moved outside. On June 24, all seedlings were thoroughly watered with a solution containing a shoot growth inhibitor Bonzi® (conc. 5 mL L^{-1} of a solution containing 4g L^{-1} of the active ingredient paclobutrazol; Syngenta, North Carolina, USA.). Regular fertigation continued until seedlings had set bud (July 12). On that date the seedlings were separated into two treatments: standard and high feed. Standard feed seedlings continued to receive the same fertilizer concentration while nutrient concentrations were doubled for high feed seedlings (156 ppm N, 154 ppm P, 322 ppm K, 92 ppm S, and twice the chelated micronutrient concentration). Accordingly, under the naming scheme used in Chapter 2, standard feed seedlings would be equivalent to 100-100 SGI while high feed seedlings would be 100-200 SGI. Fertigation was continued at these levels until September 4, after which all fertigation ceased for all seedlings and seedlings were only watered as necessary. The seedlings were allowed to naturally go dormant and harden while remaining outside. In November 2010 seedlings were lifted, bagged, and stored frozen (-3 °C) in waxed cardboard boxes until June 2011. Both seedling stock types were similar in height at the time of outplanting (36.4 cm; $p = 0.792$).

Foliar nutrient concentrations measured on seedlings in the nursery showed significantly higher concentrations of N, P, K, and S in the high feed seedling stock type (Table 3-1). An early study (see previous chapter) showed that pre-senescence late-growing season foliar nutrient concentrations are well correlated with dormant stem and root tissue nutrient concentrations (Schott et al. 2013). Foliar samples were collected in late summer of 2010 to determine the nutrient status of each stock type. An early study (see previous chapter) showed that leaf nutrient concentrations during the latter part of the growing season are well correlated with nutrient concentrations in dormant stem and root tissues (Schott et al. 2013). 5 foliar samples (each consisting of leaves from 10 seedlings) were collected from the upper 10 cm of seedlings of both high and standard feeds at the nursery.

To determine foliar nutrient concentrations all samples were washed with de-ionized water, dried to constant mass at 70°C for two days in an oven and ground to a fine powder in a Wiley Mill (Thomas Scientific, Swedesboro, NJ, USA) passing 40-mesh (0.4mm) screen. Total N concentration of foliar tissue samples was determined by Kjeldahl digestion (Kalra and Maynard 1991). Concentrations of P, K and S were determined by inductively coupled plasma optical emission spectrometry (ICP-OES) after microwave digestion (EPA Method 3051, U.S. Environmental Protection Agency, Washington, D.C.).

Permanent tree plots were established within each fertilizer block to include a total of 36 tree seedlings. Each plot included 18 high and 18 normal feed seedlings. In August of 2011 tree seedling mortality, heights and calipers were measured for each seedling in each permanent measure plot and in August 2012 mortality and height measurements were repeated. Leaf samples were taken during the growing season to assess seedling nutrient status (see also below).

In 2011, one leaf was collected from each seedling within each measurement plot and pooled by stock type, resulting in two composite samples of 18 leaves per subplot. In addition, in August of 2011 two seedlings of each stock type with approximately average height were carefully excavated in each subplot, but outside the permanent tree plot, placed into plastic bags, and stored frozen. Leaves and petioles were removed from stems and the stem and root tissues were separated. All tissues were oven dried at 70°C to constant mass and weighed to determine leaf, stem, and root dry weights.

3.2.5 Soil analysis

On May 11, 2011, pre-fertilization soil characteristics were determined for each capping material by collecting two samples from the top 20 cm of soil from each of the 40 subplots (Table 3-2). Samples were analyzed for texture, available nitrate, plant available sulfur, plant available phosphate, plant available potassium, total phosphorous, total Kjehldahl nitrogen, conductivity, and pH. Soil texture measures were calculated from hydrometer readings and percent total clay (<2μm), silt (2-50μm), and sand (>50μm) were determined (Carter and Gregorich, 2008). For nitrate measurements, a dilute calcium chloride solution was used to extract available nitrate and nitrite from the soil. The nitrate was then passed through a copperized cadmium column to quantitatively reduce the nitrate to nitrite. After being diazotized with sulfanilamide and coupled with N-(1-naphthyl) ethylenediamine dihydrochloride, the samples were colorimetrically measured at 520nm (Alberta Agriculture, 1988). Plant available sulfur and potassium, were determined by ICP-AES on soil that was extracted with a weak calcium chloride solution (Carter and Gregorich, 2008). Total Kjeldahl nitrogen (TKN) and phosphorous (TKP) were determined by using a SmartChem Discrete Wet Chemistry Analyzer (Westco Scientific Instruments Inc, Danbury, Ct) set to 660 nm to analyze samples that had been ashed with sulfuric acid (Rutherford et al, 2008). A slurry of 1 part dry soil mixed with 2 parts de-ionized water (by volume) was allowed to stand with occasional stirring for 30-60 minutes. The pH of the resulting slurry was measured with a pH meter, and a conductivity meter was used to measure the electrical conductivity of the filtered extract (Carter and Gregorich, 2008).

3.2.6 Soil nutrient supply

Plant Root Simulator probes (Western Ag Innovations Inc., Saskatoon, Canada) were used in 2011 and 2012 to measure bioavailable nutrients in the soil. Individual plant root simulator probes consist of a 10 cm^2 ion exchange resin membrane surrounded by a plastic frame and handle. Plant root simulator probes were used in pairs consisting of one anion probe and one cation probe whose membranes adsorb the associated soil nutrients. In both 2011 and 2012, two burials (June 8-July 26 & July 26-September 6) were used to avoid probe saturation and minimize any degradation of the accumulated nutrients by soil microbes. Each burial consisted of four pairs of plant root simulator probes being buried per subplot; two pairs at each of two opposite corners of the permanent tree measurement plots. In 2011 two samples were analyzed per subplot for a total of 80 samples/burial period while in 2012 (when soil nutrient supply had decreased without additional fertilizer input) all probes in each subplot were analyzed together for a total of 40 samples/burial period. In order to capture both vertical and horizontal nutrient flow, each probe was inserted into the soil by hand at a 45° angle. The entire membrane was buried with only part the handle remaining visible above-ground. After burial, the soil around the probes was pressed to ensure contact between the soil and membrane.

Upon removal, excess soil was removed and probes were placed in sealable plastic bags and stored in a cooler for less than 4 hours before being moved to a refrigerator. Probes were stored in a refrigerator (maximum 4 days) until cleaning. The membranes and plastic handles of the probes were thoroughly cleaned with de-ionized water and a toothbrush before being sealed in new, sealable bags and being sent in an insulated box to Western Ag Innovations for analysis.

At Western Ag Innovations, the samples were processed according to their established methods. First, the ions were desorbed off the ion-exchange membrane using either 0.5 N HCL. The resulting eluate was colorimetricaly analyzed for NO_3^-, NH_4^+, and P. Ca, Mg, Fe, Zn, Mn, Cu, Al, Pb and Cd were measured by atomic absorption inductively-coupled plasma spectrometry while K content results were obtained via flame emission. Results from early-season and late-season burials were pooled for each growing season and the results of plant root simulator probe analysis are expressed in 10 cm^{-2} 91 $days^{-1}$.

3.2.7 Statistical analyses

This study was set up as a 2 x 4 x 2 factorial block design with 5 replications to determine the effects of aspen stock types with different nutrient status (standard and high feed), fertilizer application (high and low IAF, CRF, and control) and capping material (FFM and PMM) and on outplanting performance. Seedling height growth; root collar diameter; RSR; leaf, stem, and root dry weight; N, P, and K foliar nutrient concentrations; and soil N, P, and K supply data were analyzed using Analyses of Variance with the GLM procedure. T-tests were performed using the univariate procedure to compare pre-planting characteristics of high and standard feed seedlings and PMM and FMM capping materials (SAS 9.2, SAS Institute, Cary, NC, USA). Student-Newman-Keuls tests, which control Type I error at an experimental level, were used to compare significant differences among treatment means. Repeated measures ANOVAs were used to compare 2011 and 2012 height growth and foliar nutrient concentrations. All data met the assumptions for normality and homogeneity of variance except for soil available P and K data which were transformed with base 10 log transformations, all descriptive statistics shown in this paper are from non-transformed data.

3.3 Results

3.3.1 First outplanting season seedling morphology and growth

High feed seedlings were, on average, taller, had larger root collar diameters and greater root, stem, and leaf dry weights than standard feed seedlings at the end of the first outplanting season (Table 3-3) across all fertilization regimes and soil types. Only root:stem ratio (RSR) was greater in standard feed than high feed seedlings (Table 3-3).

High feed seedlings had a higher leaf dry weight than standard feed seedlings on both capping materials. Furthermore, leaf dry weight was greater on PMM than FMM, though this difference was more pronounced for high feed seedlings than standard feed seedlings, resulting in a material by feed interaction ($p = 0.014$; Figure 3-1). However, none of the other growth variables showed this response to material and feed (all $p \geq 0.098$).

When unfertilized, none of the measured growth variables differed between PMM and FFM plots for both seedling types however, when fertilized a positive growth response was only recorded when seedlings were growing in PMM (all variables material by fertilizer interactions, $p \leq 0.021$) (Table 3-4). There was no growth response to fertilization on FFM. Seedlings in the fertilized PMM plots, treated with the high and low rate IAF, had greater caliper growth, and stem and lef dry mass than those treated with CRF (Table 3-4).

Overall root dry weight was greater on PMM than FFM capping materials (PMM: average 7.71, SEM 0.35; FFM: average 6.77, SEM 0.29; $p=0.016$) and was not affected by fertilizer regime ($p = 0.512$). RSR was greatest on control plots (average 1.39, SEM 0.04), followed by CRF plots (average 1.22, SEM 0.06), and least on the plots with IAF (low IAF: average 1.08, SEM 0.03; high IAF: average 1.03, SEM 0.04) but was not affected by capping material (fertilizer $p < 0.001$; material $p = 0.078$). Overall, after the first outplanting season, height growth was greater on PMM than FMM ($p > 0.001$, Table 3-4) and regardless of capping material seedlings that received IAF had the greatest height growth in the field followed by CRF and control ($p < 0.001$; Table 4).

3.3.2 Foliar nutrient concentrations after the first growing season

After the first growing season in the field, foliar N concentrations were higher in standard feed seedlings (average = 30.76 mg g^{-1}; SEM = 0.50) than in high feed seedlings (average = 29.15 mg g^{-1}, SEM = 0.48; $p = 0.015$) but did not differ between unfertilized seedlings growing on the PMM and FMM capping materials ($p < 0.001$; Table 3-5a). However when fertilized, foliar N concentrations were higher in seedlings growing on PMM compared to FMM material (fertilizer by material interaction, $p < 0.001$). The highest foliar N was measured in seedlings that had received the high IAF treatment regardless of capping material.

Foliar P in standard feed seedlings were 10.8% higher than high feed seedlings ($p < 0.001$). Foliar P concentrations were the same on FMM regardless of fertilizer regime. On PMM, seedlings fertilized with an IAF had higher foliar P concentrations than all other fertilizer and capping material combinations (Table 3-5).

Foliar K concentrations were higher for standard feed seedlings than high feed seedlings with the highest foliar K concentration observed in standard feed seedlings on PMM plots (PMM: average=9.88, SEM=0.46; FMM: average=8.89, SEM=0.28). However, high feed foliar K concentrations were lower and did not differ by capping material (average=7.85, SEM=0.33; capping material by feed interaction $p = 0.007$). On PMM capping material, all fertilized seedlings had higher foliar K concentrations than control seedlings. However, on FFM only fertilization with IAF at high levels resulted in foliar K concentrations greater than those observed on control plots (Table 3-5).

3.3.3 Growth performance in 2012 vs 2011

Over all treatments, seedlings grew 52.2% more in height in 2012 than in 2011 ($p < 0.001$). Seedling mortality was low throughout the study and over both years. In 2012 high feed seedlings continued to show greater height growth than standard feed seedlings (21%) on PMM, while there was no difference in height growth between standard and high feed stock types when growing on the FMM (material by feed interaction $p = 0.022$; Figure 3-2a). As in 2011, seedlings on PMM outperformed those on FFM in 2012; however, that disparity became more prominent, increasing from 21% in 2011 to 56% in 2012 (material by year interaction, $p < 0.001$) (Figure 3-3b). After two growing seasons 4.9% of the seedlings had died; however mortality was not influenced by fertilizer regime, material, or seedling feed (all $p > 0.385$, data not shown).

In 2012 only CRF fertilization resulted in height growth greater than that on control plots, while in 2011 all fertilized seedlings (IAF and CRF) grew more than the unfertilized seedlings (fertilizer by year interaction $p < 0.001$; Figure 3-3a). In contrast to 2011, where seedlings growing on PMM and fertilized with IAF showed the greatest height growth (Table 3-4), the greatest overall height growth in 2012 was achieved by seedlings on PMM that were fertilized with CRF (material by fertilizer interaction $p = 0.016$). Seedlings treated with IAF did not continue to show increased height growth on PMM or FMM compared to the control. In 2012, seedlings on unfertilized FMM plots performed the same as high IAF and CRF plots and better than low IAF plots (Figure 3-2b).

High feed seedlings planted on peat-mineral mix capping material and fertilized with controlled-release fertilizer had the greatest two-season outplanting growth reaching an average height of 111 cm (SD = 6.74) by the end of 2012. The treatment combination that resulted in the shortest seedlings at the end of 2012 was standard feed seedlings on FFM fertilized with low levels of IAF; these seedlings reached an average height of only 73.7 cm (SD = 1.99).

3.3.4 Soil nutrient supply

The total soil N soil supply was much lower in 2012 than 2011 for all treatments ($p < 0.001$; Table 6). However in 2011, soil N supply was greater on PMM than FFM capping materials while N supply was greatest in plots with the high IAF treatment followed by low IAF, CRF, and the unfertilized control (fertilizer $p < 0.001$; Table 3-6). In 2012, there was no difference in soil N supply by fertilizer regime or capping material (fertilizer, material, and their interaction all $p \geq 0.28$) resulting in a significant year by fertilizer by material interaction p = 0.0157 (Table 3-6).

Overall average soil P supply was 10 times higher in the high IAF and 5 times higher in the low IAF treatment than the control and CRF treatments ($p < 0.01$; Table 3-6). However, soil P supply did not vary by either year (p = 0.21) or capping material (p = 0.521; Table 3-6.)

In 2011 and 2012 FMM plots had an average of 178 μg 10 cm^{-2} 91 $days^{-1}$ soil K supply compared to 32 μg 10 cm^{-2} 91 $days^{-1}$ of K supply on PMM plots ($p < 0.001$). In 2011 soil K supply did not differ by fertilization regime (p = 0.188; Table 3-6). However in 2012, soil K supply was higher in the high IAF FFM plots compared to the other fertilizer treatments (p = 0.006; Table 6). As this was the singular significant change among the treatment combinations, overall average soil K supply measures did not change from 2011 to 2012 (p = 0.48).

In 2011, the relationship between total soil N supply and height growth followed a saturation curve where at height growth of seedlings did not increase at total available soil N levels above 450 μg 10 cm^{-2} 91 $days^{-1}$ (Figure 4a). High feed seedlings consistently outperformed the low feed seedlings regardless of soil N supply (Figure 4a). In 2012 the soil N supply was much lower and the differences in N supply among treatments much narrower. The relationship found in 2011 was not apparent in 2012; however, the treatment with the highest height growth also had the highest soil N supply with the other treatments showing only minimal differences in height growth (Figure 3-4b). There was no linear relationship between total soil N supply and height growth in 2012 (p = 0.5761) but high feed seedlings still outperformed standard feed seedlings overall.

3.4 Discussion

In the first growing season nutrient loaded aspen seedlings had greater height growth than standard feed seedlings, regardless of material and field fertilizer application. Average yearly height growth was 15.2% greater for high feed seedling than standard feed seedlings; the improved performance observed in the second outplanting season was not merely additive as height growth, rather than overall height, continued to be greater for high feed seedling into 2012. Measures of root collar diameter, root dry weight, stem dry weight, and leaf dry weight taken in 2011 were also greater in high feed seedlings than standard feed ones. Regardless of seedling feed, the aspen seedlings used in this study showed very high survival rates (95.1%), likely due to the use of the shoot growth inhibitor paclobutrazol and moving seedlings outside after only six-weeks in the greenhouse. These techniques result in improved carbohydrate reserves and RSRs, factors know to be important aspen seedling stock quality measures, as compared to conventionally produced aspen seedlings (Landhäusser et al. 2012; Schott et al. 2013).

The differing responses of seedling height growth to field fertilization regime in 2011 and 2012 parallel the release rates of the different fertilizers with IAF resulting in the greatest height growth in the first outplanting season (2011) while only plots fertilized with controlled release fertilizer performed better than the control plots in 2012. The high (500 kg/ha) and low (250 kg/ha) application rates of IAF did not result in increases of any performance measure under any conditions, indicating that the additional fertilizer applied in the high regime was either lost from the rooting zone or picked up by competing vegetation, whose competitive effects might have negated any benefit from additional fertilizer application.

In both the first and second outplanting seasons, seedlings growing on PMM outperformed seedlings on FFM (in terms of seedling height in 2011 and 2012, and leaf, stem, and root dry weight, and root collar diameter in 2011). This resulted in capping material being a factor in all significant interactions. It is likely that this response is at least partially attributable to differential herbaceous competition development on the two materials. The average percent cover of herbaceous species was greater on FMM than PMM plots with this difference being more pronounced in 2011 than 2012 (personal observation). Differences in growth parameter response to capping material were most pronounced when the sites were fertilized, especially in the first year. This is consistent with the findings of Pinno et al. (2012) who observed that aspen established from seed on both PMM and FFM capping materials exhibited only minimal differences in height growth response with no fertilization, though the application of a combination NPK fertilizer resulted in seedlings showing better height growth responses on PMM than FFM. This may be due to the higher moisture holding and nutrient adsorption capacities of PMM capping materials, which had more silt and clay, as compared to the sandier FMM. The PMM acts as a buffer to water and nutrient loss, retaining them within the rooting zone of aspen seedlings for longer than FFM. Water availability is of particular importance when aspen seedling nutrition needs are met as water and nutrients often co-limit aspen seedling growth so that an increase in one without an increase in the other may not translate to improved seedling performance (van den Driessche et al. 2003).

In 2011 capping material and fertilizer regime affected aspen seedling foliar N, P, and K concentrations in similar ways; seedlings on PMM generally had higher foliar concentrations than those on FFM when fertilized. However, lower foliar nutrient levels were observed in high feed seedlings than standard feed seedlings, likely the result of a dilution effect resulting from the greater growth and higher leaf dry weight of high feed seedlings.

Despite a wide range of available soil N levels in 2011, the marginal returns on seedling height growth with higher soil available nitrogen rates were minimal (especially at levels above 500 $\mu g\ cm^{-2}$ 94 $days^{-1}$) suggesting a poor fertilizer use efficiency at higher levels of available N corresponding to the high IAF fertilization rates on both FF and PMM and also low on PMM. Though high application levels of IAF increased soil available P and K levels into the second growing season, this increase did not result in greater seedling growth. This suggests that these nutrients may not have been limiting on the reclamation site studied. Despite the 8-9 month release rate of the CRF, in 2012 available N levels varied little by capping material or fertilizer regime and the available P and K measures of plots treated with CRF did not differ from those of control plots in either year.

The high feed seedlings produced with SGI application and high mid-season fertilization rates had low mortality and high outplanting performance on an oil sands reclamation area. Furthermore, PMM capping material results in better aspen seedling growth than FFM. Low levels of IAF application achieved the same outplanting performance as the high IAF application. Controlled release fertilizers can improve aspen seedling height growth into the second growing season and CRF application methods and rates that optimize reclamation performance should be further studied.

Tables

Table 3-1: Average foliar nutrient concentrations (standard error of the mean) of high and standard feed seedling stock types measured from foliage removed before leaf senescence in early September 2010 prior outplanting (n=10).

Nutrient ($mg\ g^{-1}$)	Seedling feed		p-value
	Standard	High	
N	18.9 (0.62)	27.0 (0.74)	< 0.001
P	2.73 (0.02)	3.17 (0.02)	< 0.001
K	5.02 (0.26)	8.46 (0.19)	< 0.001
S	2.06 (0.14)	2.45 (0.14)	0.013

Table 3-2: Average soil measures (standard error of the mean) of forest floor material and peat-mineral mix capping materials measured in June 2011 before planting and fertilization. Different letters denote significant differences between the materials (n=5).

		P-value	FFM	PMM
pH		0.858	6.64 (0.08)	6.67 (0.16)
sand	%	0.035	58.22 (1.57) a	52.28 (1.74) b
silt	%	0.070	28.02 (0.70)	33.53 (2.53)
clay	%	0.921	13.76 (1.09)	14.19 (3.99)
TKP	%	0.074	0.014 (0.00)	0.024 (0.01)
TKN	%	0.038	0.124 (0.01) b	0.318 (0.01) a
Conductivity	($dS\ m^{-1}$)	0.229	0.139 (0.01)	0.125 (0.01)
Available nitrate-N	mg/kg	0.152	3.51 (0.35)	3.75 (0.19)
Available phosphate-P	mg/kg	0.004	6.18 (0.69) a	3.41 (0.62) b
Available potassium	mg/kg	0.002	78.75 (3.36) a	65.53 (2.48) b
Available sulfate-S	mg/kg	0.004	11.04 (1.83) b	16.70 (2.80) a

Table 3-3: Seedling morphological characteristics after the first outplanting season (2011) average seedling morphological characteristics (standard error of the mean) (height growth, root collar diameter; root, leaf, and stem dry weight; and root:stem ratio) of high and standard feed seedlings. Root collar diameter and height growth were measured on all seedlings in the field while all other seedling characteristics were measured on a subset of seedlings excavated at the end of the 2011 growing season (n=5).

		Seedling feed		
		High	Standard	p-value
Height growth	(cm)	22.49 (0.89)	18.10 (0.80)	< 0.001
Root collar diameter	(mm)	8.52 (0.17)	6.94 (0.13)	< 0.001
Root dry weight	(g)	8.33 (0.31)	6.15 (0.24)	< 0.001
Stem dry weight	(g)	7.87 (0.38)	4.96 (0.21)	< 0.001
Leaf dry weight	(g)	5.47 (0.37)	3.40 (0.19)	< 0.001
Root:stem ratio		1.09 (0.03)	1.27 (0.04)	< 0.001

Table 3-4: Average (standard error of the mean) of height growth, root collar diameter, root:stem ratio, leaf dry weight, stem dry weight, and root dry weight in 2011 as influenced by capping material and fertilizer regime (n = 5). Letters within columns denote significantly different means (α = 0.05).

Capping material	Fertilizer regime	Height growth (cm)	Root collar diameter (mm)	Root:Stem Ratio	Leaf dry weight (g)	Stem dry weight (g)	Root dry weight (g)
FFM	Control	15 (1.4) b	7.2 (0.3) b	1.4 (0.06) ab	3.4 (0.5) b	6.0 (0.5) b	7.1 (0.6) a
	low IAF	21 (1.5) ab	7.7 (0.3) b	1.1 (0.05) bc	3.9 (0.4) b	6.3 (0.5) b	7.0 (0.6) a
	high IAF	21 (1.1) ab	7.0 (0.4) b	1.1 (0.05) c	3.0 (0.3) b	5.3 (0.7) b	5.6 (0.5) b
	CRF	17 (1.4) b	7.4 (0.3) b	1.3 (0.09) ab	3.7 (0.3) b	5.1 (0.5) b	7.4 (0.5) a
PMM	Control	15 (1.7) b	7.3 (0.3) b	1.4 (0.06) a	3.2 (0.3) b	6.7 (0.5) b	7.4 (0.6) a
	low IAF	26 (1.5) a	8.8 (0.4) a	1.0 (0.03) c	6.4 (0.6) a	8.1 (0.8) a	8.1 (0.9) a
	high IAF	26 (1.4) a	8.6 (0.5) a	0.95 (0.04) c	6.9 (0.6) a	8.5 (1.0) a	7.9 (0.6) a
	CRF	22 (0.9) ab	7.9 (0.5) ab	1.2 (0.07) bc	5.0 (0.6) ab	6.7 (0.9) ab	7.4 (0.6) a
p-value		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Table 3-5: First outplanting season (2011) average (standard error of the mean) foliar N, P, and K concentrations collected from seedlings growing on two capping materials (PMM and FFM) that were treated with four fertilizer regimes. Foliar nutrient concentration was measured on leaves removed in late August of 2011. Letters within columns denote significantly different means (α = 0.05) (n=5).

Capping material	Fertilizer regime	Foliar N ($mg\ g^{-1}$)	Foliar P (mg g-1)	Foliar K (mg g-1)
FFM	Control	23.00 (0.68) e	2.11 (0.05) c	7.10 (0.19) c
	low IAF	23.83 (0.58) e	2.05 (0.07) c	7.64 (0.27) c
	high IAF	28.44 (0.56) d	2.14 90.07) c	9.86 (0.31) a
	CRF	27.42 (0.58) d	2.33 (0.09) bc	8.08 (0.37) bc
PMM	Control	23.63 (1.18) e	2.09 (0.05) c	6.45 (0.33) c
	low IAF	35.09 (1.09) c	2.58 (0.08) ab	9.57 (0.61) ab
	high IAF	40.46 (0.58) a	2.85 (0.15) a	10.45 (0.47) a
	CRF	37.71 (1.05) b	2.37 (0.05) bc	9.17 (0.28) ab
p-value		< 0.001	< 0.001	< 0.001

Table 3-6: Average total soil available N, P, and K (standard error of the mean) by capping material and fertilizer regime. Values are the sum of two plant root simulator probe measurements taken during the early season (early June to late July) and the late season (late July to early September) in both 2011 and 2012 (n=5). Letters within columns denote significantly different means (α = 0.05).

Material	Fertilizer Regime	N 2011	N 2012	P 2011	P 2012	K 2011	K 2012
FFM	Control	40 (11) dc	12 (1.5) a	4.1 (1.1) bc	2.6 (0.5) b	118 (26) bc	110 (29) bc
	low IAF	232 (59) c	15 (6.0) a	16 (4.1) ab	6.8 (1.4) b	238 (72) a	162 (51) b
	high IAF	639 (86) b	23 (8.8) a	27 (6.8) a	18 (4.8) ab	190 (37) ab	338 (82) a
	CRF	196 (61) c	14 (3.8) a	3.3 (0.2) c	3.1 (0.3) b	137 (29) b	132 (40) bc
PMM	Control	41 (23) dc	25 (7.8) a	2.2 (0.2) c	1.2 (0.2) b	14 (2.7) d	14 (2.5) c
	low IAF	746 (228) b	13 (6.00) a	22 (3.4) a	8.1 (3.6) b	27 (3.9) d	24 (2.1) c
	high IAF	1459 (209) a	11 (2.9) a	23 (9.9) a	33 (16) a	43 (9.4) cd	64 (22) bc
	CRF	427 (120) bc	31 (17) a	2.9 (0.7) c	3.2 (0.6) b	33 (16) d	37 (7.8) c
P-values		< 0.001	0.608	< 0.001	0.009	< 0.001	< 0.001

Figures

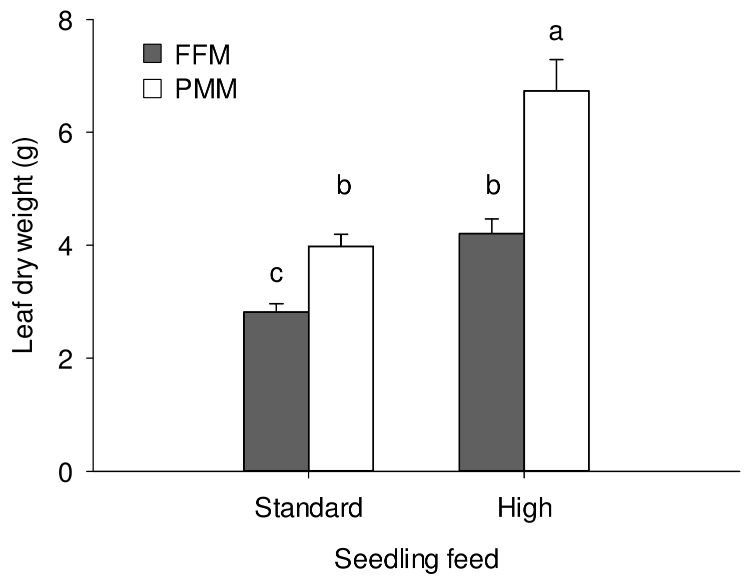

Figure 3-1: Average leaf dry weight of high and standard feed aspen seedlings planted in forest floor material (FFM) and peat-mineral mix (PMM) capping materials. Leaf dry weight measures were obtained from seedlings excavated in August of 2011. Error bars are standard error of the mean (n=5). Letters above bars denote significantly different means (α = 0.05).

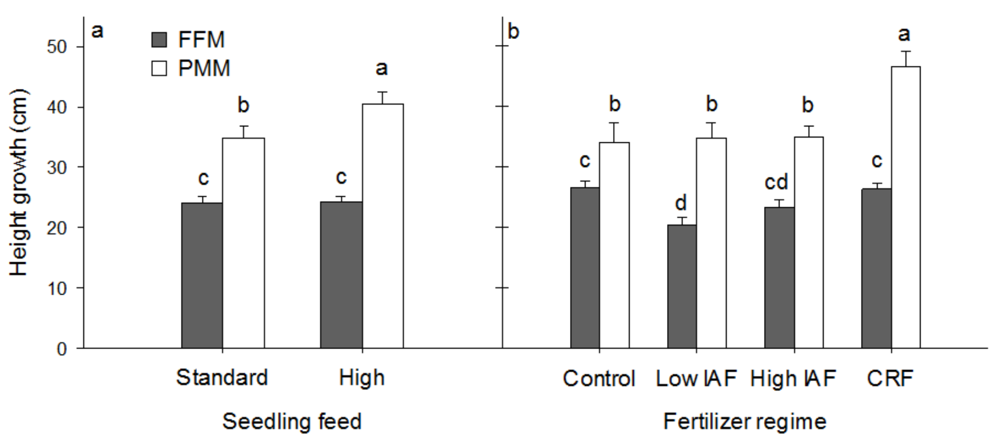

Figure 3-2: Average (standard error of the mean) aspen seedling height growth over 2011 and 2012 grown on forest floor material (FFM) and peat-mineral mix (PMM) capping materials for two seedling feed types (a) and under control, low and high immediately available fertilizer (IAF), and controlled release fertilizer (CRF) fertilization regimes (b). Error bars are standard error of the mean (n=5). Different letters above bars denote significant differences among means (α = 0.05).

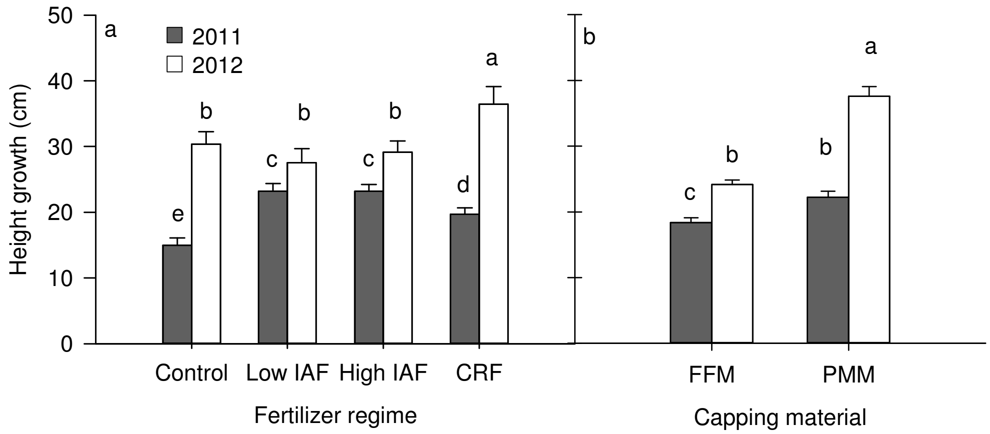

Figure 3-3: Average height growth in 2011 and 2012 of aspen seedlings under control, low and high immediately available fertilizer (IAF), and controlled release fertilizer (CRF) fertilizer regimes (a) and on forest floor material (FFM) and peat-mineral mix (PMM) capping materials (b). Error bars are standard error of the mean (n=5). Letters above bars denote significantly different means (α = 0.05).

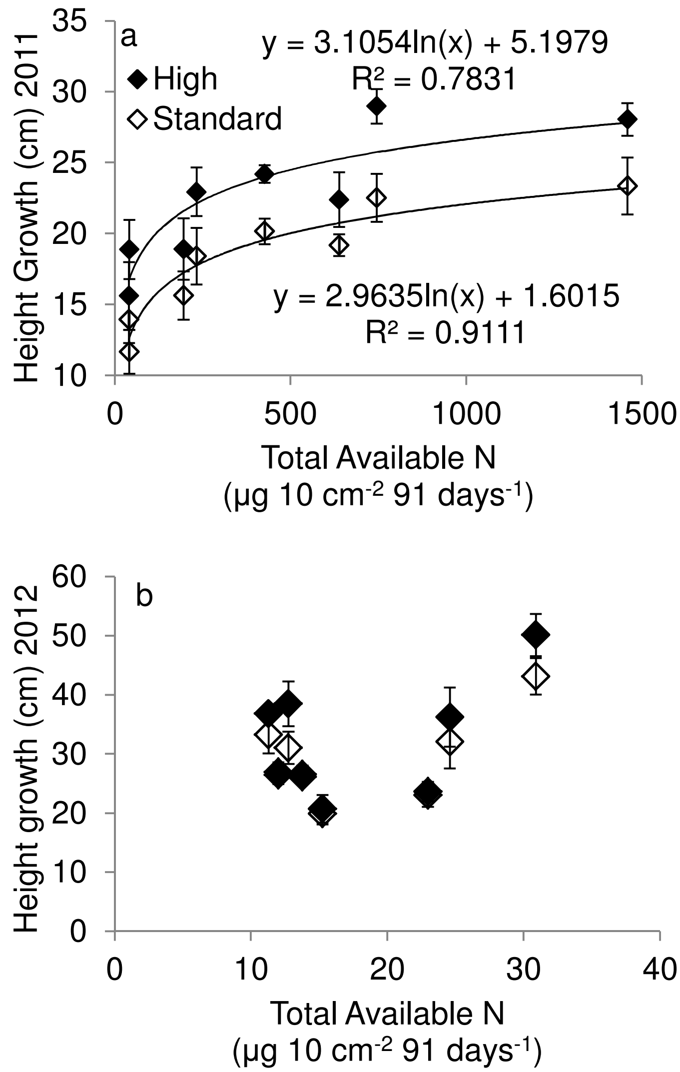

Figure 3-4: Relationship between height growth (cm) and total soil available N in years 2011 (a) and 2012 (b). Yearly soil available N values are the sum of two plant root simulator probe measurements taken during the early season (early June 8 to late July 26) and the late season (July 26 to September 6) in both 2011 and 2012. Total soil available N was calculated as the sum of two plant root simulator probe extractions during the early and late growing season with expressed as µg 10 cm^{-2} 91 $days^{-1}$ (n=5). Error bars are standard error of the mean.

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Chapter 4: General Discussion and Conclusions

4.1 Research summary

The main objectives of this thesis were to investigate methods for producing nutrient-loaded aspen seedlings and to evaluate the outplanting performance of two nutrient-loaded aspen seedling stock types on different capping materials under differing fertilization regimes.

In the first study, I tested a total of five seedling stock types (exponential, 100-100, 100-100 SGI, 100-150, and 100-150 SGI) for their morphological characteristics and nutrient and carbohydrate content and concentration. The results showed that despite having high tissue nutrient concentrations, exponentially fertilized aspen seedlings were not desirable for outplanting based on their short stature and poor root development that did not fill the seedling plug. However, seedlings treated with a SGI were able to incorporate the nutrients N, P, and S into their stem tissue and leaves at concentrations 25 to 30 % greater than non-SGI seedlings. The higher nutrient concentrations meant that despite the smaller size of SGI treated seedlings, their woody nutrient contents were similar to or only slightly lower than the contents of non-SGI seedlings. SGI-treated seedlings also met morphological measurement goals and achieved higher RSRs and carbohydrate concentrations than non-SGI seedlings. Increased mid-season fertilization rates increased seedling nutrient concentrations for non-SGI seedlings while higher fertilization rates applied to seedlings that had already received SGI only increased root tissue nutrient concentrations. Based on these findings, I conclude that treatment with a SGI is a precursor for successful nutrient loading of aspen seedlings.

The findings about the success of nutrient-loading aspen seedlings led me to begin the second study with great optimism for the outplanting success of the high (100-200 SGI) and standard (100-100 SGI) seedling stock types. The use of the SGI paclobutrazol and moving seedlings outside after only six-weeks in the greenhouse are two seedling production techniques applied to both seedling feed types that differ from the conventional methods used to produce aspen seedling stock for the Alberta oil sands region. My findings confirmed that nutrient-loading aspen seedlings in this manner did result in high seedling outplanting success and low mortality. While aspen seedling survival was 95.1% for the study presented in Chapter 3, a study located less than 500m away from that site reported the survivorship of conventionally produced aspen seedlings planted on PMM to be only 81.2% (Sloan and Jacobs, 2013).

Among the two seedling feeds tested in the study, high feed seedlings had 15.2% greater average yearly height growth than standard feed seedlings with that difference increasing to 21% on PMM capping material. After the first season, high feed seedlings had 22.8% greater root collar diameter, 35.4% greater root dry weight, 58.7% greater stem dry weight, and 68.9% greater leaf dry weight than standard feed seedlings. Lower root:stem ratios of high feed seedlings indicated that they were allocating greater proportions of their resources to above ground growth than standard feed seedlings. Overall, high feed seedlings showed better outplanting performance than standard feed seedlings.

High levels of fertilization with an IAF did not result in seedlings performing better than when low levels of IAF were applied despite resulting in dramatic increases in soil available nutrients in the first growing season and elevated soil available P and K levels into the second growing season. While first year aspen seedling performance was better with IAF application, overall height growth after two years of outplanting was greatest for seedlings fertilized with CRF. Both high and low feed seedlings showed better outplanting performance on PMM capping material than FFM and seedlings planted on PMM were more responsive to fertilization. Competing vegetation cover was dramatically influenced by both capping material and fertilizer regime (Alia Snively, personal communication) and likely played a large role in the responses of aspen seedlings to these factors.

When the findings of both studies are considered together, this thesis identifies a set of methods for producing aspen seedlings with high survivorship and outplanting performance. This research was undertaken specifically considering the methods and resources available to reclamation practitioners and there are many ways the findings can be incorporated into reclamation efforts.

4.2 Applications for reclamation

The findings that aspen seedlings can be nutrient loaded and that nutrient-loaded aspen seedlings have high outplanting success have the potential to improve restoration efforts that utilize aspen seedlings across North America. By demonstrating a replicable method by which to produce quality, nutrient-loaded aspen seedlings this study can serve as a framework for greenhouse operators to do the same. I recommend that containerized aspen seedlings be grown outside about six weeks after being planted, when greenhouse planting schedules allow. Furthermore, I recommend nutrient-loading aspen seedlings by treating them with a shoot growth inhibitor. This treatment allows seedlings to accumulate greater nutrient concentrations in their woody tissues and achieve more desirable morphological characteristics. These methods could also prove useful in the production of tree seedlings of other species that exhibit an indeterminate growth strategy. SGI treatment also results in a uniform crop of seedlings whose height can be controlled. This eliminates the need for the top-pruning of seedlings so that they fit into standard tree-planting boxes, a practice often undertaken as aspen seedlings grown in greenhouses without SGI can reach heights of more than 50 cm in a single growing season.

Both high (100-100 SGI) and standard (100-200 SGI) aspen seedling stock types performed well when outplanted in a reclamation area in the Alberta oil sands region, showing low mortality and vigorous growth. However, high feed nutrient-loaded aspen seedlings had greater outplanting performance than standard feed seedlings in both outplanting years. Therfore, I recommend high feed, nutrient-loaded aspen seedlings for use in reclamation efforts, especially on peat-mineral mix capping materials.

While IAF fertilizers did benefit aspen seedling growth in the first outplanting season their effects were fleeting and were not maintained into the second outplanting season. High IAF application levels resulted in no improvement in aspen seedling growth or morphology over low IAF application levels. Additionally, the lack of improved growth in the second growing season with IAF as compared to control plots highlights the short-lived nature of their effects on tree growth. These findings urge restraint when applying IAFs to boreal reclamation sites and suggest their use may not be beneficial to forest establishment. The beneficial effect of CRF on aspen seedling growth continued into the second growing season indicating it to be a better option for the long-term growth of aspen seedlings on reclamation sites.

Furthermore, peat-mineral mix capping material is not a detriment to the rapid establishment of aspen seedlings.

The findings of this study are limited by the two-year time frame of the outplanting study and further research and long-term monitoring will afford a better understanding of the role nutrient-loaded aspen seedlings can play in facilitating and potentially hastening the establishment of natural forest ecosystems on reclamation sites.

4.3 Future research

Additional research into the balance of nutrients applied to containerized aspen seedlings produced in greenhouse settings is necessary in order to continue improving the quality of aspen seedling stock. While the nutrient-loading study did not find any marked nutrient imbalances in aspen seedlings under the fertilizer ratio currently used, little is understood about optimum nutrient ratios for aspen seedlings. Furthermore, my findings suggest some limitations to K uptake and incorporation by seedlings treated with SGI and further attention should be dedicated to this matter.

The long-term effects of paclobutrazol on the greater ecosystem merit further investigation. While the effects of paclobutrazol on plant growth appear to be limited to the growing season in which it is applied plant tissues should be tested for paclobutrazol presence after outplanting. The potential effect of residual paclobutrazol on organisms that might consume paclobutrazol-treated tissue should also be studied.

One of the simplest concepts used in describing plant nutrient uptake and use is that of a "critical level." Though the implementation of the concept of a critical level varies somewhat in practice, a critical value can be generally described as the nutrient concentration required for optimum growth. One important limitation of the concept of critical levels is that they are calculated assuming that "no other factor is limiting or suboptimal" (Needham et al. 1990). This limitation means that critical levels can only be established for a given nutrient when all other elements can be proven to have been provided at optimum levels. That makes the concept somewhat difficult to apply to species about which only limited information is available and excludes the possibility of modeling complex nutrient interactions.

As nutrients are often co-limiting to seedlings, the interactions of such nutrients are of great importance. Thus, research evaluating two or more nutrients simultaneously by looking not only at their concentrations but also by using ratio techniques to better understand their interactions would be preferred. The simplest example of such techniques is comparing ratios of two nutrients. An example that is commonly used in plant nutrition is the comparison of N:P ratios in plant tissue to help better understand the nutrient limitations of a system (Koerselman and Meuleman 1996). One method for understanding complex nutrient interactions is the Diagnosis and Recommendation Integrated System, or DRIS. DRIS is still based in bivariate ratios of nutrient concentrations but takes into account many of these ratios (Guillemette and DesRochers 2008). An additional technique that could be applied in order to better understand aspen seedling nutrient status, Compositional Nutrient Diagnosis, uses ratios that include more than two nutrient concentrations, thus making it a multivariate analysis. In CND multivariate nutrient ratios, or CND scores, are calculated from the nutrient concentrations of key plant nutrients (Quesnel et al. 2006). The multivariate nature of CND allows for a more representative model of plant tissue nutrient composition than the bivariate CVA or DRIS (Parent and Dafir 1992; Quesnel et al. 2006).

Vector analysis is another method of evaluating and comparing plant nutrient concentration data. For vector analysis, a reference or control treatment is needed as well as a measure of dry weight of the tissue for which the nutrient concentration was analyzed. Vector analysis allows for the simultaneous analysis of the behaviors of multiple nutrients and can detect situations in which nutrients may be co-limiting. Multiple nutrient loading studies have relied on vector analysis to detect differences between fertilization regimes and indicate possible nutrient interaction causes for nutrient status results. Unfortunately, the role of dry weight in vector analysis means that vector analysis is not readily interpreted when the seedlings being compared are of drastically different dry weights, such as when seedlings are grown with and without SGI.

While aspen is an important species in North America, I hope that these findings have an international reach as they apply to the study of other trees with an indeterminate growth strategy. I propose that future research should investigate the application of shoot growth inhibitors to the production of seedlings of other species with an indeterminate growth strategy, such as eucalypts.

A better understanding of the role competing vegetation plays in the early growth of aspen seedlings planted on reclamation sites could deepen our understanding of the mechanisms behind multiple interactions observed in this study. Coordinating the results of vegetation studies with aspen seedling growth data would be one way to gain insight into this area and I hope further research in this area will be forthcoming.

Water availability is a key driver of aspen seedling growth and one of the greatest challenges facing reclamation in the Alberta oil sands region is the effective construction of landforms that are hydrologically self-sustaining. Upland forest areas are relied on to provide groundwater inflow to wetlands such as fens but resiliency of such systems to decadal-scale drought is unknown (Price et al. 2010). Furthermore, due to the large areas that must be reclaimed in the region, tree planting cannot always be scheduled to correspond to weather that minimizes seedling planting shock. Studies of other species have shown increased drought tolerance with shoot growth inhibitor treatment and it should be tested if this finding applies to aspen seedlings as well (Fletcher and Nath 1984). Accordingly, research into the relative drought tolerance and/or water use of nutrient loaded versus non-loaded seedlings could prove invaluable in creating landscapes with functioning hydrology.

While increasing seedling nutrient concentration is viewed as the goal of nutrient loading, it may make nutrient loaded seedlings more attractive as a food source. Ungulate browsing greatly limits forest regeneration in many forest types and browsing patterns have been shown to be highly systematic (Frerker et al. 2012) and the fecundity of herbivorous insects is known to be related to host plant quality (Awmack and Leather 2002). Further research could compare the rates of mammal, insect, and pathogen damage to nutrient loaded aspen seedlings to the rates observed for seedlings of lower nutrient concentrations giving us a better understanding of how nutrient loaded aspen seedlings fit into the reclamation ecosystem.

Additionally, I propose that similar experiments be carried out with directly placed capping material that has not been stockpiled. In the outplanting study the capping material was stockpiled for two years before being applied to the site. However, the negative effects of stockpiling on topsoil have long been recognized (Iverson and Wali 1982; Visser et al 1984; Miller et al. 1985; McKenzie and Naeth 2007) and the direct transfer of topsoil from the area to be mined to a reclamation area offers an opportunity to mitigate those effects. Soil structure and available nutrients are altered by stockpiling and vegetative and mychorrhizal propagules are also affected. These factors could affect responses of reclamation systems to fertilization. Therefore, I suggest further research into the effects of directly placed capping material on aspen seedling outplanting performance.

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Appendices

Appendix I. Analysis of Variance table showing the p-values for the main effects and their interactions for 2011 aspen seedling outplanting performance measures. Caliper, RSR, leaf, shoot, and root dry weights were all measured on seedlings excavated at the end of the first outplanting season. Different letters denote significant differences between main effects.

Variable	Caliper (mm)	RSR	Leaf dry weight (g)	Shoot dry weight (g)	Root dry weight (g)
Seedling feed	<0.001	<0.001	<0.001	<0.001	<0.001
High	a	b	a	a	a
Standard	b	a	b	b	b
Fertilizer regime	0.003	<0.001	<0.001	0.001	0.512
Control	c	a	d	d	X
low IAF	a	c	a	a	X
high IAF	b	c	b	b	X
CRF	b	b	c	c	X
Capping material	<0.001	0.078	<0.001	<0.001	0.016
PMM	a	X	a	a	a
FFM	b	X	b	b	b
Fertilizer x material	0.021	0.222	<0.001	0.003	0.14
Fertilizer x feed	0.474	0.234	0.236	0.171	0.392
Material x feed	0.313	0.867	0.014	0.098	0.26

Appendix II. Results of a repeated measures analysis of variance comparing the impacts of the main effects of year, seedling feed, fertilizer regime, and capping material and their interactions on aspen seedling height growth. Different letters denote significant differences between main effects.

	P-value				
Year	< 0.001	2011	b	2012	a
Seedling feed	< 0.001	High	a	Standard	b
Capping material	< 0.001	PMM	a	FFM	b
Fertilizer regime	< 0.001	Control	b	low IAF	ab
		high IAF	ab	CRF	a
Feed x material	0.022				
Feed x fertilizer	0.862				
Material x fertilizer	0.005				
Year x feed	0.332				
Year x material	< 0.001				
Year x fertilizer	< 0.001				
Feed x material x fertilizer	0.939				
Year x feed x material	0.351				
Year x feed x fertilizer	0.974				
Year x material x fertilizer	0.192				
Year x feed x material x fertilizer	0.846				

Appendix III. Average (SEM) height growth of outplanted standard and high feed seedlings on FFM and PMM capping materials fertilized under four fertilization regimes in 2011 (a) and 2012 (b). Error bars are standard error of the mean (n=5).