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

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ABSTRACT

Nutrient loading of nursery seedling stock of species with an indeterminate growth strategy is challenging and poorly understood. Here, we explore the use of two potential techniques for nutrient loading of trembling aspen (*Populus tremuloides* Michx.) seedlings: 1) exponential fertilization and 2) early shoot growth termination in order to divert assimilated nutrients and carbon to storage rather than to growth. In the first study, aspen seedlings were treated with an exponential and constant fertilization rate, both of which supplied the same amount of nutrients over the growing season. Exponential fertilization resulted in overall poor planting stock form (stunted seedling growth and weak root development) and produced only marginal improvements of nutrient status. As a result, the exponential fertilization regime studied cannot be recommended as a treatment for aspen seedlings. In the second study we treated seedlings with a 2 × 2 factorial combination of fertilization and shoot growth inhibitor (SGI) applications with the fertilizer treatments varying in terms of mid-season fertilizer concentrations. Seedlings with SGI application had much higher tissue nutrient and carbon reserve concentrations than seedlings without a SGI application. In addition, nutrient uptake appeared to be more efficient in SGI treated seedlings, which could potentially result in significant reductions of nutrient application rates during aspen seedling production in nurseries. Overall, the early shoot growth termination using a SGI appears to be an effective technique to produce nutrient loaded aspen seedlings.

Keywords: Growth-limitation hypothesis; Nursery seedling quality; Nutrient and carbon reserves; Paclobutrazol; *Populus tremuloides* ;

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.

METHODS

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). 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). 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.

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.

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).

RESULTS

Exponential vs. constant rate fertilization

Seedlings with constant rate (100-100) or exponential (EXP) fertilization exhibited very different growth patterns (Fig. 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 (Fig. 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). 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). 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 & 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 3).

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 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 4). However, regardless of fertilizer regime, SGI treated seedlings had higher RSRs than non-SGI treated seedlings ($P < 0.001$; Table 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 5). However, despite lower seedling heights, SGI did not affect woody tissue N content at either fertilizer rate ($P = 0.902$) (Table 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 5).

Foliar and stem N, P, and S concentrations increased between 25 and 30% when seedlings had their shoot growth prematurely terminated (Fig. 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 (Fig. 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 (Fig. 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 (Fig. 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 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 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 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 6).

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.

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Fig. 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.

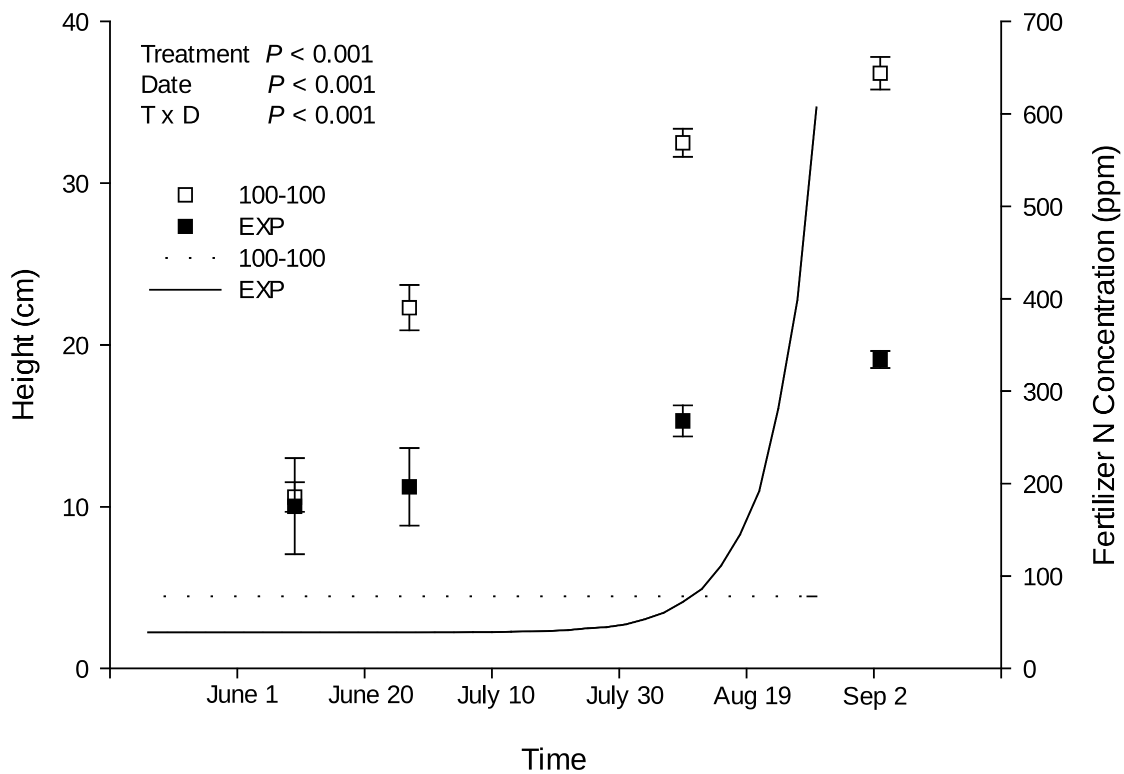

Fig. 2 Average total N, P, K, and S concentrations in stem, root, and foliar tissues of aspen seedlings that had been treated with different combinations of two fertilizer rates and a shoot growth inhibitor (SGI). Leaves were sampled in late August while stems and roots were sampled on dormant seedlings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

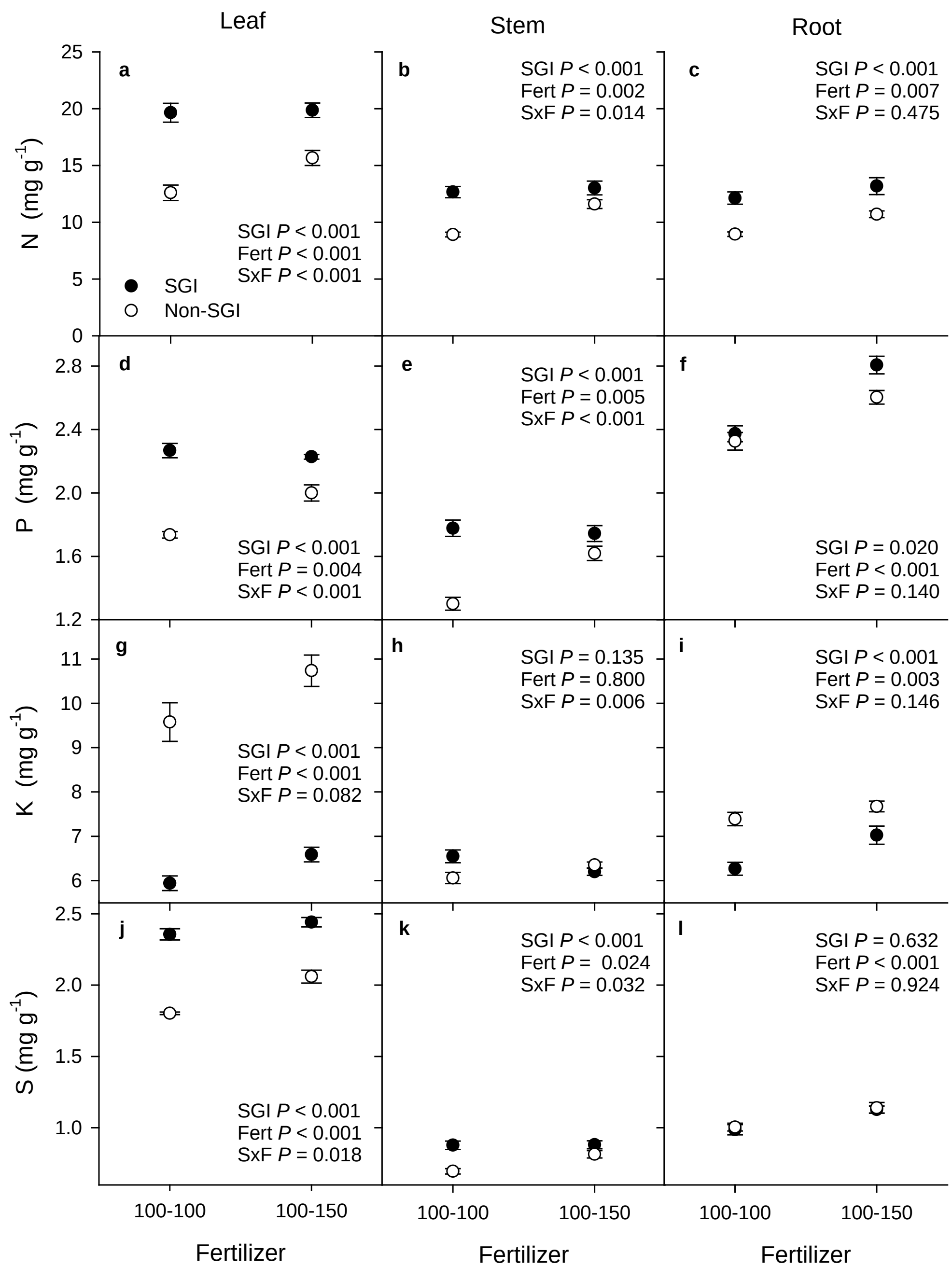

Table 1 Description of the 5 fertilization treatments used in this study. Fertilizer concentration represents a percentage of a standard nursery fertilizer regime (100% or 150%) used for commercial aspen planting stock in early (May 12 – July 12) and mid (July 12 – September 4) season. SGI is the application 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 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	(cm^3)	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 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 (mg g^{-1})			Nutrient Content (mg $seedling^{-1}$)		
	Tissue Type	EXP	100-100	*P*	EXP	100-100	*P*
N	Stem	13.83 (0.43)	8.92 (0.23)	< 0.001	5.70 (0.32)	11.7 (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)	0.695 (0.02)	< 0.001	0.41 (0.02)	0.912 (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 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 (g)	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 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	4.375 (0.20) a
100-150	50.672 (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	6.5 (0.25) c	18.45 (0.67) c	2.784 (0.08) c
100-100	37.74 (0.97) b	8.48 (0.25) b	29.46 (0.70) b	3.842 (0.14) b

Table 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