Partitioning of carbon allocation to reserves or growth determines future performance of aspen seedlings

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Abstract

Increasingly, trembling aspen (*Populus tremuloides* Michx.) is being planted in stressful situations such as forest land reclamation, afforestation and forest restoration in North America. This is due to its fast potential for growth and high resiliency, but its indeterminate growth strategy provides a special challenge in creating suitable planting stock for these sites. Clues from naturally established aspen seedlings suggest that root total non-structural carbohydrate (TNC) reserves and root:shoot ratio could be strongly related to subsequent seedling establishment and growth. Carbon allocation in plants is generally partitioned between reserve accumulation and growth and development. Under conditions in controlled nurseries, the allocation of carbohydrates to reserves could be manipulated in order to produce seedlings designed for specific environmental conditions. To manipulate seedling characteristics we attempted to induce premature bud set during nursery culture while allowing continued photosynthesis. Treatments included applying different fertilizer regimes, light intensities, reducing photoperiod and use of a shoot growth inhibitor. The shoot-growth inhibitor was the most reliable treatment and resulted in complete bud set, while blackout was successful only when seedlings were grown outside. Low nutrient treatments resulted in early bud set; however, leaves were abscissed much earlier. There was a distinct trade-off between growth and reserve accumulation with the late bud set. A longer period of height growth reduced root and total TNC reserves, while early bud set allowed for continued photosynthesis and produced the highest levels of root and total TNC reserves in seedlings. Increased TNC reserves of aspen planting stock was positively related to height growth after outplanting, with root TNC reserves as high as 33% of dry weight and root:shoot ratios greater than 2 associated with the best growth.

Keywords

Carbon reserve allocation, *Populus tremuloides*, seedling morphology, outplanting success, paclobutrazol, seedling quality

1. Introduction

There is increasing interest in the planting of trembling aspen (*Populus tremuloides* Michx.) seedlings for the restoration of degraded aspen stands, afforestation of heavily disturbed areas such as in mining reclamation and short-rotation plantations in the boreal forest zone. However, the quality of aspen planting stock and early plant performance has traditionally been questionable and inconsistent (Van den Driessche et al., 2003). Currently there are no morphological or physiological characteristics of aspen seedlings identified that correlate well with subsequent outplanting performance. In other tree species morphological characteristics such as root volume, seedling height or total mass have been shown to be good predictors of future growth (Davis and Jacobs, 2005; Jacobs et al., 2005). These variables are currently being used for aspen and appear to be poor predictors for the quality of aspen planting stock. Stored carbohydrates reserves are important for the establishment and growth of seedlings, particularly in deciduous species that need to rely on the stored reserves to initiate leaf area and new root growth without current photosynthesis (Loescher et al., 1990; Turgeon, 1989; Chapin et al., 1990; Kozlowski, 1992; Kozlowski and Pallardy, 2002; Sprugel, 2002; Landhäusser, 2011).

The size of planting stock is often positively correlated with growth and this response is often associated with greater nutrient and carbohydrate reserve content, but this has rarely been quantified or confirmed particularly for species with indeterminate growth habits such as aspen. Naturally established aspen seedlings which were very short in stature after the first growing season had high growth rates in the second growing season, which were associated with high concentrations of carbohydrate reserve and high root:shoot ratios (Martens et al. 2007). Since carbohydrate reserves are not easily determined, we are not aware of any studies that have explored the linkage between carbohydrate reserve concentration and content, nursery growing conditions, and/or morphological features of aspen seedlings and how those affect subsequent outplanting performance.

Trembling aspen, like most early successional and shade intolerant species in the Salicaceae, has an indeterminate growth strategy that allows it to grow significantly in height during favourable growing conditions. Under less favourable conditions, such as drought, low nutrient availability or low soil temperatures, however, aspen reduces photosynthesis and terminates height growth and the production of new leaf area, i.e. terminal bud set (Hogg and Hurdle, 1995; Landhäusser and Lieffers, 1998; Landhäusser et al., 2001; Galvez et al., 2011). Although photosynthesis is reduced in aspen under stress conditions, some carbon continues to be assimilated and is used to build carbohydrate reserves rather than structural growth (Galvez et al., 2011; Martens et al., 2007). Under these stress conditions an asynchrony between carbon supply and the immediate carbon demand for growth develops as a result of the termination of height growth (Chapin et al., 1990), allowing the plant to divert photosynthates to reserves instead of growth (Körner, 1991; Galvez et al., 2011). This change in allocation towards reserves could be useful in manipulating the reserve accumulation and status in aspen seedling planting stock as higher concentrations or content of reserves appear to positively influence the outplanting performance of the aspen planting stock (Martens et al., 2007).

In this study we investigate whether early termination of shoot growth, as a result of abiotic stresses can be used to control the amount of carbohydrate reserves in aspen seedlings. To induce bud set we limited nutrients and N, used a chemical shoot growth retardant or reduced the photoperiod by using blackout and grew these seedlings either under greenhouse or outside conditions. In addition, we explored how carbohydrate reserves affect subsequent initial growth performance and whether more easily-measured characteristics such as root:shoot ratio can be useful predictors for reserve status and growth performance after outplanting. We hypothesise that applying the above stresses will induce bud set in the seedlings and that bud set will increase carbohydrate reserves and root:shoot ratio in seedlings and improve outplanting performance. We further hypothesise that seedlings established under outside conditions will accumulate more carbohydrate reserves than seedlings growing under greenhouse conditions.

2. Materials and methods

2.1 Seedling planting stock production

In the late spring of 2007, aspen seedlings were grown at the University of Alberta from seed in styroblock containers (5-12, Superblock, Beaver Plastics Ltd., Edmonton, Alberta, Canada) with cavities 5 cm in diameter and 12 cm deep for a soil volume of 220 ml. Seed was collected from a wide range of open pollinated aspen clones of the boreal mixedwood region of west central Alberta, Canada. Before seeding, styroblocks were cut into strips containing six cavities each, for a total of 120 strips. Each strip was considered to be an experimental unit that contained six cavities (subsamples). Six strips each were combined and placed in twenty wooden racks (blocks). All cavities were filled with Pro-mix (Sunshine, SunGro Horticulture Canada Ltd., Seba Beach, Alberta, Canada) containing 55-65% sphagnum peat moss, perlite, dolomitic limestone, gypsum and a wetting agent.

Cells were hand-seeded with three to five aspen seeds per cavity in the second week of May and allowed to establish for seven weeks in a greenhouse. In the greenhouse photoperiod was ca. 17 hours, with an air temperature ranging between 20 and 24 °C. Soil moisture was checked daily and the blocks were watered if necessary. All blocks were fertilized once at the beginning of week 4 (at the beginning of June) with a 10% strength of the standard fertilizer concentrations (see below for description) to provide the seedlings with some initial nutrients until treatments were implemented after week 7. In weeks 5 and 6, cells were thinned to one seedling each where the tallest seedling was left and empty cells were filled with transplanting some of the healthy thinned seedlings.

After the seven-week establishment period, each of the six strips in each block was treated randomly with one of six shoot termination treatments. To prematurely terminate shoot growth, selected seedling strips were exposed to a reduced nutrient regime, reduced photoperiod (blackout), a shoot growth retardant or a combination of reduced nutrients and reduced photoperiod (Table 1). Ten of the twenty blocks were randomly chosen and moved to a location outside for the remainder of the growing season, while the remaining ten blocks were left growing inside the greenhouse resulting in a total of 12 differently treated seedling types (Table 1).

2.2 Fertilizer, photoperiod, and growth inhibitor treatments

All fertilizer treatments were applied twice a week. To achieve even fertilization, the seedlings strips common to a fertilization treatment were bundled together from all the racks and the root systems were submerged in the fertilizer solution until saturation. After fertilization, strips were placed back into their respective racks. Fertilization continued for the next 7 weeks until the end of week 14. The fertilizer solutions were prepared from a commercially used fertilizer blend that is considered standard for the production of aspen planting stock. The commercial blend contains 54 mg L^{-1} nitrogen (N), 57 mg L^{-1} phosphorous (P), 71 mg L^{-1} potassium (K), 66 mg L^{-1} calcium (Ca) and micronutrients consisting of boron, copper, iron, magnesium, manganese, molybdenum, sodium, sulphur and zinc. Seedlings treated with a low nutrient regime were subjected to a nutrient concentration at 10% the standard rate for either N (including N and Ca) or all nutrients (including N, P, K and Ca).

Blackout treated seedlings were exposed twice to short days (8 hours of light) for 6 consecutive days during week 10 and 14 of the growing season, which corresponded to the third week of July and the third week of August. Over these six days, seedling strips assigned for blackout treatment were moved daily to a dark room for 16 hours. The seedling strips assigned to the shoot growth retardant treatment were treated once in week 10, with 20 mg L^{-1} paclobutrazol (Bonzi®, Syngenta, Wilmington, DE, USA) by adding 5 ml Bonzi per litre to the fertilizer solution in which the root systems were submerged during the fertilization treatment.

Starting from week 15 (fourth week of August), all seedlings were fertilized twice weekly with standard fertilization rates until end of week 16. At week 17 (first week of September) all inside-grown seedlings were placed outside the greenhouse to allow them to induce dormancy naturally, and no more fertilizer was applied to any seedlings. All seedlings had completely hardened by week 21 (first week of October). The four tallest seedlings from each seedling strip in each block were selected for further study. Two seedlings were placed into over-winter storage at a commercial storage facility at -3°C. The other two seedlings were assessed for shoot height, root collar diameter, terminal bud volume (calculated as for an ellipsoid using length and diameter measurements), root volume and dry weight, coarse root dry weight, shoot mass, shoot N, and root and shoot total non-structural carbohydrates (TNC). For chemical analyses, tissue samples were dried at 70°C and ground through a 40-mesh screen using a Wiley mill. Nitrogen concentration of shoot materials was determined by Kjeldahl digestion (Kalra and Maynard, 1991). For TNC analysis, water soluble sugars were extracted three times from each sample with 80% ethanol at 95°C. The ethanol extract was analyzed for total sugar concentration using phenol-sulfuric acid. The residue obtained after extraction was analyzed for starch content by digestion using an enzyme mixture of α-amylase and amyloglucosidase followed by the colorimetric measurement of the glucose hydrolysate using a peroxidase-glucose oxidase-o-dianisidine reagent (Chow and Landhäusser, 2004).

2.3 Field performance of seedling stock

In the spring of 2008, the cold-stored seedlings were planted at the Ellerslie Research Station, Edmonton, Alberta, Canada (N 53º 24’ 44.07”; W 113º 32” 31.01”). The planting sites had been treated with a herbicide application of glyphosate (Roundup®, Monsanto, St. Louis, MO, USA) in late August of 2007 and then rotary tilled in the fall. In the outplanting study, seedlings were planted in a completely randomized design in a 25-m by 6-m plot. In total 240 seedlings (20 seedlings/treatment × 12 treatments) were planted. Seedlings were spaced 50 cm apart on a square grid. A buffer row of aspen seedlings was planted at the same spacing around the perimeter of the experimental plot. Immediately after planting vegetation mats (60-cm × 60-cm Brush Blanket, Arbortec Industries Ltd., Mission, BC, Canada) were placed around every seedling. Initial seedling height was also measured at this time. Weed problems became apparent in early July and the plot was weeded once.

In mid-September 2008, seedling height, the length of the longest new shoot, and root collar diameter were measured on all seedlings. Overall, mortality was less than 1% and not attributable to any treatment. From each treatment, 10 seedlings were carefully excavated by hand and care was taken to follow and gather all portions of the root system that had grown away from the original plug during the 2008 growing season. In the lab, the soil was carefully washed from the roots and roots growing away from the original root plug were separated from the plug. All samples were dried at 70°C. After drying, the new roots from 2008, the roots contained in the plug, and the shoots were weighed.

2.4 Analyses

To analyse differences in planting stock, a wooden rack containing one strip of each of the 6 treatments was considered a block. Each block was repeated 10 times inside and 10 times outside. As a result, there were 12 treatment combinations (6 shoot termination combinations in 2 locations) replicated 10 times. To assess differences in the seedling characteristics prior to planting, two seedlings (subsamples) were taken from each block. Differences among seedling characteristics were analysed as a split plot analysis of variance. Differences among specific treatments were determined using Tukey's test. Regression analysis, for all stocktypes together and for stocktypes grouped by fertilization (low and standard) or location (inside greenhouse and outside), was used to examine the relationship between physiological characteristics such as TNC reserves with morphological characteristics such as seedling size. The best relationship, based on r^2 value, is presented. Separate equations are shown for each grouping where there are clearly non-continuous relationships.

For the field performance study, seedlings were planted in a completely randomized design. Seedling stocktype field performance was analyzed using a split plot analysis of variation. The main response variable tested was height growth, which was strongly correlated to other growth variables such as dry mass production ($p = 0.012$). Regression analysis, for all stocktypes together and for stocktypes grouped by fertilization or location, was used to relate field performance to the seedling stock morphological and physiological characteristics. Statistical significance was assessed using $\alpha = 0.05$ and all statistics were completed using JMP 8.0 (SAS Institute Inc., Cary, NC, USA).

3. Results

The low N and low fertilizer treatments effectively resulted in early shoot growth termination and the formation of buds that did not break during the rest of the growing season. However, these seedlings were very small, had poor plug fill, and lost their leaves as much as three weeks earlier than seedlings of any of the other shoot termination treatments. When fertilized with a standard nursery fertilization regime, terminating shoot growth was 100% successful with the application of the shoot growth inhibitor regardless of whether seedlings were inside-grown or outside-grown. On the other hand, the blackout treatment worked well only when seedlings were outside-grown (98% of seedlings terminated shoot growth and held bud). When inside-grown, the blackout treatment resulted in a reflush of terminal buds in over 60% of the seedlings after they had initially terminated shoot growth. Similarly, untreated seedlings under the standard nutrient regime continued to grow in height late into the growing season.

Growing seedling planting stock inside the greenhouse produced taller seedlings with greater aboveground shoot mass ($p < 0.001$, Table 2). However, the inside-grown seedlings had lower root mass compared to outside-grown seedlings ($p < 0.001$) and as a result, root:shoot ratios ($p < 0.001$) were much lower in the inside-grown seedling stock compared to the outside-grown stock (Table 3). Outside-grown planting stock had higher root TNC concentrations (33.1% on average) compared to inside-grown stock (26.9%) of the same treatment ($p < 0.001$, Table 3). The highest root TNC concentrations were associated with the shoot growth inhibitor treatment in either inside or outside-grown seedlings and the blackout treatment when outside-grown only. Shoot TNC concentration varied much less among stock types and was only slightly higher in outside-grown stock (18.7% on average) compared to inside-grown stock (17.9%) (Table 2). Total root TNC content followed the same pattern as root TNC concentration but shoot TNC content was not significantly different between outside and inside-grown seedlings ($p = 0.195$, Table 3). In addition, outside-grown seedlings with standard fertilization and treated with a shoot growth inhibitor had almost twice the N concentrations in stem tissues (1.45%) than outside-grown seedlings not treated with a shoot growth inhibitor (0.73%) (Table 2).

Root TNC concentration of seedling planting stock was positively related to root:shoot ratio across all treatments (Figure 1). Both root TNC content and concentration were negatively related to seedling stock height (Figure 2). However, common measures of stock quality such as height:diameter ratio (p = 0.108), root collar diameter (RCD) (p = 0.751) and root weight (p = 0.119) were not related to root TNC concentration. Overall, taller seedlings had higher shoot TNC content but lower root and total TNC reserve content than seedlings that had their shoot growth terminated earlier. (Tables 2 and 3).

First growing season growth after outplanting varied by treatment and was generally favoured by the seedlings that had grown outside under standard nutrient regimes and had their shoot growth prematurely terminated (Table 4). Height growth was the most responsive variable and was most positively related to initial root TNC concentration and content, terminal bud volume, and root:shoot ratio (Figure 3). For other seedling morphological characteristics, height growth after outplanting was negatively related to initial seedling stock height and height:diameter ratio (Figure 4) and was not related to RCD (p = 0.648) .

4. Discussion

Total non-structural carbohydrate reserves can be manipulated in seedling stock of deciduous species that have indeterminate growth strategies. To be successful, however, shoot elongation needs to be terminated or slowed and it is essential that photosynthesis continues although shoot growth has ceased (i.e. no leaf abscission). Premature terminal bud set will result into overall shorter seedling stock. In this study, we used reduced nutrient availability, reduced day length, and an artificial shoot growth inhibitor to induce terminal bud set. Although the low nutrient treatments induced bud set, it was closely followed by leaf senescence, which did not allow for an extended period of photosynthesis and resulted in low seedling reserves. With adequate standard fertilization, shortened day length was only successful in fully terminating height growth when seedlings were outside-grown, while many of the inside-grown seedlings reflushed. Reflushing is a common response in species with indeterminate growth strategy, such as aspen, where episodic shoot growth is possible if supply of a limiting resource becomes more favourable later in the growing season (Deppong and Cline, 2000); however, if resources are not limiting throughout the growing season these plants will grow continuously and allocate most resources towards growth.

Growing seedlings inside or outside of a greenhouse had a significant impact on seedling characteristics and reserves. Outside-grown seedlings responded more strongly to the shoot termination treatments indicating that outside conditions add another layer of complexity to growing nursery stock. Outside conditions such as wind, greater swings in vapour pressure deficits and temperature, in addition to different light intensities and light quality are very different from greenhouse conditions. Light conditions in particular might be related to the generally higher root reserves detected in outside grown seedlings even in those without a shoot termination treatment. Therefore, only the shoot growth inhibitor treated seedlings or those seedlings with shortened day length, when grown outside, stopped height growth while maintaining leaf area and photosynthesis. Overall, however, the shoot growth inhibitor treatment resulted in the most uniform and reliable bud set and was the simplest procedure to induce bud set.

There is a direct trade-off in plants between photosynthate allocation to growth and TNC reserve accumulation (Chapin et al., 1990), which results in faster and taller growing plants having reduced reserves (Wyka, 2000). Since height growth of aspen seedlings in our study was inhibited using abiotic stresses or an application of a shoot growth inhibitor (paclobutrazol), photosynthates were not allocated to height growth, making more of it available for the accumulation of TNC reserves. A similar accumulation of TNC reserves has been found in *Oxytropis sericea,* a perennial herb, where slower growing plants allocated proportionally more C to root reserves than faster growing plants (Wyka, 2000). Our study also revealed a clear partitioning of reserves between shoot and root tissues in aspen seedlings. Shoot TNC reserve storage appears to be limited by the capacity of stem tissues to store reserves, since TNC reserves in stems represented approximately 18% of the stem mass, regardless of treatment. In roots, TNC reserves reached up to 37% of dry mass and showed much greater variation in both concentration and content, indicating that roots can store more TNC reserves and appear to be a more sensitive measure of accumulated C reserves in aspen seedlings than the stems. This prioritization of photosynthates to the root system over shoots in aspen seedlings may be an ecological adaptation for a species which naturally regenerates from its root system after disturbances such as fire, drought and defoliation kill the aboveground portion of the plant (Frey et al., 2003). Further, this difference in allocation pattern might also indicate that, although shoot TNC content may be higher in larger seedlings, it may not be a good indication of accumulated reserves available for future growth, as overall seedling TNC content in our seedlings was mostly driven by root reserves. Although shoot TNC content was positively correlated with seedling height, it was not related to outplanting performance, indicating that a larger aspen seedling does not necessarily provide a growth benefit upon outplanting in aspen (but see below). This finding differs from most other seedling outplanting studies which have found that larger seedlings, both conifers and hardwoods with periodic growth strategies, perform better. For example, the best predictor of ponderosa pine (*Pinus ponderosa*) seedling height after one growing season at a mesic site was the initial height of the seedling stock (Pinto et al., 2011) while initial stock size of red and white oak (*Quercus rubra* and *Q. alba*) was the best predictor of height after two years (Jacobs et al., 2005).

The relationship between carbohydrate reserves and seedling survival and performance is a common relationship found among tree species worldwide, from naturally established tropical understory trees (Myers and Kitajima, 2007) to planted conifer and deciduous seedlings in temperate and boreal environments. For example, planted Douglas-fir (*Pseudotsuga menziesii*) seedlings require TNC reserves of 10-12% dry mass for adequate seedling survival and initial growth (Ritchie, 1984). It appears that aspen planting stock need much higher root TNC reserves (greater than 33%) for improved growth in northern environments. Upon outplanting, root TNC reserves are important for seedlings to survive the stressful environmental conditions, such as high water stress encountered immediately after planting (Grossnickle, 2005). New root growth in the closely related European aspen (*Populus tremula*) was dependent on current photosynthates (Eliasson, 1968) so adequate root TNC reserves could allow for early root growth in seedlings while stem reserves are adequate to support the flush of the new foliage in the spring (Landhäusser, 2011).

Different morphological or physiological seedlings characteristics might be beneficial depending on the growing environment. In situations where resources such as water and nutrients are readily available, vegetative competition can be very intense and larger seedlings could offer a competitive advantage in the acquisition of light and, therefore, TNC reserve status might not play such a large role (Rodriguez-Alvarez, 2011). In more stressful growing environments, such as reclaimed mining sites and logging roads where nutrients and water could be limiting but competition from other species is not so intense, characteristics that allow the seedling to develop a larger root system to explore more resources might be more appropriate. Increased root:shoot ratio is beneficial in providing seedlings with relatively more root surface area with which to immediately take up water, thereby reducing water stress which is a major contributor to planting check (Grossnickle, 2005). Shorter planting stock may also be beneficial in stressful environments by reducing drought stress after planting by having a reduced transpiring surface as has been shown in hybrid poplars (*Populus* X) (DesRochers and Tremblay, 2009) and black spruce (*Picea mariana*) (Jobidon et al., 1998). The low-nutrient seedlings in our study did have increased relative carbon allocation to their root systems which has been seen before in aspen (Coleman et al., 1998; Pinno et al., 2012). However, height growth after planting was still lower in these seedlings than in those treated with standard nutrient concentrations, likely due to the lower nutritional status in these seedlings which would put them at a disadvantage. In addition, other planting stock characteristics, such as inadequate plug-fill makes these stock types difficult to handle during storage and planting. All these specific seedling stock characteristics might give seedlings a greater chance to establish and survive after outplanting; however, in the long-term, site conditions are likely to drive future performance and productivity of these trees.

Nursery production of aspen seedlings should focus on producing seedling stock with relatively high levels of root TNC reserves. Height growth greater than 30 cm in the first year after outplanting was associated with root TNC concentrations greater than 33% dry mass. Morphologically, seedlings with a root:shoot ratio greater than 2 and stock height less than 40 cm performed the best. These morphological characteristics are easily measured and are relatively cheaper to determine than TNC reserves, making them ideal indicators of aspen seedling quality. However, it is important to note, that in our study seedling stock height was actually related to the length of time the seedlings were allowed to grow before bud set, while root TNC was related to the length of time seedlings were allowed to photosynthesize after bud set. Therefore, one could conceive a situation where seedlings were started earlier in the nursery to allow for a longer growth period and then had their shoot growth terminated which could potentially produce a taller seedling with high levels of TNC. As a result, height alone would not be a good predictor for seedling reserve status. In addition, artificially creating seedlings with these morphological characteristics through the late pruning of shoots is unlikely to be associated high root TNC reserves (Rodriguez-Alvarez, 2011).The recommended nursery treatments for aspen to ensure earlier bud set followed by a period of sustained photosynthesis late in the growing season, involve stopping height growth with either an artificial shoot growth inhibitor, such as paclobutrazol, or by artificially shortening day length combined with growing the seedlings outside, while continuing a standard fertilization regime.

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Figure captions

Fig. 1. Root total non-structural carbohydrate (TNC) concentration in relation to seedling stock root:shoot ratio. Circles are standard nutrient fertilization regime and squares are low nutrient fertilization regime. White symbols are inside-grown stock and black symbols are outside-grown stock. Values are mean and standard error (n = 10).

Fig. 2. Root total non-structural carbohydrate (TNC) content (a) and concentration (b) of aspen seedling stock in relation to seedling stock height. Circles are standard nutrient fertilization regime and squares are low nutrient fertilization regime. White symbols are inside-grown stock and black symbols are outside-grown stock. Values are mean and standard error (n = 10).

Fig. 3. Height growth of outplanted seedlings in relation to: (a) seedling root total non-structural carbohydrate (TNC) concentration, (b) root TNC content, (c) terminal bud volume and (d) seedling root:shoot ratio. Circles are standard nutrient fertilization regime and squares are low nutrient fertilization regime. White symbols are inside-grown stock and black symbols are outside-grown stock. Values are mean and standard error (n = 10).

Fig. 4. Height growth of outplanted seedlings in relation to: (a) stock height and (b) seedling height:diameter ratio. Circles are standard nutrient fertilization regime and squares are low nutrient fertilization regime. White symbols are inside-grown stock and black symbols are outside-grown stock. Values are mean and standard error (n = 10).

Table 1

Table 1: Range of treatments used to produce seedling with different levels of carbohydrate reserves and root:shoot ratios. Fertilization solutions were prepared from a commercial blend with 100 representing the standard nutrient supply and 10 representing one-tenth of the standard nutrient concentrations.

Treatment		Fertilization			
Location	Shoot termination	N	P	K	Micro
Outside	None	100	100	100	100
Outside	Blackout	100	100	100	100
Outside	Bonzi	100	100	100	100
Outside	Low N	10	100	100	100
Outside	Low N, Blackout	10	100	100	100
Outside	Low N, Low fertility	10	10	10	100
Inside	None	100	100	100	100
Inside	Blackout	100	100	100	100
Inside	Bonzi	100	100	100	100
Inside	Low N	10	100	100	100
Inside	Low N, Blackout	10	100	100	100
Inside	Low N, Low fertility	10	10	10	100

Table 2

Table 2: Average of shoot variables (SD) of planting stock at the end of the nursery period. Different letters indicate significant differences among all treatments (n = 10 for all variables except for shoot N n = 4). To indicate differences between outside and inside-grown stock, *** = $p < 0.001$, ** = $p < 0.01$, * = $p < 0.05$ and NS = not significant.

Treatment		Height	Shoot dry weight	Terminal bud volume	Shoot TNC	Shoot TNC	Shoot N
Location	Shoot termination	(cm)	(g)	(mm^3)	(%)	(g)	(%)
		***	*	***	*	NS	***
Outside	None	44 (6) bc	1.88 (0.48) a	13.7 (4.6) ab	17.5 (0.6) a	0.33 (0.08) a	0.73 (0.03) cd
Outside	Blackout	34 (4) de	1.22 (0.20) b	14.0 (4.1) ab	17.8 (2.2) a	0.22 (0.04) bc	0.91 (0.13) c
Outside	Bonzi	29 (8) ef	0.87 (0.40) bc	17.9 (11.0) a	19.4 (0.7) a	0.17 (0.07) cd	1.45 (0.11) a
Outside	Low N	21 (6) gh	0.47 (0.25) cd	7.9 (3.5) bc	19.1 (1.1) a	0.09 (0.05) de	0.67 (0.04) de
Outside	Low N, Blackout	18 (8) h	0.33 (0.26) d	6.2 (3.6) c	19.1 (2.7) a	0.06 (0.05) e	0.70 (0.01) de
Outside	Low N, Low fertility	17 (6) h	0.34 (0.19) d	8.6 (1.7) bc	19.3 (1.4) a	0.06 (0.03) e	0.60 (0.08) def
Inside	None	53 (8) a	1.76 (0.55) a	3.6 (2.0) c	14.4 (3.4) b	0.25 (0.11) abc	0.57 (0.08) def
Inside	Blackout	49 (10) ab	1.72 (0.55) a	4.2 (1.9) c	17.8 (1.0) a	0.30 (0.10) ab	0.72 (0.10) cde
Inside	Bonzi	39 (4) cd	1.03 (0.15) b	7.6 (4.0) bc	19.5 (0.8) a	0.20 (0.03) c	1.15 (0.13) b
Inside	Low N	24 (7) fgh	0.43 (0.24) cd	5.9 (2.5) c	17.8 (1.2) a	0.08 (0.04) de	0.52 (0.10) def
Inside	Low N, Blackout	24 (4) fgh	0.48 (0.21) cd	6.8 (3.7) c	19.0 (1.6) a	0.09 (0.04) de	0.52 (0.04) ef
Inside	Low N, Low fertility	26 (7) efg	0.53 (0.28) cd	7.2 (2.8) c	18.6 (1.4) a	0.10 (0.05) de	0.46 (0.06) f

TNC = total non-structural carbohydrates

N = nitrogen

Table 3

Table 3: Average of root variables (SD) of planting stock at the end of the nursery period. Different letters indicate significant differences among all treatments (n = 10). To indicate differences between outside and inside-grown stock, *** = $p < 0.001$, ** = $p < 0.01$, * = $p < 0.05$ and NS = not significant.

Treatment		Root collar diameter	Root dry weight	Coarse root dry weight	Root:shoot ratio	Root TNC	Root TNC
Location	Shoot termination	(mm)	(g)	(g)		(%)	(g)
		NS	***	***	***	***	***
Outside	None	5.12 (0.4) a	2.78 (0.76) a	1.33 (0.28) a	1.6 (0.4) efg	29.9 (1.9) bcd	0.83 (0.22) ab
Outside	Blackout	4.47 (0.4) ab	2.47 (0.49) a	1.21 (0.21) a	2.1 (0.5) bcde	34.2 (2.8) ab	0.85 (0.19) ab
Outside	Bonzi	3.98 (0.7) bc	2.66 (0.89) a	1.17 (0.45) a	3.4 (0.8) a	37.0 (2.8) a	0.98 (0.34) a
Outside	Low N	3.11 (0.7) de	1.17 (0.81) bcde	0.52 (0.41) bcd	2.7 (0.8) abcd	31.5 (2.8) bc	0.39 (0.30) cd
Outside	Low N, Blackout	2.82 (0.7) de	0.87 (0.53) cde	0.35 (0.23) cd	2.9 (0.6) abc	32.3 (2.7) abc	0.28 (0.17) d
Outside	Low N, Low fertility	2.70 (0.6) e	0.79 (0.39) cde	0.37 (0.17) cd	2.9 (0.8) ab	33.5 (2.2) ab	0.26 (0.12) d
Inside	None	4.51 (0.7) ab	1.29 (0.49) bcd	0.56 (0.24) bcd	0.8 (0.2) g	20.6 (8.6) e	0.28 (0.17) d
Inside	Blackout	4.56 (0.8) ab	1.44 (0.47) bc	0.69 (0.25) bc	0.9 (0.1) fg	27.1 (2.9) cd	0.39 (0.12) cd
Inside	Bonzi	3.63 (0.4) cd	1.78 (0.36) b	0.73 (0.28) b	1.9 (0.3) cde	34.0 (2.6) ab	0.61 (0.16) bc
Inside	Low N	2.92 (0.7) de	0.60 (0.24) e	0.23 (0.13) d	1.8 (0.7) def	25.9 (3.5) de	0.16 (0.08) d
Inside	Low N, Blackout	3.09 (0.7) de	0.71 (0.33) de	0.30 (0.18) d	1.9 (1.0) bcde	28.1 (2.3) cd	0.20 (0.10) d
Inside	Low N, Low fertility	3.01 (0.7) de	0.71 (0.25) de	0.30 (0.10) d	1.9 (1.4) def	25.6 (3.5) de	0.18 (0.07) d

TNC = total non-structural carbohydrates

Table 4

Table 4: Average growth response (SD) one growing season after outplanting of the different planting stock types. Different letters indicate significant differences among all treatments (n = 10 for all variables except height growth n = 20). To indicate differences between outside and inside-grown stock, *** = $p < 0.001$, ** = $p < 0.01$, * = $p < 0.05$ and NS = not significant.

Treatment		Height growth	Diameter growth	Stem mass growth	Root mass growth	Total mass growth
Location	Shoot termination	(cm)	(mm)	(g)	(g)	(g)
		***	***	***	**	***
Outside	None	28.3 (17.1) bcd	2.9 (1.0) bc	8.0 (2.9) abc	6.2 (3.0) ab	14.1 (5.6) abc
Outside	Blackout	42.6 (17.2) ab	4.2 (1.1) ab	10.9 (4.0) a	7.7 (4.4) a	18.6 (8.2) a
Outside	Bonzi	45.1 (14.4) a	4.4 (1.2) a	9.4 (3.5) ab	6.1 (3.3) ab	15.5 (6.3) ab
Outside	Low N	28.4 (18.0) bcd	3.2 (1.0) abc	4.4 (1.8) cdef	3.3 (1.5) b	7.6 (3.2) cd
Outside	Low N, Blackout	24.4 (15.4) cd	3.5 (1.2) abc	3.8 (2.2) def	4.4 (2.9) ab	8.2 (4.9)cd
Outside	Low N, Low fertility	32.5 (21.2) abc	4.4 (0.7) a	6.0 (1.7) bcdef	5.4 (1.6) ab	11.4 (2.8) bcd
Inside	None	15.2 (10.5) d	2.7 (0.8) c	4.8 (2.9) cdef	5.9 (2.3) ab	10.7 (4.2) bcd
Inside	Blackout	20.0 (13.5) cd	2.6 (0.9) c	6.5 (2.7) bcde	4.0 (2.5) b	10.5 (5.0) bcd
Inside	Bonzi	30.5 (17.1) abcd	4.4 (0.9) a	7.1 (2.3) bcd	4.9 (2.1) ab	12.0 (3.9) abcd
Inside	Low N	20.7 (13.7) cd	2.9 (0.6) bc	3.5 (1.1) def	3.9 (1.5) b	7.4 (2.3) cd
Inside	Low N, Blackout	22.2 (12.3) cd	2.1 (0.7) c	2.7 (1.2) f	2.5 (1.2) b	5.2 (2.2) d
Inside	Low N, Low fertility	19.3 (15.2) cd	2.3 (1.1) c	2.8 (1.9) ef	2.7 (1.6) b	5.5 (3.3) d

Figure 1

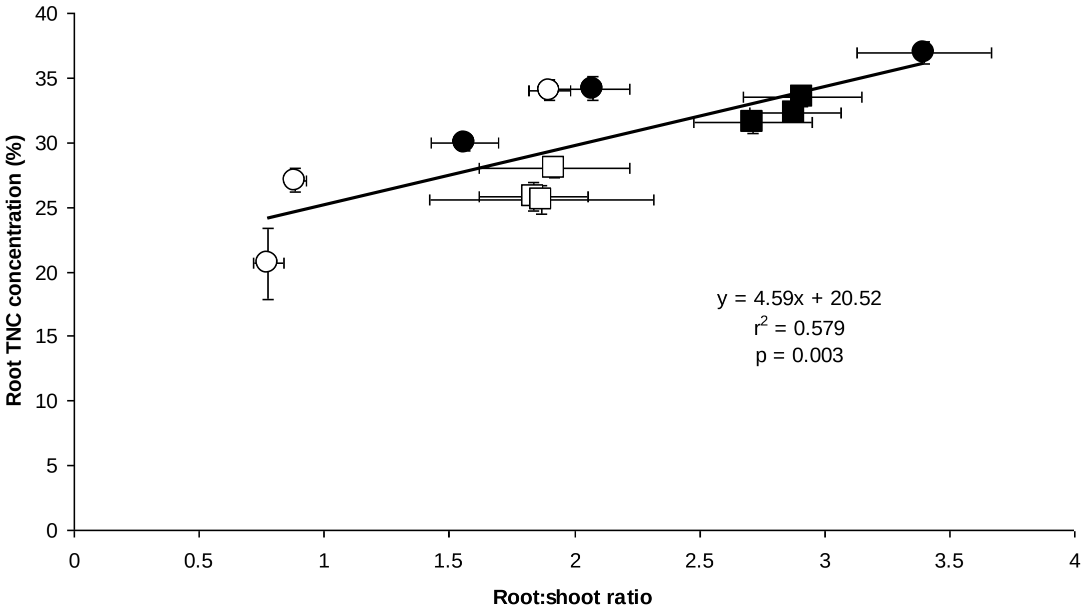

Figure1

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Figure 2

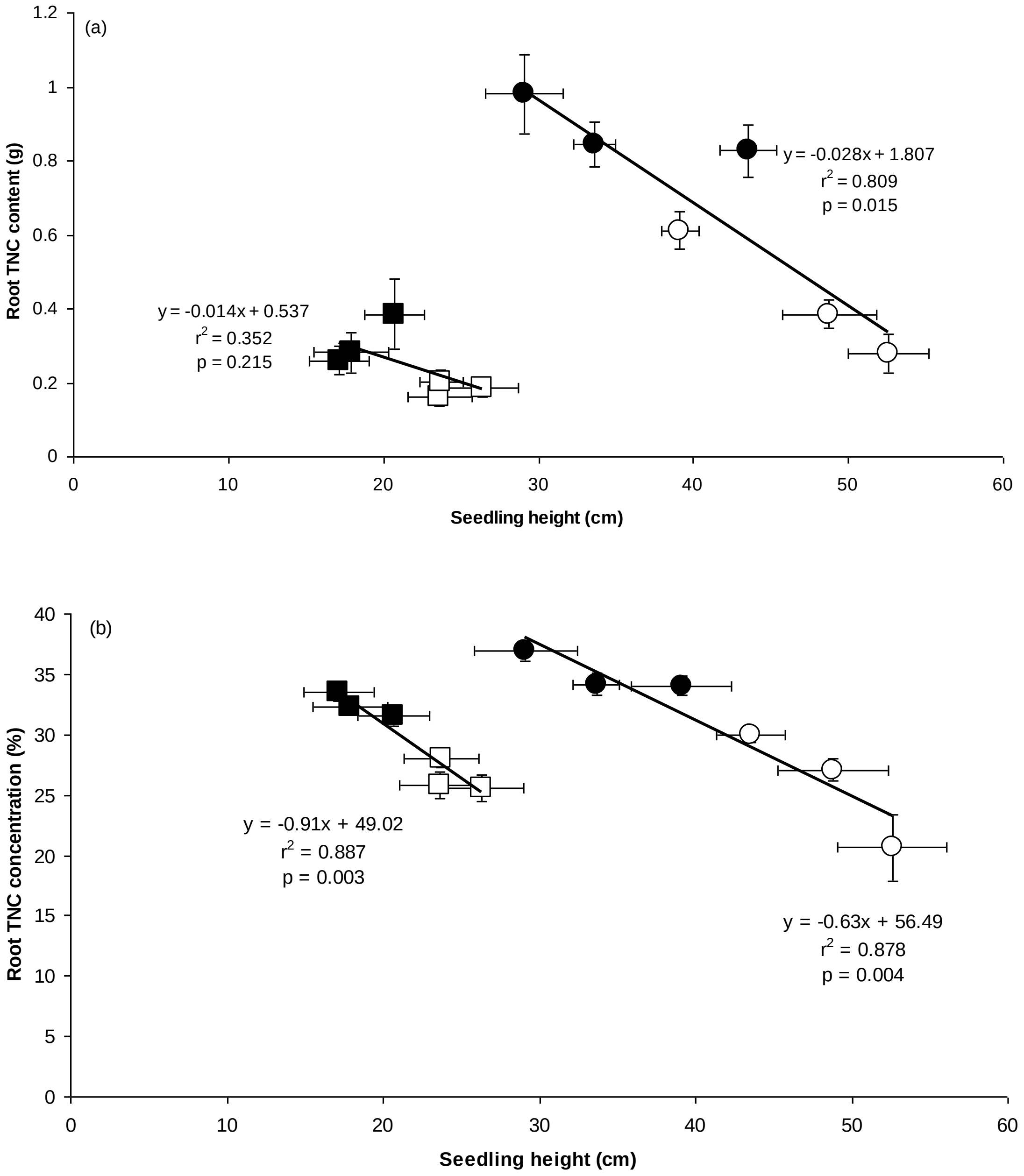

Figure 2

Figure 3

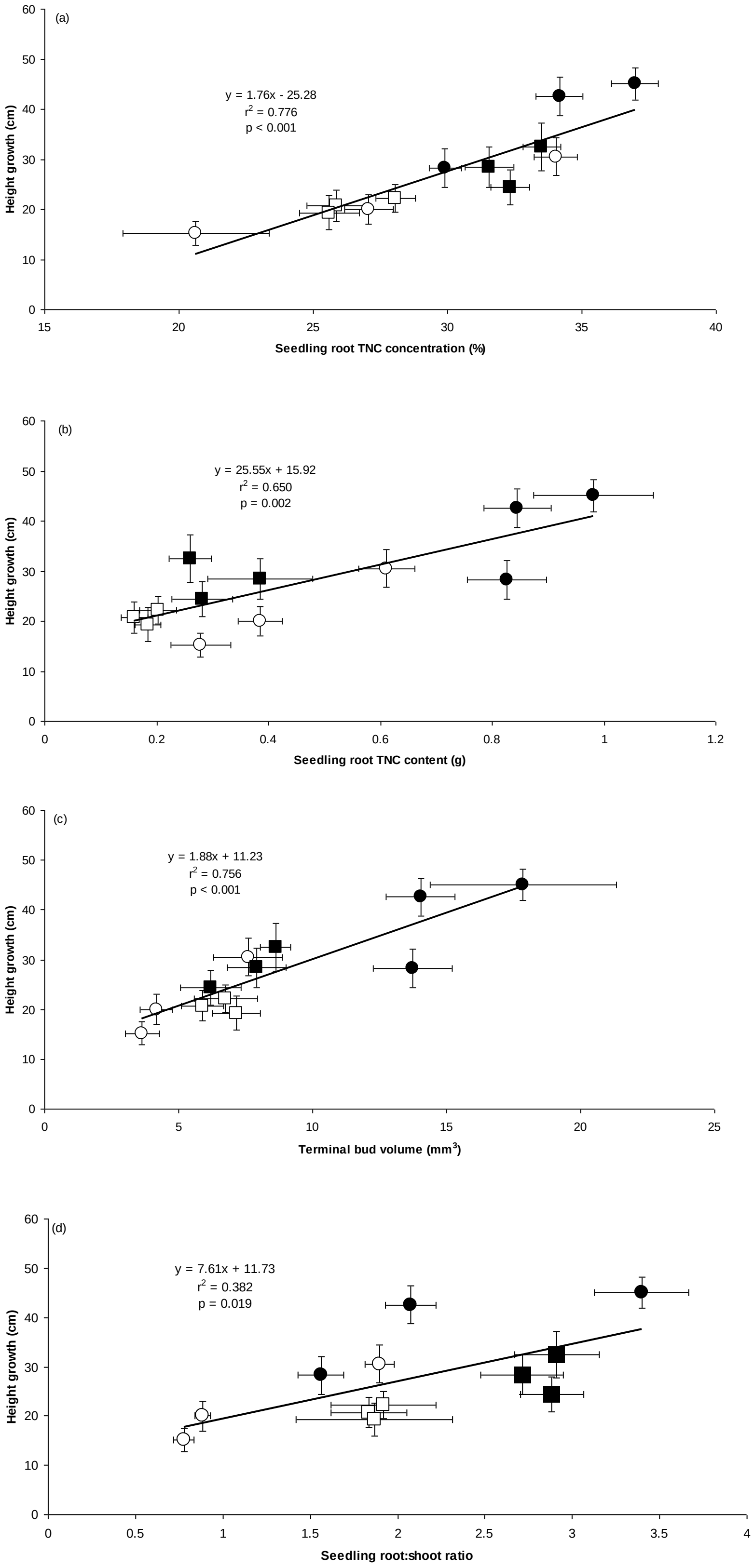

Figure 3

Figure 4

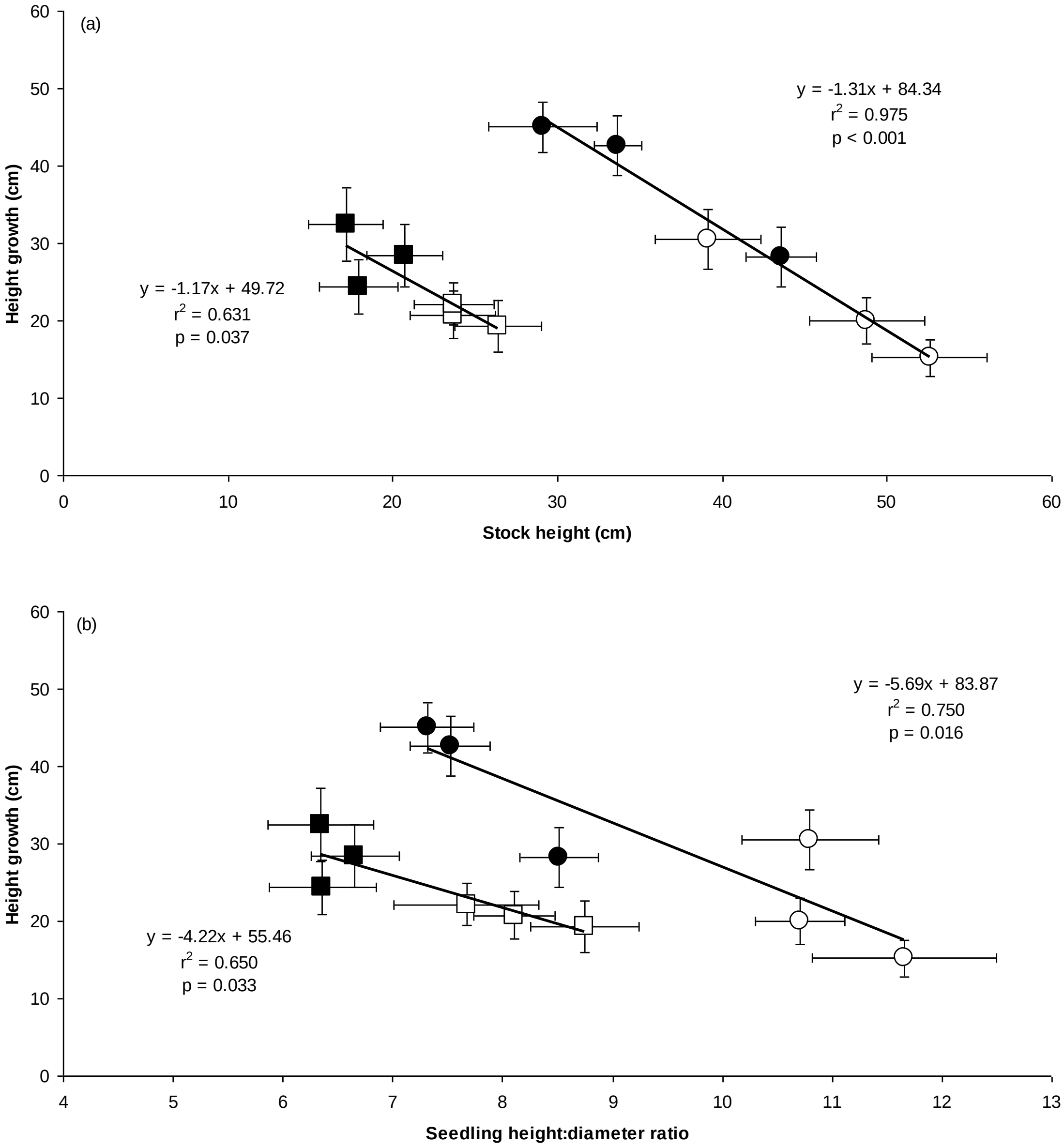

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Figure 4