Effect of stock type characteristics and time of planting on field performance of aspen (*Populus tremuloides* Michx.) seedlings on boreal reclamation sites

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Abstract

Aspen (*Populus tremuloides* Michx) has great potential as a reclamation species in mining sites in the boreal forest, but planting stock has shown poor field performance after outplanting. In this study we tested how different aspen seedling characteristics and planting times affect field outplanting performance on reclamation sites. We produced three different types of aspen planting stock, which varied significantly in seedling size, root-to-shoot ratio (RSR), and total non-structural carbohydrate (TNC) reserves in roots, by artificially manipulating shoot growth during seedling production. All three stock types were then field-planted either in late summer, late fall, or early spring after frozen storage. Seedlings were outplanted onto two reclaimed open-pit mining areas in the boreal forest region of central and east-central Alberta, Canada, which varied significantly in latitude, reclamation history, and soil conditions. Overall, height growth was better in aspen stock types with high RSR and TNC reserves. Differences in field performance among aspen stock types appeared to be more strongly expressed when seedlings were exposed to more stressful environmental site conditions, such as low soil nutrients and moisture. Generally, aspen seedlings planted with leaves in the summer showed the poorest performance, and summer- or fall-planted seedlings with no shoot growth manipulation had much greater stem dieback after the first winter. This indicates that the dormancy and hardening of the stem as a result of premature bud set treatments could improve the outplanting performance of aspen seedlings particularly during summer and fall plants.

Keywords: Growth and carbon allocation; Nursery stock; Root carbohydrate reserves; Root-to-shoot ratio; Seedling quality

Introduction

Trembling aspen (*Populus tremuloides* Michx.) is a widely distributed tree species native to North America. Its natural range extends from Alaska in the west, across Canada into north eastern United States and in higher elevations in the western and south-western areas of the United States and into northern Mexico (Little 1971; Perala 1990). This early successional, fast-growing species grows over a wide range of climatic and soil conditions and is considered relatively drought tolerant as compared to other boreal forest species (Lieffers et al. 2001). This makes it an ideal species for reclamation of disturbed mine sites in the boreal region, where there is a limited number of native tree species available for the reclamation and restoration of disturbed sites (Macdonald et al. in press). Currently, most aspen reclamation programs in the boreal forest region of Canada use nursery-grown seedlings. However, the outplanting success of aspen seedlings has been limited on boreal sites, where aspen seedlings often suffer from transplant shock and exhibit several years of slow growth after outplanting (Van den Driessche et al. 2003; Martens et al. 2007).

There is a lack of knowledge in identifying seedling characteristics and planting techniques that could be beneficial to improving aspen seedling establishment (Puttonen 1997). In the past, aspen seedling quality was assessed similarly to conifer planting stock (Sutton 1979) based upon height, root collar diameter (RCD), and terminal bud size (Chavasse 1980; Thompson 1985; Navarro et al. 2006). The current choice of aspen seedling attributes might not be adequate for assessing seedling quality and subsequent field performance since it has been recognized that seedling height and RCD are not always associated with field performance of broadleaf seedlings (Jacobs et al. 2005; Martens et al. 2007; del Campo et al. 2010). For example, there is a negative correlation between seedling initial height and field performance in northern red oak seedlings

(*Quercus rubra* L.) (Thompson and Schultz 1995) as well as a negative relationship between survival and initial height in sawtooth oak (*Quercus acutissima* Carruth.) seedlings (Hashizume and Han 1993). Martens et al. (2007) observed that, after the first growing season, naturally-regenerating aspen seedlings were short in stature but had very high root-to-shoot ratios (RSR) and total non-structural carbohydrate (TNC) root reserves. However, these much shorter natural seedlings outperformed the nursery-produced seedling stock in height and root growth in the following growing season (Martens et al. 2007). The importance of TNC reserves in roots has also been shown for conifers (Grossnickle 2005) and aspen root cuttings (Snedden et al. 2010), which grew more new roots upon transplanting when higher reserves were present. TNC reserves in seedlings are also important for maintaining respiration from the time of lifting until restarting photosynthesis after outplanting (Marshall 1985). Since the balance between the water absorbing roots and the transpiring leaf area of newly planted seedlings is important (Haase 2008), large seedling stock with proportionally lower RSR and TNC reserves might cause poor outplanting success on reclamation sites, where a larger root system and greater root reserves may become critical to seedling survival.

The selection of a planting time for aspen on reclamation sites is generally based upon local climatic conditions and/or operational constraints such as planter availability. In northern boreal climates, fall-lifted and spring-planted aspen seedlings are generally stored frozen at − 3°C for up to seven months. In the spring, site conditions are generally moist (shortly after snowmelt), and seedlings have a full growing season for growth and root system development before the following winter. Summer planting uses aspen seedlings that have set a terminal bud and stopped height growth and/or are top-pruned if too tall; however, these seedlings are not fully dormant. These seedlings can be considered “hot”-planted because they still have green leaves at the time of planting and can potentially re-flush if conditions are favorable. Summer planting also allows for the expansion of the root system during the remainder of the growing season after planting and results in a more intimate contact of the root system with the adjacent soils, potentially providing an advantage to the seedling in the following spring (Taylor and Dumbroff 1975; Good and Corell 1982). Seedlings planted in the fall are generally considered to be fully dormant, which eliminates the need for prolonged winter storage, but here seedlings do not have time to develop much contact between the root system and the soil prior to winter. Different planting times have been investigated for conifers (Barber 1989; Dierauf 1989; Adams et al. 1991) and temperate hardwoods (Seifert et al. 2006), but the effect of planting time for aspen in a boreal climate has not been tested.

The objectives of this study were to determine the effect of planting time and different stock type characteristics on the establishment and growth of regionally-sourced aspen seedlings. In particular we were interested in the impact of seedling and root system size and the root reserves on the early outplanting performance of aspen on reclamation sites.

Materials and methods

Planting stock production

The aspen seed used in this study was collected from two open-pollinated seed sources near Edmonton, AB, Canada (53°34′ N; 113°31′ W; elevation 668 m asl) and near Fort McMurray, AB, Canada (56°43′ N; 111°22′ W; 370 m asl). Government regulations required different seed lots to be used for seedling stock production as the planting sites are in different seed zones. Seeds were sown mid-May 2008 into 615A Styroblock™ (Beaver Plastics Ltd, Acheson, AB, Canada) containers with a cavity volume of 340 cm^3. Seedlings were grown in a mixture of peat and vermiculite (9 to 1 by volume) and were germinated under greenhouse conditions at a commercial tree nursery (Smoky Lake Forest Nursery, Smoky Lake, AB, Canada 54°6′ N; 112°28′ W; 598 m asl). During germination the greenhouse had a mean temperature of 21°C, with a minimum of 18°C and maximum of 28°C; relative humidity was maintained at greater than 70%. After seeding, styroblocks were irrigated using multiple automated mists per day for 4 weeks. Fertilization began 4 weeks after seeding using a standard nursery protocol used for growing commercial aspen seedling stock. The fertilizer solution consisted of 83 ppm of N, 76 ppm of P, 160 ppm of K, and chelated micronutrients; fertilizer was applied with every watering. Seedlings were moved to outside conditions after 8 or 11 weeks, depending on the stock type produced. Once outside, the fertilization regime was changed to 54 ppm N and 95 ppm K, while P remained at 76 ppm; this was done to limit height growth and the potential of re-flush in the seedlings that had set bud (see below). Seedlings continued to be fertilized with every watering cycle over the next 12 weeks.

Prior to moving seedlings to outside conditions, the styroblocks had been assigned to three different shoot treatments to create stock types with different seedling characteristics. The three stock types were labeled after the treatments used to terminate shoot growth: blackout, shoot growth inhibitor, and control with no artificial shoot growth termination. Generally, nursery-grown aspen seedlings for boreal forest climates are sown in early May, grown over the summer, and lifted in late summer or fall, and stored frozen over the winter months to be planted the following spring. Depending on the planting time, premature bud set can be artificially induced in aspen by shortening day length by means of blackout cloths, or by using a chemical shoot growth inhibitor (Rietveld 1988; Landhäusser and Lieffers 2009). Naturally, shoot growth terminates in aspen seedlings in early fall as a result of shortened day length and cooler nights. In an earlier study, the blackout and shoot growth inhibitor treatments had been found to be two reliable methods to induce premature bud set in aspen (Landhäusser et al. in press). In the blackout treatment, bud set was induced after 8 weeks of growth. These seedlings were moved outside and subjected to an artificial shortening of day length for 7 consecutive days, by covering them with a black plastic tarp for a portion of the day to shorten the photoperiod to 8 hours from the ambient 17 hours. The same treatment was repeated again two weeks later. In the shoot growth inhibitor treatment, premature bud set was induced after 8 weeks of growth by treating seedlings with the plant growth regulator paclobutrazol (Bonzi®, Syngenta, North Carolina, USA). Paclobutrazol is absorbed by roots and shoots and inhibits gibberellin biosynthesis (Hedden and Graebet 1985) reducing internode expansion and apical dominance. This growth regulator was applied to the roots by soaking the styroblocks in a water bath with a concentration of 5 mL of Bonzi per L of water (0.02 g of paclobutrazol/L of water) as recommended by the manufacturer. Seedlings in the untreated control were moved outside the greenhouse after 11 weeks, to continue to grow and harden off naturally in the fall.

Planting time and planting sites

For the summer outplanting treatment, a third of the seedlings were lifted after the third week of August (14 weeks since seeding). The remaining seedlings stayed outside until another third of the seedlings were lifted at the end of September for the fall outplanting (18 weeks after seeding). The remaining third of the seedlings was lifted in November and stored frozen at -3 °C until the following spring (May 2009).

Seedlings were outplanted in two different reclamation sites in central and east-central Alberta. One site was located on a coal mine (Sherritt Coal) near Warburg, AB, Canada (53°10′ N, 114°19′ W; 820 m asl) and the other site was on an oil sands mine (Suncor Energy) near Fort McMurray, AB, Canada) (56°43′ N, 111°22′ W; 370 m asl). Generally, weather conditions between 2008 and 2010 were not very different between the two sites except that winter precipitation and winter mean air temperature was lower in Fort McMurray than at Warburg (Table 1). However, the reclamation history and the soil conditions of the two reclamation areas were very different. The Warburg area had been reclaimed in 1998 by placing 1 m of subsoil over saline sodic overburden and capping it with 20 cm of salvaged topsoil. After placing the cover, the area had been used as an agricultural reclamation area seeded with alfalfa (*Medicago sativa* L.) for the last five years. A research site approximately 1000 m^2 in size was selected in the area and, prior to planting, the alfalfa was killed using herbicide (Glyphosate, Roundup, Monsanto, St Louis, MO, USA) and was incorporated into the soil using a rototiller. At Fort McMurray, the area had been reclaimed in 2007 by placing 1 m of subsoil over a saline sodic overburden and capped with a 30 cm layer of peat-mineral mix (70% organic – 30% mineral component). No cover crop was seeded prior to planting. A similar sized research site (1000 m^2) was selected in the area.

To characterize the soil conditions in both research sites, 12 random soil samples were collected from the top 20 cm of the capping soil across each site and four samples were randomly pooled and analyzed as a single sample (total of n=3 soil samples per site). Soil texture was estimated using a graduated cylinder and hydrometer (Carter and Gregorich 2008). Soils were analyzed for K^+, Na^+, Mg^{2+}, and Ca^{2+} concentrations using the 1M NH_4OAc method (Page 1982), for NO_3^- and NH_4^+ using the 2N KCl method (Jones 2001), and for PO_4^{3-} with the

Kelowna method (Carter and Gregorich 2008). Total N and total P were analyzed with the Kjeldahl digestion method (Carter and Gregorich 2008).

The soil conditions were very different between the two research sites due to differences in soil properties and the reclamation and re-vegetation prescriptions. Soil at Warburg was a silty-clay loam with 28% clay, 52.5% silt, and 19.5% sand while the soil at McMurray was a sandy-clay loam with 25% clay, 27% silt, and 48% sand. Soil nutrient concentrations at Warburg were higher than at Fort McMurray, particularly for N, P, and K (Table 2).

To conform to agricultural regulations pertaining to the spread of noxious weeds, plastic mulch blankets commonly used in tree plantations to suppress competition (90 × 90 cm) (Arbortec Industries Ltd, Mission, BC, Canada) were placed around the seedlings at the Warburg site in May 2009 after the spring-planting treatment had been completed. Due to the dimensions of the plastic mulch, seedlings had been planted at a slightly tighter spacing at Warburg (0.85 × 0.85 m) than at Fort McMurray (1 × 1 m) where no plastic mulch was required. The use of plastic mulch in Fort McMurray is not required and operational, as the spread of noxious agronomic weed species is currently not considered a critical issue in this area. All seedlings were planted by hand.

Prior to planting, each site was divided into 72 plots, which were randomly assigned to one of 9 treatment combinations of three planting times (summer, fall, spring) and three stock types (blackout, growth inhibitor, control) and replicated 8 times. Each plot contained 16 seedlings (subsamples) of the same treatment combination; the plot was considered the experimental unit in this study. In total 1152 seedlings were planted at each site. Both sites were surrounded by a 2 m buffer planted with aspen seedlings to ensure seedlings growing at the periphery of each site experienced similar conditions to those growing in more central locations.

Seedling measurements

Prior to each planting time (summer, fall, spring), 10 seedlings of each stock type were randomly selected to determine pre-planting seedling characteristics. After measuring height and root collar diameter (RCD) of the seedlings, root systems were carefully washed to remove the soil. Stems and roots were separated and dried to constant weight at 70°C for 2 to 3 days. To determine water-soluble sugar and starch concentrations in root and stem tissues, samples were ground to 40-mesh (0.4 mm) with a Wiley Mill (Thomas Scientific, Swedesboro, NJ, USA). Water-soluble sugars were extracted using hot ethanol (80%) and concentrations were measured colorimetrically using the phenolsulfuric acid method (Chow and Landhäusser 2004). Remaining starch in residues were solubilized with sodium hydroxide and hydrolyzed using an enzyme mixture of α-amylase (ICN 190151, from *Bacillus licheniformis*) and amyloglucosidase (Sigma A3514, from *Aspergillus niger*) and then measured colorimetrically using peroxidase glucose-o-dianisidine solution (Sigma Glucose Diagnostic Kit 510A) (Chow and Landhäusser 2004). Initial measurements of the seedling characteristics are summarized in Table 3.

After planting, initial height (height at time of planting) was measured on all field-planted seedlings. In the spring of 2009 prior to bud flush, two seedlings of each stock type planted in the summer and fall of 2008 were excavated to determine whether root growth had occurred during the previous partial growing season. Shoot dieback was determined on each seedling once bud flush had occurred in the spring. At the end of the first and the second full growing seasons (August 2009 and 2010), total height (from ground level to the highest terminal bud) was measured. After the first and second growing season, seedling mortality was also assessed.

To gain more detailed information on seedling growth and growth partitioning in the first growing season, two seedlings of the 16 seedlings planted in each plot were excavated (total 144 seedlings). Shoot growth, RCD, and root and stem dry mass were measured. RCD growth and root growth were estimated by subtracting the average initial RCD and root mass measured on the 10 seedlings prior to planting from the RCD and root mass determined from the excavated seedlings at the end of the first growing season (2009).

Experimental design and data analysis

Initial planting stock characteristics were combined for Warburg and Fort McMurray, as seedling stock type characteristics were not different between the two seed sources. Initial characteristics were analyzed using a two-way ANOVA with three stock types and three lifting times as the fixed factors. The field study was designed as a completely randomized 3 × 3 factorial design, with the three stock types (blackout, GI, control) and three planting times (spring, summer, fall) as the fixed factors. Each treatment combination was replicated 8 times at each planting site (Warburg and Fort McMurray). Planting sites were analyzed separately as seed source, reclamation operations, and planting procedures were very different between sites (see above) and a comparison of these geographically distant locations was not an objective of this study; however, qualitative comparisons were made between the two sites when reasonable. The effect of stock type and planting time on shoot dieback, RCD increment, and root growth was tested with a two-way ANOVA. Because height growth was measured in 2009 and 2010, height growth was analyzed as a three-way ANOVA with planting time, stock type, and measurement year as main factors. Prior to analyses, the variables were examined for normality (Shapiro-Wilk test) and homogeneity of variances (Levene test). Variables not conforming to normality or homogeneity of variances were transformed. All analyses used the MIXED procedure of SAS (SAS 9.2, SAS Institute, Cary, NC). Data presented in graphs are the non-transformed means. A significance level of $\alpha=0.05$ was used for all analyses. When significant treatment effects were detected differences among means were determined using LSD multiple comparisons. Linear regression analysis was used to relate field performance of all nine treatment combinations to average initial seedling stock morphological and physiological characteristics.

Results

Stock type characteristics prior to planting

The control stock type was twice as tall as seedlings of the blackout and growth inhibitor stock types regardless of planting time ($P<0.01$) (Table 3). However, the control stock type also tended to have the lowest root volume (Table 3) and root mass (data not shown) particularly when lifted in the summer. The control stock type had a root volume similar to seedlings treated with growth inhibitor only in the spring, which resulted in a significant planting time by stock type interaction term ($P<0.001$) (Table 3). Generally, summer-planted stock types had lower root volumes, root dry mass, and root-to-shoot ratio (RSR) compared to fall- and spring-planted seedlings; however, differences in RSR among stock types became larger in the fall- and spring-lifted seedlings resulting in a significant interaction term ($p<0.001$) (Table 3). Overall, the seedlings treated with the growth inhibitor had the highest RSR, followed by the blackout stock type and the control stock type ($P<0.001$). Root TNC in fall- and spring-planted seedlings was high among all stock types, while summer-planted stock types had the lowest root TNC, particularly in the control stock type. This also resulted in a significant interaction between planting time and stock type (P<0.001) (Table 3). Generally, the TNC concentration in stems was about half of those in the roots, and stem TNC concentrations showed little difference among stock types (data not shown).

Field performance at Warburg

After two growing seasons, overall seedling mortality at Warburg was 4% and was not significantly different among stock types or planting times (data now shown). After the first growing season, there was a significant difference in height growth among stock types (P<0.001), while planting time had no effect on height growth (P= 0.737) (Fig. 1). The growth inhibitor and blackout stock types grew an average of 39 cm, which was almost double the height growth of the control stock type (21 cm). After the second growing season all three stock types had grown a similar amount resulting in a significant year by stock type interaction term (P<0.001). There were no differences in RCD and root growth among the three stock types and the three planting times after the first growing season (data not shown). Shoot dieback after the first winter was about 10 times greater in the summer- and fall-planted control stock type compared with the other two stock types; however, the control stock type had similar dieback than the other two stock types when planted in the spring (Fig. 2). This resulted in a significant interaction term between planting time and stock type (P<0.001).

Field performance at Fort McMurray

At Fort McMurray, seedling mortality after two growing seasons was not significantly different among stock types or planting times and was less than 4.5% of all seedlings planted (data now shown). The selection of stock type had a significant influence on height growth in the first growing season (P<0.001); the growth inhibitor-treated stock types grew the most with 16 cm, followed by blackout stock (11 cm) and then control stock (6 cm) (Fig. 3). However, in the second growing season the differences seen in height growth in 2009 were reduced among stock types, which resulted in a significant stock type by year interaction (P<0.001). In addition, shoot dieback was different for the stock types after the first growing season (P=0.04) with the control stock type experiencing three times more shoot dieback than the other two stock types (Fig. 3).

The spring- and fall-planted stock types had 44% greater height growth than the summer-planted stock types (P=0.001) (Fig. 4). Spring- and fall-planted seedlings grew on average 13 cm in height while the summer-planted seedlings grew only 9 cm over both growing seasons. Overall, seedlings grew more in the second growing season than in the first growing season (P=0.043) (Fig. 4).

Impact of initial seedling characteristics on field performance

When exploring which seedling characteristics explained most of the subsequent growth in the field we found that initial root TNC concentrations and RSR played a significant role in height but not in root growth performance. There was a significant positive relationship between the first season's height growth and initial root TNC concentrations at the Fort McMurray site, but this relationship was not detectable at the Warburg site (Fig. 5a). However, initial RSR of the seedlings had a significant positive influence on first-growing-season height growth at both planting sites (Fig. 5b).

Discussion

Differences in the growth performance of aspen seedlings in response to the different planting times and stock types can be related to initial seedling characteristics, particularly root TNC concentration and RSR (Fig. 5), but they were also likely influenced by planting site conditions, such as fertility, soil texture, and climatic conditions. Seedlings with high TNC reserves and RSR (e.g., fall- and winter-planted and blackout and growth inhibitor-treated stock types) grew the best, indicating that RSR and TNC reserves are important characteristics to consider for describing aspen seedling quality. This might become more important for seedlings planted on sites with potentially limiting resources such as Fort McMurray, where both TNC reserves and RSR showed stronger relationships with height growth than at Warburg (Fig. 5). At Warburg, the above seedling characteristics might not have played such a prominent role due to the potentially higher resource availability. Apart from the initial transplant check (stem dieback, see below), seedlings at Warburg were exposed to more suitable soil moisture and nutrient conditions over the first growing season, which resulted in much better aspen growth than at Fort McMurray. These improved growing conditions were likely in part due to the use of the plastic mulch, but also due to the higher soil nutrient conditions (Table 2). The high nutrient content was likely a result of the alfalfa crop, which had occupied the site prior to becoming a research site, and had been incorporated into the soil. Secondly, in addition to reducing competition, the plastic mulch also reduced water evaporation (Allen et al. 1998; Mamkagh 2009) and increased soil temperature, which might have amplified N mineralization (Truax and Gagnon 1992). The greater percentage of sand in the soil at Fort McMurray may also have negatively influenced growth due to reduced water holding capacity and faster drainage.

Carbohydrate reserves have been found to be important in newly establishing seedlings (Farmer 1978; Wilson and Jacobs 2006) as an energy source between planting and the restart of photosynthesis (Marshall 1985; Carlson and Miller 1990; Landhäusser and Lieffers 2002). The lifting and planting of seedlings during the summer coincides with a phenological stage of low carbohydrate reserves (Kozlowski and Pallardy 2002). During summer planting, seedlings had green leaves and in general their TNC reserves were low as compared to fall- and spring-planted seedlings. This could have exposed them to greater water stress after outplanting, reducing their ability to photosynthesis and accumulate additional reserves for the winter and the following growing season (Rietveld 1989; Carlson and Miller 1990; Kozlowski 1991; Martens et al. 2007).

Interestingly, root TNC reserves, particularly in the spring-planted seedlings, did not differ much between the control and growth-inhibitor stock type; however, the growth-inhibitor stock type still had twice the height growth at the Fort McMurray site. This response appears somewhat puzzling and might indicate that either the paclobutrazol affects other physiological mechanisms related to stress tolerance, or that the size of shoot tissue relative to the root system (RSR) could have an effect on root performance (Wan et al. 2006; Percival and AlBalushi 2007).

There was a very strong indication that the RSR played a significant role in the ability of aspen seedlings to establish and grow in the following year. The stock types that had a much smaller shoot relative to its root mass (high RSR) at the time of planting showed consistently better height growth regardless of planting time and location. A larger root system relative to the shoot should increase the capacity to supply more water to the shoot, which initially carries fewer leaves, and, in combination with high TNC reserves, this could result in greater growth over the growing season (Galvez et al. 2011; Landhäusser et al. in press). Further, the smaller shoots of the growth inhibitor and blackout seedlings could potentially be beneficial in coping with the greater transpirational demands imposed on the root system when outplanted on harsh sites (Ritchie 1984).

Shoot dieback regardless of stock type and planting time was much more severe at Warburg than at Fort McMurray. However, only the summer- and fall-planted control seedlings at Warburg were severely affected. This could have been the result of seed source selection or site conditions. Seedlings planted at the Warburg site were produced from a more southern seed source; therefore, seedlings might require stronger queues for stem hardening to occur during seedling production than seedlings grown from a more northern seed source. Bud dormancy and cold hardening in plant populations are known to be influenced by latitude and altitude, where longer night lengths are required to induce dormancy in more southern populations (Dormling et al. 1968; Heide 1974; Ledgard and Norton 1988). During the early stages of stem hardening, and after bud set, seedlings are able to withstand air temperatures close to 0ºC and can withstand stem cavitation (Levitt 1980). During that time, seedlings still maintain cambial activity (Timmis and Worrall 1974), root growth (Day and Butson 1989), and reserve accumulation (Landhäusser and Lieffers 2003). However, if stems are not woody enough, drought or frost might affect the planted seedlings more severely. At Warburg the summer and fall stock types were planted into a recently rototilled field and the freshly disturbed soil likely provided poor root contact, which increased the risk of drought. At Fort McMurray, these stock types were planted into soil that had last been disturbed a year prior to planting, which allowed it to settle. In addition, shortly after planting in the fall, a night killing frost event occurred at Warburg (-6ºC) which was less severe at Fort McMurray (-2ºC). Regardless, this shoot dieback appeared not to be a significant impediment for future seedling growth, as seedlings grew well in the following growing seasons, likely as a result of the high resource availability at the Warburg site.

The results of this study suggest that aspen seedling planting stock with high initial RSR and TNC reserves will perform much better on reclamation sites. This effect appears to become more prominent under resource-limiting site conditions. Summer seedling stock, which had the lowest TNC and RSR, performed the poorest, particularly on the harsher reclamation site at Fort McMurray. Artificially inducing bud dormancy increases TNC reserves and RSR in seedlings and these are linked to improved seedlings outplanting performance. When artificially inducing bud and shoot dormancy during seedling production, seed provenance locations should also be considered, where seedlings grown from a more northern seed source appear to achieve a higher state of shoot dormancy earlier than seedlings grown from a more southern seed source.

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References

Adams DL, Graham RT, Wenny DM (1991) Effect of fall planting on survival and growth of three coniferous species of container seedlings in Northern Idaho. Tree Plant Note 42:52– 55

AgroClimatic Information Service (ACIS) Live station data. Agriculture and Rural Development, Gov Alta, Canada. www.agric.gov.ab.ca/app116/stationview.jsp. Accessed 28 February 2012

Allen RG, Pereira LS, Raes D, Smith M (1998) Crop evapotranspiration – Guidelines for computing crop water requirements. In: FAO Irrigation and Drainage Paper 56. FAO Food and Agriculture Organization of the United Nations, Rome, www.fao.org/docrep/X0490E/x0490e00.htm#Contents. Accessed 04 June 2011

Barber H (1989) Planting western larch: a comparison of stocktypes and season of planting in Northeast Washington. Tree Plant Note 40:20– 24

Carlson WC, Miller DE (1990) Target seedling root system size, hydraulic conductivity, and water use during seedling establishment. In: Rose R, Campbell SJ, Landis TD (eds) Proceedings, Western Forest Nursery Association, Roseburg, OR. General Technical Report RM-200, USDA For Serv, Rocky Mtn Res Stat, Fort Collins, CO, pp 53– 65

Carter MR, Gregorich EG (2008) Soil sampling and methods of analysis, 2nd edn. CRC Press, Taylor & Francis, Boca Raton

Chavasse CGR (1980) Planting stock quality: a review of factors affecting performance. New Zeal J For 25:144–171

Chow PS, Landhäusser SM (2004) Method for routine measurements of total sugar and starch content in woody plant tissues. Tree Physiol 24:1129–1136

Day RJ, Butson R (1989) Seedling-water relationships after outplanting. In: MacIvar DC, Street RB, Auclair AN (eds) Climate Applications in Forest Renewal and Forest Production, Proceedings of Forest Climate ’86. Environ Canada, Can For Serv, Orillia, Ont, pp. 55-62

del Campo AD, Navarro RM, Ceacero CJ (2010) Seedling quality and field performance of commercial stocklots of containerized holm oak (*Quercus ilex*) in Mediterranean Spain: an approach for establishing a quality standard. New For 39:19–37

Dierauf T (1989) Early planting, over-winter storage, and late planting of white pine seedlings. Virginia Dept For, Occasional Report 83, pp 1–7

Dormling I, Gustafsson A, von Wettstein D (1968) The experimental control of the life cycle in *Picea abies* (L.) Karst. I. Some basic experiments on the vegetative cycle. Silvae Genet 17:44–120

Farmer RE Jr (1978) Seasonal carbohydrate levels in roots of Appalachian hardwood planting stock. Tree Plant Note 29:22–24

Galvez DA, Landhäusser SM, Tyree MT (2011). Root carbon reserve dynamics in aspen seedlings: does simulated drought induce reserve limitation? Tree Physiol 31:250–257.

Good GL, Corell TE (1982) Field trials indicate the benefits and limits of fall planting. Am Nursery 155:31–34

Grossnickle SC (2005) Importance of root growth in overcoming planting stress. New For 30:273–294

Hacke UG, Sperry JS, Wheeler JK, Castro L (2006) Scaling of angiosperm xylem structure with safety and efficiency. Tree Physiol 26:689–701

Hashizume H, Han H (1993) A study on forestation using large-size *Quercus acutissima* seedlings. Hardwood Res 7:1–22

Haase DL (2008) Understanding Forest Seedling Quality: Measurements and Interpretation. Tree Plant Note 52:24–30.

Hedden P, Graebet JE (1985) Inhibition of gibberellin biosynthesis by paclobutrazol in cell-free homogenates of *Cucurbita maxima* endosperm and *Malus pumila* embryos. J Plant Growth Regul 4:111–122

Heide OM (1974) Growth and dormancy in Norway spruce ecotypes (*Picea abies*). I. Interaction of photoperiod and temperature. Physiol Plant 30:1–12

Jacobs DF, Salifu KF, Seifert JR (2005) Relative contribution of initial root and shoot morphology in predicting field performance of hardwood seedlings. New For 30:235–251

Jones JB Jr (2001) Laboratory guide for conducting soil tests and plant analysis. CRC Press, pp 122–124

Kozlowski TT (1991) Effects of environmental stresses on deciduous trees. In: Mooney HA, Pell E, Winner WE (eds) Response of plants to multiple stresses. Acad Press, San Diego, California, pp 391–411

Kozlowski TT, Pallardy SG (2002) Growth control in woody plants. Academic Press, San Diego

Landhäusser SM, Lieffers VJ (2002) Leaf area renewal, root retention and carbohydrate reserves in a clonal tree species following aboveground disturbance. J Ecol 90:658–665

Landhäusser SM, Silins U, Lieffers VJ, Liu W (2003) Response of *Populus tremuloides*, *Populus balsamifera*, *Betula papyrifera*, and *Picea glauca* seedlings to low soil temperature and waterlogged soil conditions. Scan J For Res 18:391–400

Landhäusser SM, Lieffers VJ (2009) Improvement of planting stock for short-rotation aspen plantations in Alberta. FRIAA-OF-05-P008, For Res Improv Assoc of Alberta, Edmonton, AB, 32 pp

Landhäusser SM, Pinno BD, Lieffers VJ, Chow PS. (In press). Partitioning of carbon allocation to reserves or growth determines future performance of aspen seedlings. For Ecol Manage http://dx.doi.org/10.1016/j.foreco.2012.03.010

Levitt J (1980) Responses of plants to environmental stresses. Vol. 1. Chilling, freezing, and high temperature stress. Acad Press, New York, 697 p.

Ledgard NJ, Norton DA (1988) Shoot growth in 2–3 year old Nothofagus seedlings. NZ J Ecol 11:105–108

Lieffers VJ, Landhäusser SM, Hogg EH (2001) Is the wide distribution of aspen a result of its stress tolerance? In: Shepperd WD, Binkley D, Bartos DL, Stohlgren TJ, Eskew LC (comps) Sustaining aspen in western landscapes. Proceedings, RMRS-P-18, Fort Collins, CO. USDA For Serv, Rocky Mtn Res Stat, pp 311–323

Little EL Jr (1971) Atlas of United States Trees. Washington, DC. US Dept Agric For Serv 1146, 9 pp

Macdonald SE, Quideau SA, Landhäusser SM. (In press). Rebuilding boreal forest ecosystems after industrial disturbance. In: Dale Vitt D, Bhattia J (eds) Reclamation and restoration of boreal ecosystems: Attaining sustainable development. Cambridge University Press.

Mamkagh AMA (2009) Effect of tillage time and plastic mulch on growth and yield of okra (*Abelmoschus esculentus*) grown under rain fed conditions. Int J Agric Biol 11:453–457

Marshall JD (1985) Carbohydrate status as a measure of seedling quality. In: Duryea ML (ed) Proceedings, Evaluating seedling quality: principles, procedures, and predictive abilities of major tests. For Res Lab, Oregon State University, Corvallis, pp 49–58

Martens LA, Landhäusser SM, Lieffers VJ (2007) First-year growth response of cold-stored, nursery-grown aspen planting stock. New For 33:281–295

Navarro RM, Retamosa MJ, López J, del Campo A, Ceaceros C, Salmoral L (2006) Nursery practices and field performance for the endangered Mediterranean species *Abies pinsapo* Boiss: 5-year results. Ecol Eng 27:93–99

Page AL (1982) Methods of Soil Analysis: Part 2 – Chemical and microbiological properties. 2nd edn. Madison, Am Soc Agron, pp 416–418

Perala DA (1990) Quaking aspen. In: Burns RM, Honkala BH (eds) Silvics of North America. Volume 2. Hardwoods. USDA For Serv, Washington D.C., Agric Hbook 654, pp 555–569

Percival GC, AlBalushi AHM (2007) Paclobutrazol-induced drought tolerance in containerized English and Evergreen Oak. Arb Urb For 33:397–409

Puttonen P (1997) Looking for the “silver bullet” – can one test do it all? New For 13:9–27

Rietveld W (1988) Effect of paclobutrazol on conifer seedling morphology and field performance. In: Landis TD (ed) Proceedings, Combined Meeting of the Western Forest Nursery Associations, USDA For Serv, Rocky Mtn Res Stat, Fort Collins, pp 19–23

Rietveld WJ (1989) Transplanting stress in bareroot conifer seedlings: its development and progression to establishment. North J Appl For 6:99–107

Ritchie GA (1984) Chapter 23: Assessing seedling quality. In: Duryea ML, Landis TD (eds) Forest nursery manual: Production of bareroot seedlings. Kluwer Academic Pub, pp 243–259

Seifert JR, Jacobs DF, Selig MF (2006) Influence of seasonal planting date on field performance of six temperate deciduous forest tree species. For Ecol Manag 223:371–378

Snedden J, Landhäusser SM, Lieffers VJ, & Charleson L (2010) Propagating trembling aspen from root cuttings: impact of storage length and phenological period of root donor plants. New For 39:169–182.

Sutton RF (1979) Planting stock quality and grading. For Ecol Manag 2:123–132

Taylor JS, Dumbroff EB (1975) Bud, root, and growth regulator activity in *Acer saccharum* during dormant season. Can J Bot 53:321–331

Thompson JR, Schultz RC (1995) Root system morphology of *Quercus rubra* L. planting stock and 3-year field performance in Iowa. New For 9:225–236

Thompson BE (1985) Chapter 6: Seedling morphological evaluation – what you can tell by looking. In: Durvea ML (ed) Proceedings, Evaluating seedling quality: principles, procedures, and predictive abilities of major tests. Forest Research Laboratory, Oregon State University, Corvallis, pp 59–71

Timmis R, Worrall J (1974) Translocation of dehardening and bud-break promoters in climatically split Douglas-fir. Can J For Res 4:229–237

Truax B, Gagnon D (1992) Effects of straw and black plastic mulching on the initial growth and nutrition of butternut, white ash and bur oak. For Ecol Manag 57:17–27

Van den Driessche R, Rude W, Martens L (2003) Effect of fertilization and irrigation on growth of aspen (*Populus tremuloides* Michx.) seedlings over three seasons. For Ecol Manag 186:381–389

Wan X, Landhäusser SM, Zwiazek JJ, Lieffers VJ (2006) Signals controlling root suckering and adventitious shoot formation in aspen (*Populus tremuloides*). Tree Physiol 26:681–687

Wilson BC, Jacobs DF (2006) Quality assessment of temperate zone deciduous hardwood seedlings. New For 31:417–433

List of Figures:

Figure 1. Height growth of growth inhibitor, blackout, and control aspen seedling stock types in 2009 and 2010 at the Warburg planting site. Bars with the same letter are not considered significantly different (n=24).

Figure 2. Shoot dieback after the first winter at the Warburg planting site for growth inhibitor, blackout, and control aspen stock types planted in the summer and fall of 2008 and spring of 2009. Shoot dieback was measured after leaf flush during the first growing season. Bars with the same letter are not considered significantly different (n=8).

Figure 3. Height growth in 2009 and 2010 and shoot dieback after the first winter of the growth inhibitor, blackout, and control aspen seedling stock types at the Fort McMurray planting site. Shoot dieback was analyzed separately from height growth. Bars with the same letter are not considered significantly different (n=24).

Figure 4. Height growth after the 2009 and 2010 growing season of aspen seedlings planted in the summer and fall of 2008 and the spring of 2009 at the Fort McMurray planting site. Different letters for the 2009 and 2010 growing season combined indicate that there was a significant planting time effect in both years, but not a significant year by season interaction (n=48).

Figure 5. Height growth of the nine different planting stock types after the first growing season in relation to initial root total non-structural carbohydrate (TNC) concentrations (a) and initial root-to-shoot ratio (RSR) (b) at the Fort McMurray and Warburg planting sites (n=10). At the

Warburg site the relationship between initial root TNC and height growth was not significant (p=0.384).

Figure 1

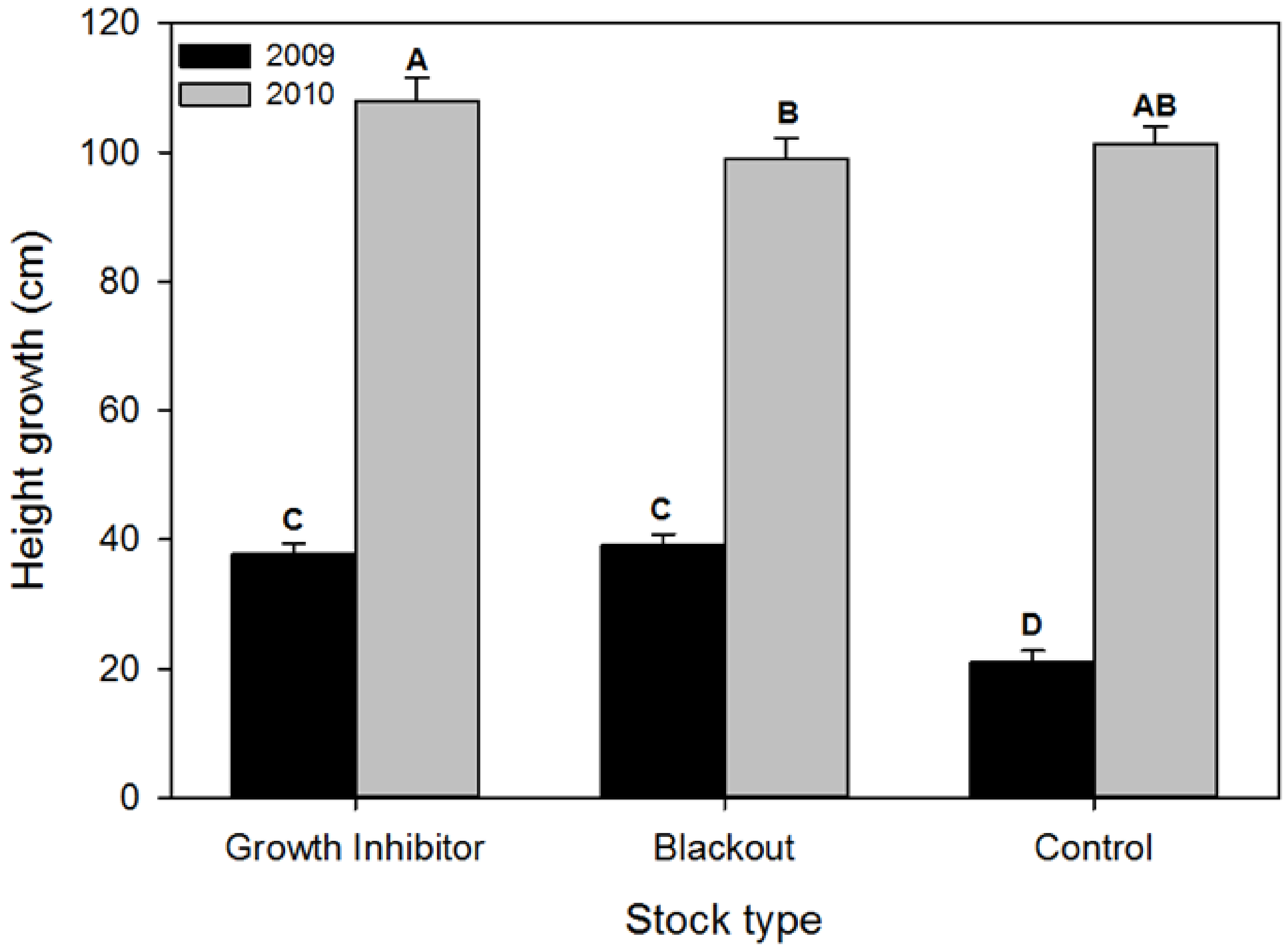

120
100
80
60
40
20
0
Height growth (cm)
2009
2010
A
B
AB
C
C
D
Growth Inhibitor
Blackout
Control
Stock type

Figure 2

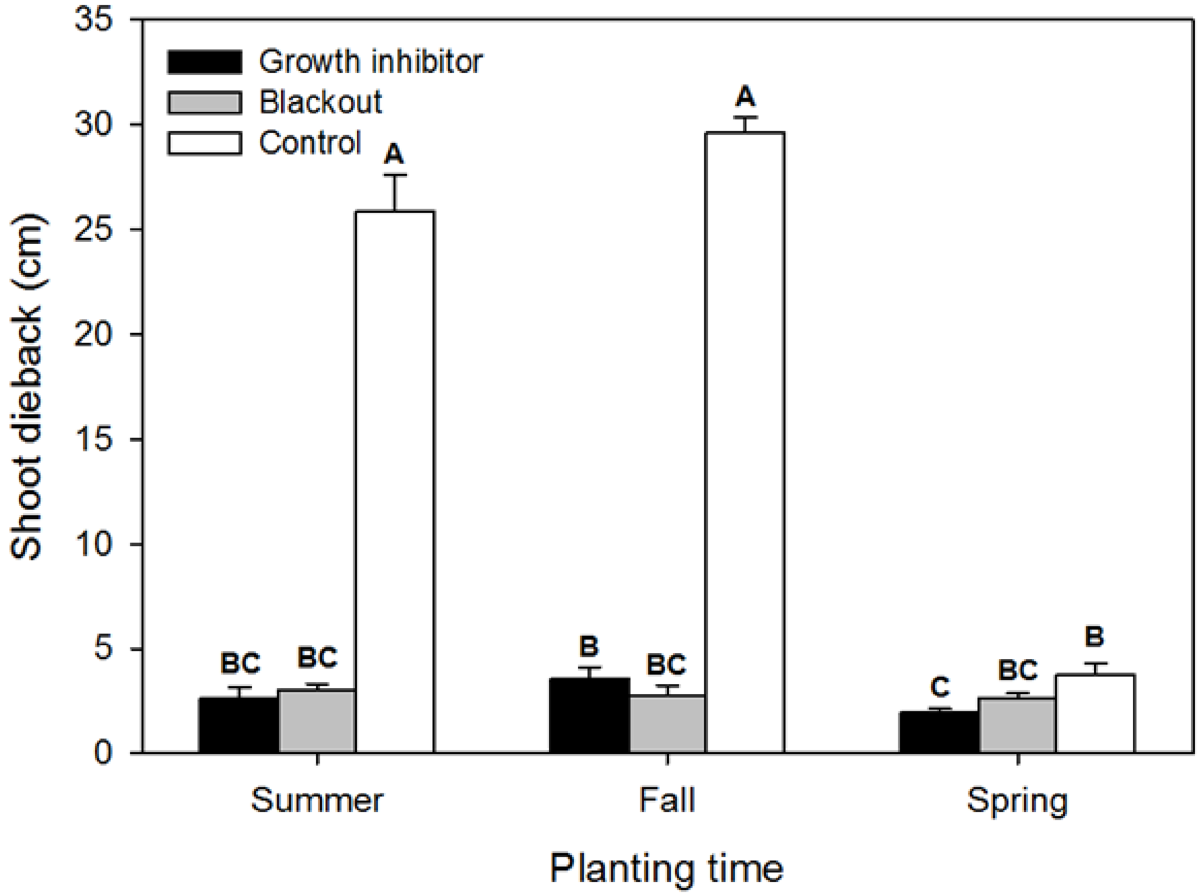
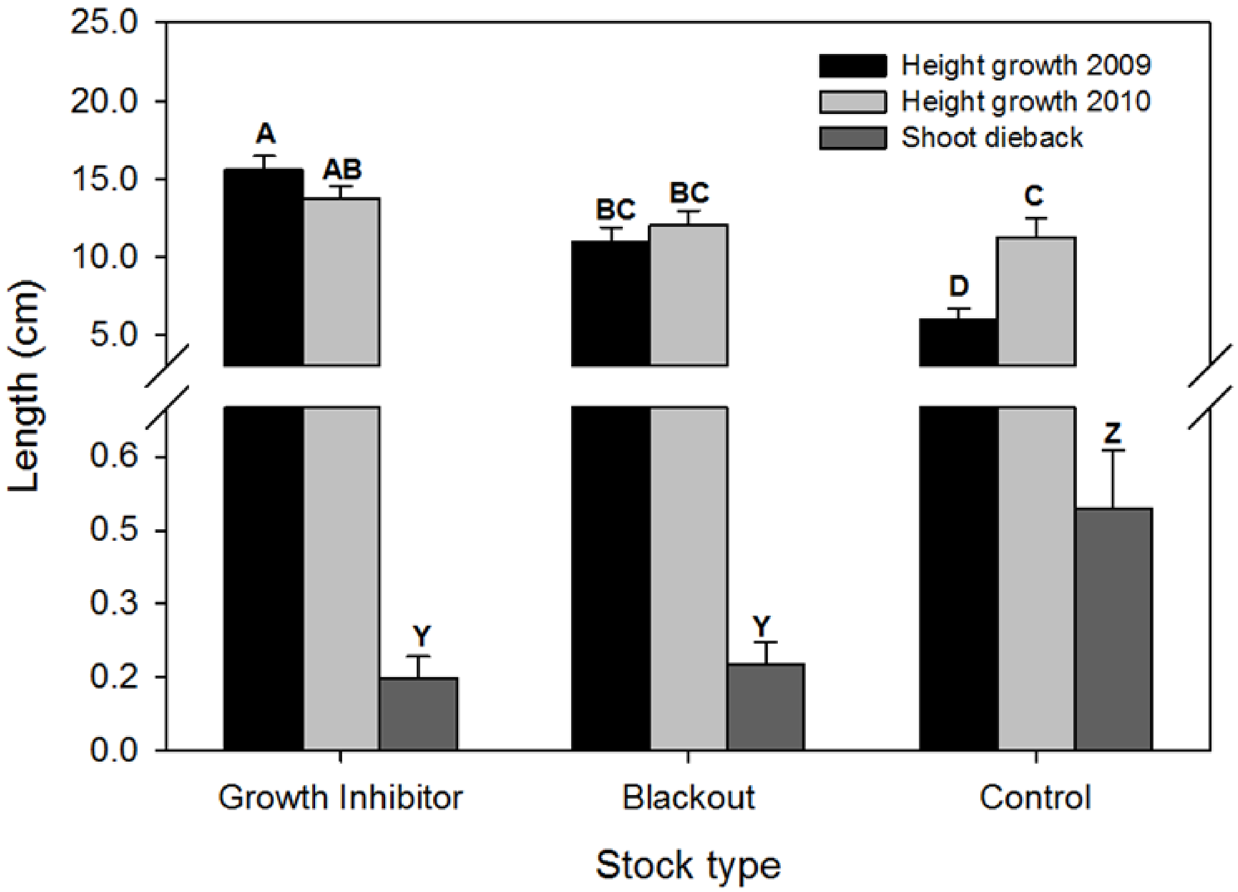

35
30
25
20
15
10
5
0
Shoot dieback (cm)
Growth inhibitor
Blackout
Control
BC
BC
A
B
BC
A
C
BC
B
Summer
Fall
Spring
Planting time

Figure 3


Length (cm)
25.0
20.0
15.0
10.0
5.0
0.6
0.5
0.3
0.2
0.0
Height growth 2009
Height growth 2010
Shoot dieback
A
AB
Y
BC
BC
Y
D
C
Z
Growth Inhibitor
Blackout
Control
Stock type

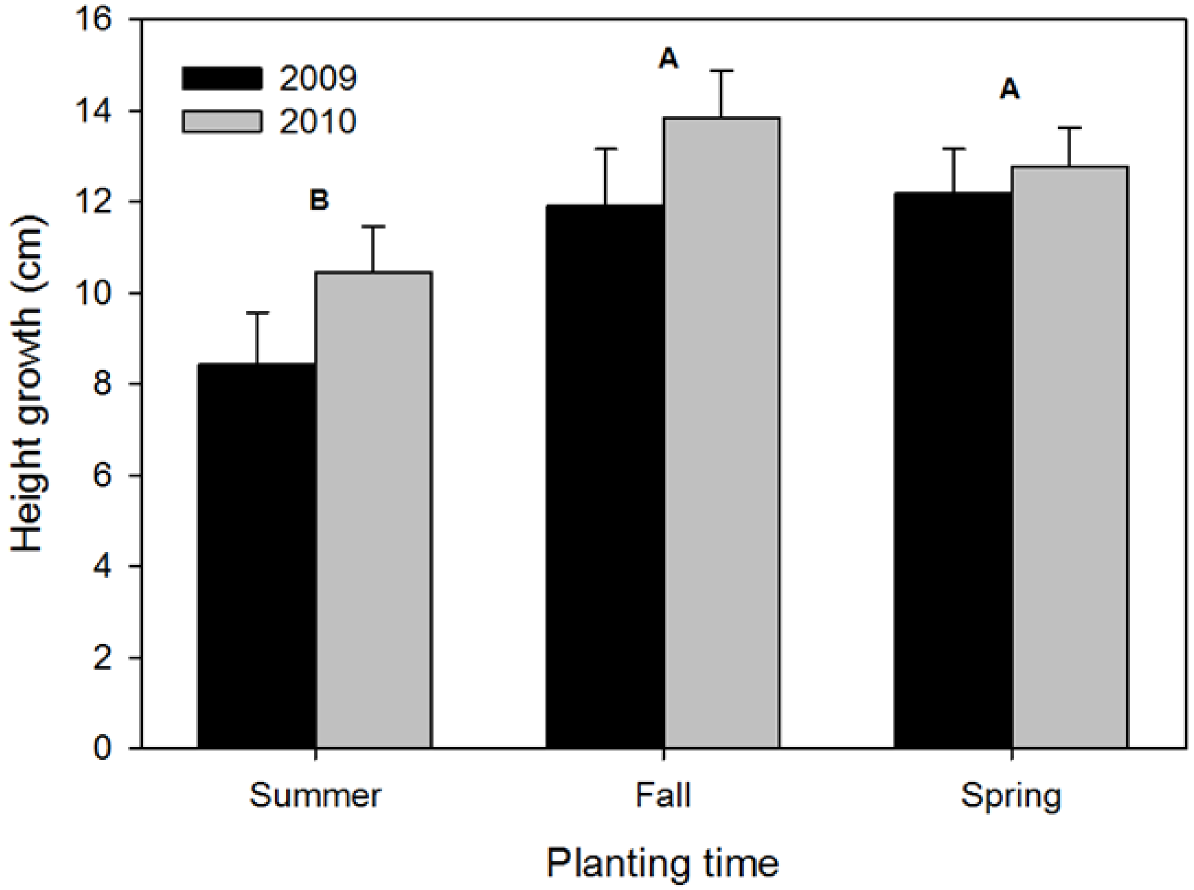

16
14
12
10
8
6
4
2
0
Height growth (cm)
2009
2010
B
A
A
Summer
Fall
Spring
Planting time

Figure 5

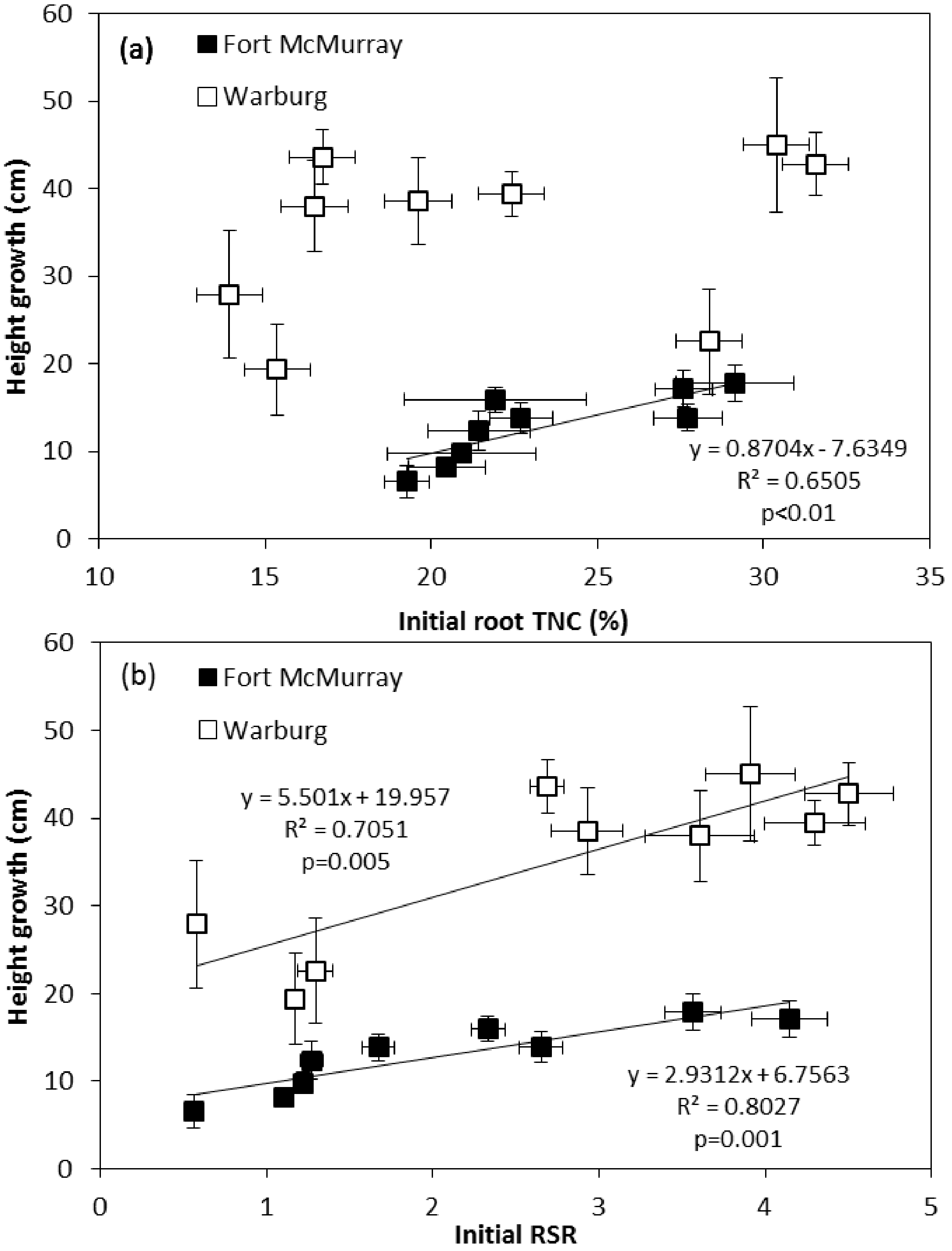

(a)
■ Fort McMurray
☐ Warburg
Height growth (cm)
0
10
20
30
40
50
60
10
15
20
25
30
35
Initial root TNC (%)
y = 0.8704x - 7.6349
R² = 0.6505
p<0.01
(b)
■ Fort McMurray
☐ Warburg
Height growth (cm)
0
10
20
30
40
50
60
0
1
2
3
4
5
Initial RSR
y = 5.501x + 19.957
R² = 0.7051
p=0.005
y = 2.9312x + 6.7563
R² = 0.8027
p=0.001

Table 1. Winter (September [previous year] to April) / summer (May to August) precipitation and mean air temperatures for 2008, 2009, and 2010 at Warburg and Fort McMurray planting sites (AgroClimatic Information Service).

Planting site	2008		2009		2010	
	Precipitation (mm)	Air Temp. (°C)	Precipitation (mm)	Air Temp. (°C)	Precipitation (mm)	Air Temp. (°C)
Warburg	87.6/262.2	-1.4/15.1	102.3/217.5	-3.4/14.3	306.9/288.9	-0.3/13.8
Fort McMurray	26.2/305	-9.7/16.2	131.8/229	-5.8/14.4	24.4/219	-2.6/15.4

Table 2. Average nutrient concentrations (standard deviation) of capping soil collected at Warburg and Fort McMurray planting sites. Numbers in a column followed by the same letter are not significantly different (n=3).

Planting site	Na^{+} [a]	K^{+} [a]	Mg^{2+} [a]	Ca^{2+} [a]	NH4^{+} [a]	NO_3^{-} [a]	PO_4^{3-} [a]	Total N [b]	Total P [b]
Warburg	31 **a**	194 **a**	475 **a**	2779 **b**	14.2 **a**	79 **a**	32.1 **a**	4.1 **a**	1.8 **a**
	(13)	(26)	(68)	(119)	(2.1)	(30)	(5.8)	(0.8)	(0.7)
Fort McMurray	58 **a**	68 **b**	544 **a**	5357 **a**	12.8 **a**	1.8 **b**	18.3 **b**	2.9 **b**	1.1 **a**
	(31)	(24)	(35)	(682)	(1.5)	(0.7)	(6.4)	(1.1)	(0.9)

[a] Values of Na^{+}, K^{+}, Mg^{2+}, Ca^{2+}, NH^{4+}, NO3^{-}, and PO_4^{3-} are expressed in mg Kg^{-1} of oven dried soil.

[b] Values of Total N and Total P are expressed in mg L^{-1} of soil.

Table 3. Average (standard deviation) of pre-planting characteristics of aspen seedling stock grown from Edmonton and Fort McMurray seed sources combined. Numbers in a column followed by different letters indicate significant difference among treatment means (Fisher LSD test; n=20). Initial seedling height was only significant for the stock type which is indicated by an asterix.

Planting time	Stock type	Height (cm)	Root volume (ml)	Root-to-shoot ratio	Root TNC (%)
Summer	Blackout	22.80	3.02 **c**	1.98 **d**	24.88 **d**
		(4.27)	(0.79)	(0.78)	(5.36)
	Growth Inhibitor	21.31	4.50 **b**	2.64 **c**	26.97 **d**
		(3.84)	(1.76)	(1.24)	(3.18)
	Control	46.91 *****	2.52 **c**	0.57 **f**	14.52 **e**
		(3.80)	(0.56)	(0.11)	(2.45)
Fall	Blackout	22.05	4.43 **b**	2.79 **bc**	37.15 **bc**
		(2.34)	(0.94)	(0.57)	(2.83)
	Growth Inhibitor	21.63	6.00 **a**	4.22 **a**	39.92 **a**
		(2.56)	(1.32)	(0.83)	(3.22)
	Control	42.73 *****	4.64 **b**	1.14 **e**	37.06 **bc**
		(4.78)	(1.14)	(0.17)	(2.63)
Spring	Blackout	22.63	4.46 **b**	3.12 **b**	35.34 **c**
		(6.12)	(0.88)	(1.02)	(4.13)
	Growth Inhibitor	22.78	6.15 **a**	4.03 **a**	38.82 **ab**
		(2.74)	(1.62)	(0.84)	(2.77)
	Control	45.43 *****	5.82 **a**	1.26 **e**	35.00 **c**
		(3.28)	(1.04)	(0.27)	(4.70)