2010

Propagating trembling aspen from root cuttings: impact of storage length and phenological period of root donor plants

Jessica Snedden

Simon M. Landhausser

Victor J. Lieffers

Lee R. Charleson

New Forests (2010) 39:169–182
DOI 10.1007/s11056-009-9162-7

Propagating trembling aspen from root cuttings: impact of storage length and phenological period of root donor plants

Jessica Snedden · Simon M. Landhäusser · Victor J. Lieffers · Lee R. Charleson

Received: 28 January 2009 / Accepted: 14 July 2009 / Published online: 26 July 2009

Abstract Two experiments were conducted to examine the effects of growing conditions, duration of cold storage, and distinct phenological periods of root donor plants on the propagation success of aspen rootlings. Root donor plants were produced either under greenhouse or open grown conditions. Root cuttings were periodically collected from donor plants that had been stored for various lengths of time in cold storage (up to 180 days), or that were stored dormant in cold storage (up to 150 days) and then grown for another full growing season. Longer storage of donor plants produced only slightly smaller rootlings and resulted in slightly lower establishment success. Rootling establishment success was severely depressed (down to 18% establishment success) when cuttings were collected during the active growth period of donor plants. Carbohydrate reserves did not influence rootling establishment success but did affect root and shoot growth performance. It appears that other factors, such as hormone levels, may be more important in rootling establishment success.

Keywords *Populus tremuloides* · Clonal propagation · Cold storage · Phenological period · Root cutting · Carbohydrate reserves · Vegetative regeneration

Introduction

Aspen (*Populus tremuloides* Michx.) is an early successional, fast growing clonal species that has a broad distribution in North America, and as such is adapted to a wide range of environmental and biotic stresses (DeByle and Winokur 1985). Forest managers have become interested in the short-rotation plantation culture of aspen due to its high fiber quality and productivity. In addition, native aspen is more likely to be accepted as a plantation species by local environmental groups and regulators of forestry activities than exotic species

J. Snedden · S. M. Landhäusser (✉) · V. J. Lieffers
Alberta School of Forest Science and Management, Department of Renewable Resources, University of Alberta, Edmonton, AB T6G 2H1, Canada
e-mail: Simon.Landhausser@ualberta.ca

L. R. Charleson
Western Boreal Aspen Corp., 11420-142 Street, Edmonton, AB T5M 1V1, Canada

such as hybrid aspens and poplars. Although aspen production from seed is generally more economical, vegetative propagation of aspen growing stock, especially stock derived from superior-performing clones, is desirable since growers can replicate clones with high quality wood fiber, fast growth rate, drought tolerance and/or potential for vegetative propagation (Noel et al. 2002; Zufa 1971). Over the last decade, there have been traditional selection, breeding, and testing programs of aspen in North America (Richardson et al. 2007); however, difficulties related to the propagation and establishment of these aspen clones are slowing the deployment of this superior genetic material (Haapala et al. 2004).

Several vegetative propagation techniques have been developed and tested for poplar species. However, many of these techniques have had poor success when applied to clonal aspen propagation (Haapala et al. 2004; Hartmann et al. 2002; Yu et al. 2001). Aspen is in the *Populus* section of the *Populus* genus, and does not root well from stem cuttings, unlike species in the *Tacamahaca* and *Aigeiros* sections (Eckenwalder 1996). Micropropagation has been considered to be a more reliable method for clonal propagation of aspen (Haapala et al. 2004), but is expensive and requires a sterilized environment, thus is susceptible to mortality from microbial contamination (Hartmann et al. 2002; Vinocur et al. 2000). The propagation of aspen from root cuttings in soil media has mostly been used operationally to produce stecklings (rooted stem cuttings) (Richardson et al. 2007), and little research has been undertaken to evaluate the direct use of small root segments for aspen propagation (rootling) (Stenvall et al. 2006; Brouard et al. 2005; Vinocur et al. 2000). There is limited information on how the rearing condition of root donor plants affects carbohydrate reserves in the roots, and how this in turn might affect the establishment success of rootlings. In addition, it is uncertain how rootling establishment and growth is affected by the duration in cold storage and the physiological condition at different phenological stages of root donor plants at the time of root collection. Understanding how growing condition, duration in storage and phenological stage affect rootling growth and establishment, may increase rootling propagation success, and reduce costs of propagation, making vegetative propagation more economical (Stenvall et al. 2004). Generally, aspen root donor plants are placed into cold storage in late fall, and remain there until the following spring when root cuttings are collected (Wang and Zwiazek 1999).

Non-structural carbohydrate (TNC) reserves appear to play a significant role in the vegetative regeneration success of aspen (Frey et al. 2003; Landhäusser et al. 2006). Root TNC reserves in aspen are known to fluctuate with growing conditions, storage time (Farmer 1963; Martens et al. 2007), and phenological stage (Landhäusser and Lieffers 2003; Schier and Zasada 1973); however, it is not clear what role they play in the establishment success of aspen rootlings.

The purpose of this study was to examine how the production and condition of the root donor plants (determined by the root carbohydrate reserves) influences the success of rootling propagation. In particular we investigated the impact of (A) the growing condition of donor plants; (B) the length of cold storage of donor plants; and (C) the phenological period of the root donor plant on rootling propagation success.

Materials and methods

Donor plant production

The aspen root donor plants were produced from root cuttings of four selected genotypes (1006, 1042, 3118 and 3149) from the Western Boreal Aspen Corporation. These four

genotypes had been identified as superiorly performing clones and were used in this study to increase our inference space on aspen rootling regeneration, thus, were not considered a fixed treatment effect. A total of 864 root donor plants were grown by a commercial nursery (Woodmere Forest Nursery, Fairview Alberta, 56°04′N, 118°24′W, 664 m a.s.l.). Donor plants were produced from root cuttings that were planted on March 6, 2006 into Superblock 5–12a, 60 mL container (Beaver Plastics LTD., Edmonton, Alberta). Genotype 1042 showed poor establishment success on first planting; therefore, a second batch was planted on April 14, 2006. On June 5 and 6, 2006, the rootlings of all four genotypes were transplanted into 9L pots (22 cm diameter and 22 cm depth) filled with 5:1 peat moss : vermiculite mixture by volume, amended with 9 g l_{soil}^{-1} dolomite lime and 3 g l_{soil}^{-1} of micronutrients (Scott 90505c). The 9L pots allowed for the increased development of plants with larger root systems. At that time, half of the aspen root donor plants were grown outside for the remainder of the growing season (outside treatment) and the other half were grown in a greenhouse (23 h of light (photosynthetically active radiation (PAR) was 100–700 $\mu mol_{photons}\ m^{-2}\ s^{-1}$ depending on the sky conditions) and 20°C day and 18°C night) (inside treatment).

All root donor stock received the same fertilizer regime, which consisted of full strength nutrient solution used by Woodmere Forest Nursery containing a blend of nitrogen, phosphorous and potassium, as well as micronutrients consisting of boron, copper, iron, magnesium, and zinc. During the hardening phase in late summer, the nitrogen concentration of the fertilizer blend and the frequency of fertilization were reduced; fertilization was discontinued by October. Inside-grown donor plants were hardened off in October by decreasing the air temperature to 15°C during the day and 5°C at night, and exposing the trees to the natural photoperiod (approximately 8 h day, 16 h night). These plants set bud in August and lost their leaves by mid-October, and remained under greenhouse conditions until December 5, 2006. Root donor plants grown outside received the same fertilizer treatments, but were subjected to the natural fluctuations in temperature and photoperiod, and remained outside until moved to cold storage. Outside plants set bud in July and lost their leaves in September. Roots of the outside donor plants did not completely freeze until November 4, 2006, whereas inside donor plant roots did not freeze until placed into cold storage at −3°C on December 5, 2006. For the storage time experiment, all root donor plants had their shoots cut off before the donor plants were bagged and placed in cold storage. Donor plants used for the phenology experiment were all stored in cold storage in large plastic bags with their stems attached.

Growth of root cuttings

Root donor plants taken out of cold storage were thawed for 2 days at 20°C before root cuttings (5 cm long) could be collected. For all collection periods, soil was shaken from the root ball, and roots were carefully cleaned under cold running water. Ten root cuttings (subsamples), ranging in diameters from 0.9 to 10.6 mm, were cut from each individual donor plant root. When harvesting root sections, the distal and proximal ends of the cutting were noted. Root cuttings for all experiments were planted into (Superblock 2–11) styroblock containers (Beaver Plastics LTD., Edmonton, Alberta); cells were 2 cm wide and 11 cm deep, and had a volume of 40 ml. Styroblocks (8 by 10 rows of cells) were filled with Pro-mix (Sunshine, SunGro Horticulture Canada Ltd., Seba Beach, Alberta) containing 55–65% sphagnum peat moss, plus perlite, dolomitic limestone, gypsum and wetting agent. The soil was thoroughly watered, and root cuttings were carefully planted to ensure good contact with the soil.

All root cuttings were planted leaving 1.0–1.5 cm of the proximal end above the soil level. For all experiments, root cuttings were grown for 31 days in a growth chamber. Growth chamber conditions throughout the experiments were kept on an 18 h/6 h light/dark cycle, with daytime air temperatures of 20°C and night temperature of 18°C. Relative humidity was maintained at 60%. Light intensity was maintained at 350–400 $\mu mol_{photons}$ m^{-2} s^{-1} photosynthetically active radiation at the styroblock level. Rootlings were watered every second day, and after leaf flush were fertilized weekly with 1 g/L of 10-52-10 (N-P-K) commercial fertilizer with chelated micronutrients (Plant Products, Brampton, Ontario).

Measurements and TNC sample analysis for all experiments

At each root collection time, three additional samples of root cuttings (of varying diameters) were collected from each donor plant and bulked to determine total soluble sugar, starch, and non-structural carbohydrate concentrations (TNC) in the root tissue. In the phenology experiment (see below), a sample of the stem of the donor plant was also analyzed for TNC. For TNC analysis, samples were dried at 68°C and ground in a Wiley mill to 40-mesh. For each sample, tissues were extracted three times with 80% hot 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).

Measurements of total root mass, shoot height, caliper and root total non-structural carbohydrates were taken on a subsample of ten root donor plants from each clone to describe the root donor stock (Table 1). Planted rootlings were harvested and measured after a 31 day growing period. The total number of roots originating from each cutting was counted, along with the height of the tallest shoot. A rootling was considered "established" if the cutting had produced both roots and shoots within the 31 day period.

Experimental design and analysis for all experiments

Both experiments (impact of storage time and donor plant phenological period) used a randomized block design, with the styroblock as the block factor (replicated nine times),

Table 1 The average (± SD) of shoot height, root dry weight, and root carbohydrate reserves per dry mass of root donor plants of the four selected genotypes grown for one season inside in a greenhouse and outside in the open ($n = 10$)

Growing condition	Genotype	Shoot height (cm)	Root dry mass (g)	Root carbohydrate reserves (% dm)
IN	1006	154 ± 24.8^{A}	35 ± 5.5^{ABC}	28 ± 6.8^{BC}
IN	1042	104 ± 9.6^{C}	18 ± 6.6^{D}	25 ± 4.2^{CD}
IN	3118	162 ± 14.3^{A}	24 ± 7.2^{CD}	21 ± 3.5^{D}
IN	3149	159 ± 18.7^{A}	45 ± 12.3^{A}	32 ± 5.9^{BC}
OUT	1006	126 ± 29.7^{BC}	46 ± 15.7^{A}	32 ± 4.8^{ABC}
OUT	1042	122 ± 26.8^{BC}	28 ± 9.5^{BCD}	29 ± 2.4^{BC}
OUT	3118	134 ± 7.1^{AB}	39 ± 6.4^{AB}	32 ± 2.9^{AB}
OUT	3149	114 ± 11.4^{BC}	47 ± 7.5^{A}	38 ± 3.6^{A}

Different letters indicate significant differences among means ($\alpha < 0.05$)

where each block contained 10 root cuttings (subsamples) each from the four genotypes, that had been grown either inside or outside (growing condition). In both experiments the donor plants consisted of the four genotypes (1006, 1042, 3118 and 3149), and the two growing conditions (inside and outside). Each donor plant was only used once. Since clonal differences were not within the scope of this study, and there were few interactions among the clones and the treatments, data were not analyzed for a clonal effect; however, the clonal variation was included in the analysis as part of the random effect. Depending on the particular experiment, another fixed treatment variable was added (see experiments below). Blocks were re-randomized every five days in the growth chamber to minimized potential spatial differences in growth chamber conditions. Differences among the blocks were tested, and no significant differences between blocks were found, and were left out of the linear model as a result. The treatment effects on rootling establishment success and growth were analyzed using the mixed model procedure in SAS (Ver. 8.1 SAS Cary, NC), using a complete randomized block design. The linear model for the time in storage experiment was $Y_{ijk} = \mu + G_i + S_j + GS_{ij} + \varepsilon_{ijk}$, where Y_{ijk} is the response variable, μ is the overall average, G_i is the effect of the ith level of growing condition, S_j is the effect of the jth level of time in storage, GS_{ij} is the interaction between growing condition and time in storage and ε_{ijk} is the random error in the experimental design, which included the effect of clone, which was not analyzed separately as a factor. The linear model for the phenological period experiment is $Y_{ijk} = \mu + G_i + P_j + GP_{ij} + \varepsilon_{ijk}$, where Y_{ijk} is the response variable, μ is the overall average, G_i is the effect of the ith level of growing condition, P_j is the effect of the jth level of phenological period, GP_{ij} is the interaction between growing condition and phenological period and ε_{ijk} is the random error in the experimental design, which also included the affect of clone. All data met the statistical assumptions of normality and homogeneity of variance. Statistical significance was assessed using $\alpha = 0.05$. To compare collection times T-tests were performed and the experiment-wise error was adjusted for the number of comparisons.

Donor plant storage time

The effect of storage time on establishment success of root cutting was tested on root donor plants that had been cold stored for 0, 30, 90, 120, 150 or 180 days starting in December 2006. At each storage time nine donor plants from each of the four genotypes that had been grown either inside or outside were removed from cold storage (total of 72 donor plants per collection). Ten root cuttings (subsamples) were taken from each donor plant and planted into one of nine styroblocks (replicates). As a result each styroblock contained a total of 80 root cuttings.

Donor plant phenology

To test the effect of some distinct phenological periods during the dormant and growing season of root donor plants on root cutting establishment success, six different stages of root donor plant conditions were identified. Three stages during cold storage: (1) immediately prior to cold storage (December 5, 2006); (2) after 75 days of storage (February 18, 2007); and (3) after 150 days of storage (May 4, 2007). After 150 days in storage all remaining root donor plants were moved outside, divided into three groups and allowed to grow to one of three stages: (4) leaf flush and shoot extension (June 5, 2007); (5) when leaf senescence initiated (September, 2007); and (6) fully dormant (immediately prior to

freezing) (November, 2007). For each stage, ten root cuttings (subsamples) were collected from nine donor plants of each genotype that were grown either inside or outside, and were planted into one of nine styroblocks each (replicate).

Results

Genotype differences among root donor plants

Genotype 1042 had half the average root mass and notably less height than the other three genotypes as a result of the later start (Table 1); however, the rootlings of this genotype responded to the treatments in similar patterns as the other genotypes, thus all four clones were used in the experiments and subsequent analyses. Overall, outside-grown donor plants were shorter ($P < 0.001$), had more root mass ($P = 0.001$) and total root carbohydrate reserves ($P < 0.001$) than donor plants that were grown inside a greenhouse.

Donor plant storage time

Length of cold storage of donor roots affected the rootling establishment success ($P < 0.001$), their average shoot height ($P < 0.001$), and the average number of roots produced from each root cutting ($P < 0.001$). On average, rootlings from inside-grown donor plants had lower establishment success (82%) than those from outside-grown plants (87%) ($P < 0.001$). This was especially true for rootlings from inside-grown donor plants stored for 90–150 days, thus resulting in a significant interaction between the storage time and the growing condition of donor plants ($P = 0.01$) (Fig. 1a). Rootling success of inside-grown donor plants was highest (above 85%) when donor plants were not stored, or stored for only 30 days. Rootling establishment success was the lowest (65%) when donor root systems had been stored for 120 days; however, establishment success increased again after storage of 150 and 180 days. Shoot height was greater in rootlings produced from outside-grown donor plants compared with inside-grown donor plants. Shoots were tallest when outside donor plants were not stored (4.43 cm), and showed a linear decline in height with duration of storage, up to 120 days (Fig. 1b). After 150 days in storage, shoot height growth recovered somewhat but was still lower than the height growth after 0 days of storage. The number of roots produced on rootlings from outside-grown donor plants was always higher than on rootlings produced from inside-grown donor plants ($P < 0.001$). The average number of roots produced from rootlings from outside-grown donor plants was greatest after 30 days in storage (4.0 roots), then decreased to 2.6 roots by day 90. After 90 days, root numbers increased slightly to 3.4 roots by 150 and 180 days (Fig. 1c).

Outside-grown donor plants had consistently higher total non-structural carbohydrates (TNC), soluble sugars, and starch concentrations than inside-grown plants (all $P < 0.001$) (Fig. 2a). The duration of cold storage affected TNC, soluble sugars, and starch in the roots of donor plants (all $P < 0.001$); however, there was no clear decline in TNC with storage time. Changes in TNC levels were accompanied by variations in soluble sugar and starch concentrations within the roots of donor plants at each time. Percent sugar stayed close to 25% when donor plants were stored for 90 days or less, then dropped below 20% when stored for more than 120 days (Fig. 2b). Percent starch decreased after 30 days in storage,

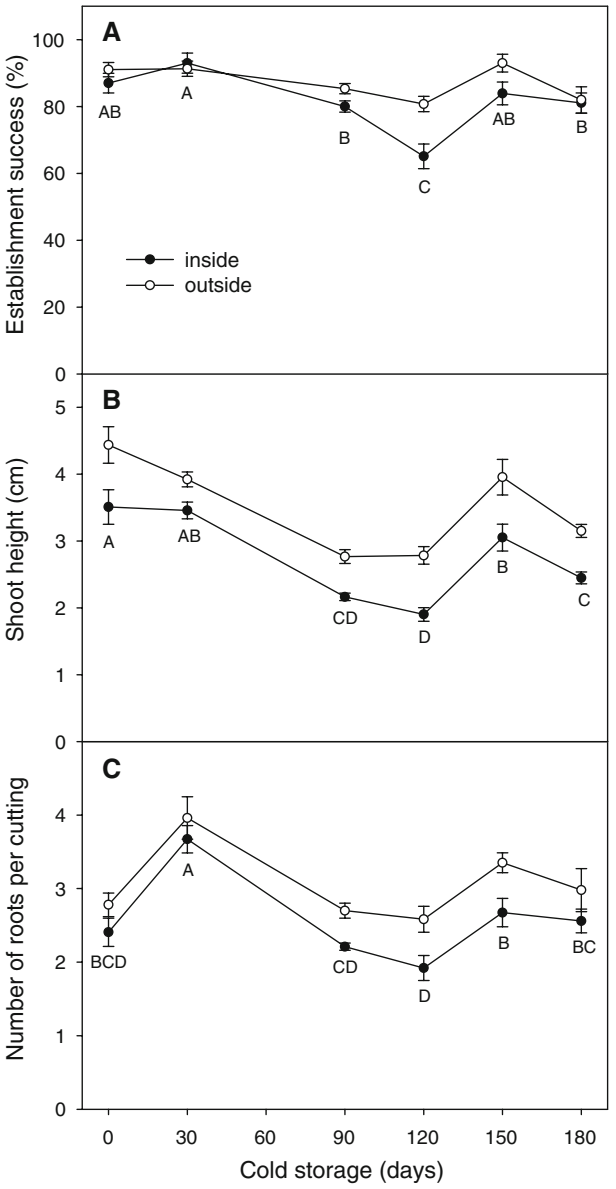

Fig. 1 The effect of cold storage duration of inside- and outside-grown donor plants on (**a**) percent establishment success of rootlings (**a**), (**b**) average rootling shoot height (**b**), and (**c**) average number of roots produced by rootlings. *Symbols* with *different letters* are significantly different at $\alpha = 0.05$ for storage time only (uses the mean for inside and outside for each date). *Error bars* represent the standard error of the mean ($n = 9$)

then increased gradually as time in storage increased, resulting in approximately a 45% increase after 180 days of storage (Fig. 2c).

Rootling growth rate was positively correlated with the diameter of root segments, but rootling establishment success was not related to root diameter. Root segments with larger diameters (across all four genotypes) produced rootlings with taller shoots over the 31 day growing period ($R^2 = 0.269$, $P < 0.001$; Fig. 3); however, there was no influence of root diameter on the number of roots produced ($R^2 = 0.004$; $P = 0.09$), or on the overall establishment success of rootlings ($R^2 < 0.001$, $P = 0.978$).

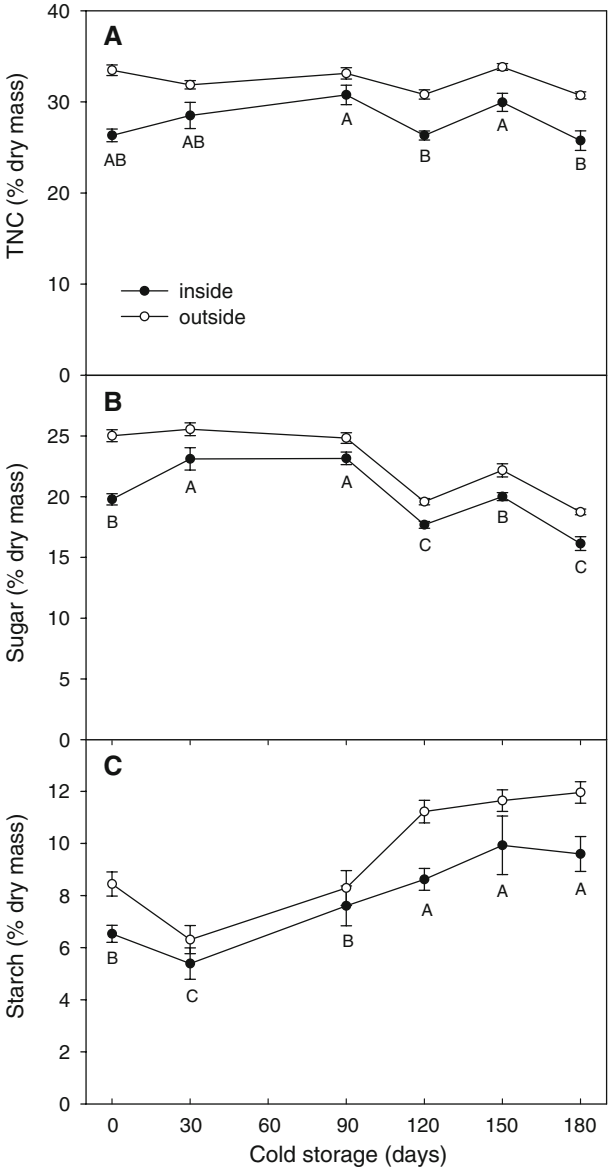

Fig. 2 Total nonstructural carbohydrate (**a**), sugar (**b**), and starch (**c**) concentration of inside- and outside-grown root donor plants over a period of 180 days of cold storage. *Symbols* with *different letters* are significantly different at $\alpha = 0.05$ for storage time only. *Error bars* represent the standard error of the mean ($n = 9$)

Donor plant phenology

The phenological period of donor plants affected rootling establishment success ($P < 0.001$), average shoot height ($P < 0.001$) and the average number of roots produced on a cutting ($P < 0.001$) (Fig. 4). Over the whole experiment, rootling establishment success from inside- and outside-grown donor plants were similar, but there were some differences over time; outside-grown plants initially had greater establishment success than inside-grown donor plants (Fig. 4a). Rootling establishment was greater than 80% when cuttings used to produce rootlings were collected on the three dates that occurred while the

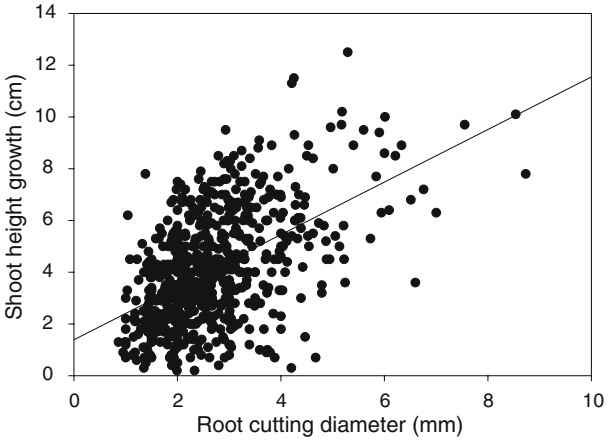

Fig. 3 Relationship between root cutting diameter and height growth of sprouts of four aspen clones grown under inside and outside conditions ($y = 1.016 \times x + 1.389$; $P < 0.001$; $R^2 = 0.269$; $n = 663$)

donor plants were dormant (December 5, February 18, and May 4). Whereas establishment success fell sharply to 18% when root segments were collected when donor plants were in leaf flush and early shoot extension (June 5). However, establishment increased again when cuttings were collected in late summer when donor plants senesced (September 4), and when the donor plants once again became dormant in the fall of 2007 (November 19). Rootling establishment success on September 4 and November 19 was lower than at the December 5th collection the previous year, which was at the initiation of this experiment. Initially, rootlings grown from outside-grown donor plants had greater shoot height than those from inside-grown plants; however, these differences disappeared during the growing season (Fig. 4b). It is noteworthy, that the height growth of rootlings from senesced donor plants collected on September 4 was as low as those during leaf flush (June 5), while cuttings collected from fully dormant donor plants in November 19 were similar to those cuttings collected during the previous dormancy cycle (February 18 and May 4). Root cuttings grown from outside-grown donor plants initially produced more roots than inside-grown donor plants, but as time went on these differences became negligible (Fig. 4c). In addition, shoot height and number of roots decreased steadily when root segments were harvested between December and the beginning of May, and were lowest when donor plants were flushing (June 5th).

Total TNC levels in the donor roots were affected by the different phenological periods of the donor plant ($P < 0.001$) (Fig. 5a). Prior to the experiment outside-grown donor plants had higher root and stem TNC concentrations than inside-grown donor plants; however only root data are presented here. Throughout the three collection times during the dormant period prior to bud flush/shoot extension, TNC levels in roots were between 26 and 33%; however, during leaf flush and shoot extension (June 5), root TNC levels dropped dramatically to between 10 and 14%. By late summer, during senescence (September 4) and when the donor plants were once again fully dormant (November 19, 2007), root TNC had recovered to levels similar to those measured in the initial dormant period (Fall 2006) (Fig. 5a). Soluble sugar concentration in roots dropped throughout the dormant periods, and was lowest (10%) during the early growing season. Soluble sugar concentration in roots collected in September and November 2007 had recovered to 22%, i.e., levels similar to the first collection in December 2006 (Fig. 5b). Changes in root TNC levels appear mostly driven by seasonal changes in starch concentrations. During the dormant period, root starch concentrations increased from 8% in December to about 10% in early May.

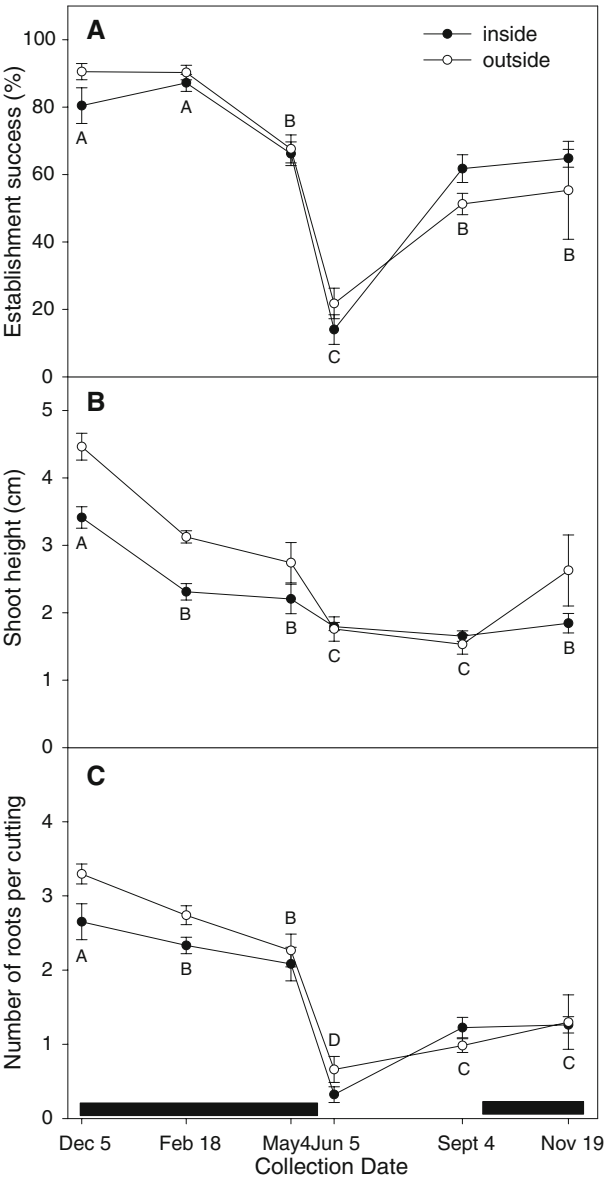

Fig. 4 The rootling establishment success (**a**), shoot height (**b**), and average number of roots produced per cutting (**c**) of rootlings collected from root systems of donor plants grown inside and outside at different phenological periods. Horizontal black bar indicates dormant periods. *Symbols* with *different letters* are significantly different at $\alpha = 0.05$ for storage time only (uses the mean for inside and outside for each date). *Error bars* represent the standard error of the mean ($n = 9$)

During leaf flush (June 5) the starch concentration decreased significantly to 2% (Fig. 5c). Root starch concentration had a prominent peak (25% of dry mass) in September 2007; although, by November 2007 root starch concentrations had decreased to levels similar to those measured in the previous dormant season (Fig. 5c).

Discussion

Overall, root carbohydrate reserves appear to be a poor indicator of the initiation of buds and thus, the success of rootling propagation in aspen. Although factors such as growing

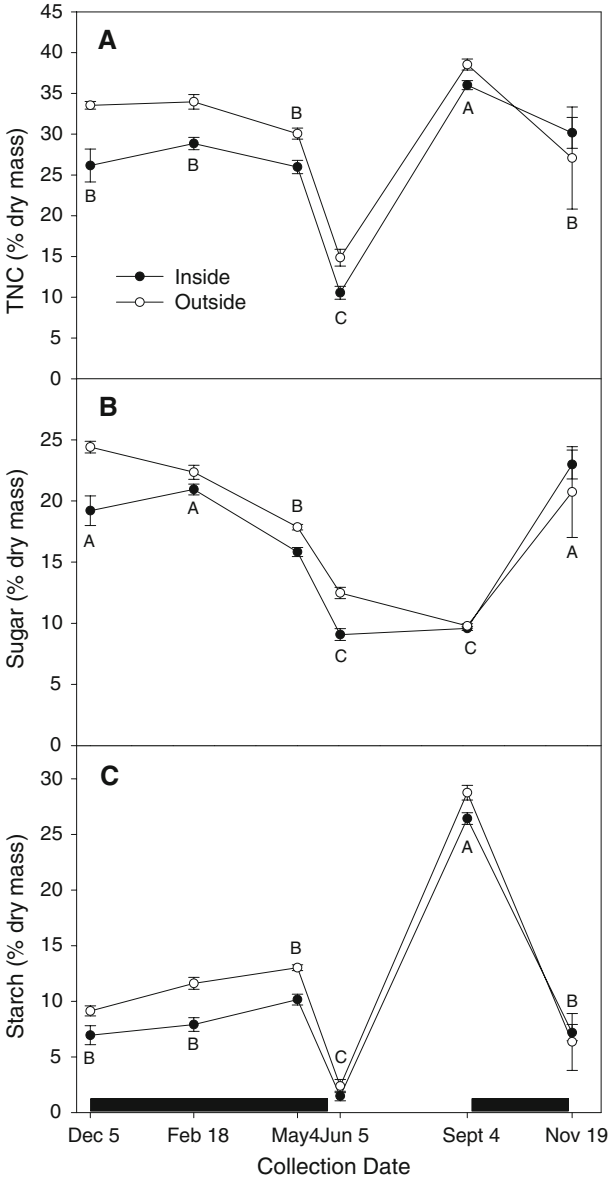

Fig. 5 Total non-structural carbohydrates (**a**), sugar (**b**), and starch in roots of inside- and outside-grown root donor plants (**c**) at different phenological periods. *Horizontal black bar* indicates dormant periods. *Symbols* with *different letters* are significantly different at $\alpha = 0.05$ for storage time only (uses the mean for inside and outside for each date). *Error bars* represent the standard error of the mean ($n = 9$)

conditions of donor plants (inside and outside), and phenological period clearly affected root carbohydrate reserves, there was no consistent relationship between the root carbohydrate reserves and subsequent establishment success of rootlings. Only in the donor-plant phenology experiment was there an indication of such a relationship, where the lowest level of establishment success was found from donor plants harvested in spring at the time of leaf flush (Fig. 4), and very low carbohydrate reserves (Fig. 5). However, the fact that at the September sampling period the TNC concentration was the highest recorded, but establishment success was only moderate does not lend much support to idea that carbohydrate levels are the primary controlling factor for establishment success.

Shoot height growth, however, was well linked to carbohydrate reserves. Root donor plants grown outside had higher root carbohydrate reserves than inside-grown donor plants (Table 1). This is likely due to the earlier budset occurring under outside conditions, as a result of fluctuating air and soil temperature, exposure to wind, higher vapor pressure deficits, and shortening day length. This resulted in a shift in allocation of carbohydrate reserves away from stem growth, towards storage and root growth (Martens et al. 2007). The differences between inside- and outside- grown root donor plants were carried through the cold storage period. While the rootlings produced from roots of outside-grown plants were taller and had greater numbers of roots than rootlings from inside-grown donor plants, the rate of establishment success of rootlings was similar except for the period between 120 and 150 days of cold storage (Fig. 1a). It is interesting to note that the difference in establishment success and growth between inside- and outside-grown donor plants in the phenology experiment disappeared quickly during the following growing season where all donor plants were grown outside (Fig. 4a). Coincidentally, the difference in root carbohydrate reserves between the formerly inside- and outside-grown donor plants also disappeared at that time.

We expected a decline in rootling performance with long periods in storage because of the respiration losses of carbohydrates during storage (Zasada et al. 1994; Landhäusser and Lieffers 1997), and the fact that root cuttings depend on carbohydrate reserves to establish the buds and first leaves and roots prior to initiation of photosynthesis (Schier and Zasada 1973). However, the average rootling establishment success for all treatments was above 80% throughout the cold storage experiment, indicating that the length of cold storage and the growing conditions of the root donor plants were not major factors affecting propagation success. However, the decline in height and root growth of rootlings after 120 days of storage is somewhat puzzling especially since shoot height and root growth recovered with the longer storage times of 150 and 180 days. The reasons for this are unlikely to be directly linked to root carbohydrate reserves, since there was no detectable change in total nonstructural carbohydrate (TNC) supply with time in storage. Over the course of long-term storage, there tended to be a gradual reduction in the amount of sugars, offset by a gain in the amount of starch. As the sugars were high at the beginning of storage and low at the end of storage, and performance was better at 0–30 and at 150 days of storage, the form of the carbohydrate reserve (sugar vs. starch) does not appear to be directly associated with bud initiation and early rootling establishment success; however, subsequent growth appears to be affected. There are likely other mechanisms affecting rootling establishment when root donor plants are stored for various lengths of time such as various growth hormone and possibly nutrient concentrations within the plant (Eliasson 1969, 1971; Schier and Zasada 1973) or other physiological processes such as dormancy and hardening (Lang et al. 1987; Lindqvist 2001).

The most critical stage where rootling establishment success was severely limited (down to 18%) was during the period of leaf and shoot extension early in the summer. This period coincided with very low root carbohydrate concentrations. However, given that the establishment success of rootlings was generally not strongly related to root carbohydrate reserves, as described above, we speculate that due to the extremely low root TNC reserves (10–15%) at the time of active shoot growth, roots and shoots on the cuttings might have been initiated but their continued development was impeded (Schier and Zasada 1973). Plant hormones play a critical role in the initiation of shoots and roots in aspen (Eliasson 1969, 1971; Schier and Zasada 1973; Frey et al. 2003), and likely have a larger effect on establishment success than root carbohydrate reserves (Wan et al. 2006). Newly expanding leaves and shoots produce large amounts of auxins (Cline 1991, 1994), which can severely

suppress the initiation of shoots on aspen roots (Wan et al. 2006). This is especially important in our donor plants where root to shoot ratios were low (data not shown), likely resulting in auxins dominating in the root system (Cline 1991). This is also supported by the observation that the bud initiation and rooting was not linked to the diameter of root cuttings, while shoot height growth was. This suggests that initiation of stems and roots are likely related to hormonal signals, while the initial growth rate of the roots and sprouts is likely related to the carbohydrate reserves in the root segment. Larger root segments have greater capacity for reserve storage and therefore more energy for increased growth rates, but they do not necessarily have the correct physiological signal to initiate stems or roots (Stenvall et al. 2006). The relatively good establishment success of rootlings at the end of the growing season, coupled with relatively poor height growth of shoots and roots is also interesting. At that time, TNC reserves were high, but most of the TNC were tied up in the form of starch; starch reserves were twice as high as sugar reserves at any time during the phenology experiment. It is possible that the starch was not as easily available for growth of the rootling as starch needs to be converted to soluble sugars to be readily available for shoot and root growth (Landhäusser and Lieffers 2003).

In conclusion, donor plants that were grown under outside conditions had higher root carbohydrate reserves, produced larger and had slightly higher establishment success of rootlings than donor plants grown and hardened-off in the greenhouse. Second, there was only a small negative effect due to long periods of cold storage of donor roots on the establishment success of rootlings. Thus, nursery operators could store donor plants for long periods and start crops of rootlings at any time during the winter and early spring. Third, collecting from donor plants near the end of the growing season, produced rootlings that were smaller and had fewer roots but it did not affect establishment success. Fourth, while there is evidence that high levels of root carbohydrates are beneficial to the growth of rootlings, there are other unmeasured factors such as hormone and nutrient levels at the time of collection that likely contribute to the establishment success of rootlings and require further investigation.

Acknowledgments We thank Jill Fraser, Jennifer Langhorst, Geoffrey Kershaw, Mike Wagner, Tomasz Gradowski, Kevin Renkema, Chris Williams and Dominique Deshaies for their help with this study. Pak Chow for TNC analyses, Jeff Hoyem (Woodmere Nursery) for donor planting stock production and Larry Lafleur (Smoky Lake Nurseries) for providing cold storage facilities. Financial support is gratefully acknowledged from Western Boreal Aspen Corporation, Woodmere Forest Nursery, and Forest Resource Improvement Association of Alberta (FRIAA).

References

Brouard JS, Niemi F, Charleson LR (2005) The effect of root segment origin, size, and orientation in aspen rootling propagation. In: McKeand SE, Li B (eds) Proceedings 28th Southern forest tree improvement conference. Raleigh, NC. National Technical Information Service, Springfield, Virginia, USA, pp 80–91

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

Cline MG (1991) Apical dominance. Bot Rev 57:318–358. doi:10.1007/BF02858771

Cline MG (1994) The role of hormones in apical dominance: new approaches to an old problem in plant development. Physiol Plant 90:230–237. doi:10.1111/j.1399-3054.1994.tb02216.x

DeByle NV, Winokur RP (1985) Aspen: ecology and management in the Western United States. United States Department of Agriculture-Forest Service, Fort Collins, CO, USA

Eckenwalder JE (1996) Systematics and evolution of *Populus*. In: Stettler RF, Bradshaw HD, Heilman PE et al (eds) Biology of *Populus* and its implication for management and conservation. NRC Research Press, Ottawa Ontario, Canada, pp 7–32

Eliasson L (1969) Growth regulators in *Populus tremula* I. distribution of auxin and growth inhibitors. Physiol Plant 22:1288–1301. doi:10.1111/j.1399-3054.1969.tb09119.x

Eliasson L (1971) Growth regulators in *Populus tremula*, IV. Apical dominance and suckering in young plants. Physiol Plant 25:263–267. doi:10.1111/j.1399-3054.1971.tb01440.x

Farmer RE (1963) Vegetative propagation of aspen by greenwood cuttings. J For 61:385–386

Frey BR, Lieffers VJ, Landhäusser SM et al (2003) An analysis of sucker regeneration of trembling aspen. Can J Res 33:1169–1179. doi:10.1139/x03-053

Haapala T, Pakkanen A, Pulkkinen P (2004) Variation in survival and growth of cuttings in two clonal propagation methods for hybrid aspen (*Populus tremula* x *P. tremuloides*). For Ecol Manage 193: 343–354

Hartmann HT, Kester DE, Davies FT Jr et al (2002) Hartmann and kester's plant propagation: principles and practices, 7th edn. Prentice-Hall, Upper Saddle River, NJ, pp 278–694

Landhäusser SM, Lieffers VJ (1997) Seasonal changes in carbohydrate storage and regrowth in rhizomes of and stems of four boreal forest shrubs. Scand J For Res 12:27–32. doi:10.1080/02827589709355380

Landhäusser SM, Lieffers VJ (2003) Seasonal changes in carbohydrate reserves in mature northern *Populus tremuloides* clones. Trees (Berl) 17:417–476. doi:10.1007/s00468-003-0263-1

Landhäusser SM, Lieffers VJ, Mulak T (2006) Effects of soil temperature and time of decapitation on sucker initiation of intact *Populus tremuloides* root systems. Scand J For Res 21:299–305. doi:10.1080/02827580600813313

Lang GA, Early JD, Martin GC et al (1987) Endodormancy, paradormancy and ecodormancy–physiological terminology and classification for dormancy research. Hortsci 22:371–377

Lindqvist H (2001) Effect of different lifting dates and different lengths of cold storage on plant vitality of silver birch and common oak. Sci Hortic (Amsterdam) 88:147–161. doi:10.1016/S0304-4238(00)00197-7

Martens LA, Landhäusser SM, Lieffers VJ (2007) First-year growth response of cold-stored, nursery-grown aspen planting stock. New For 33:281–295. doi:10.1007/s11056-006-9027-2

Noel N, Leple JC, Pilate G (2002) Optimization of in vitro micro propagation and regeneration for *Populus* x *interamericana* and *Populus* x *euramericana* hybrids (*P. deltoides*, *P. trichocarpa*, and *P. nigra*). Plant Cell Rep 20:1150–1155. doi:10.1007/s00299-002-0465-9

Richardson J, Cooke JEK, Isebrands JG et al (2007) Poplar research in Canada–a historical perspective with a view to the future. Can J Bot 85:1136–1146. doi:10.1139/B07-103

Schier GA, Zasada JC (1973) Role of carbohydrate reserves in the development of roots suckers in *Populus tremuloides*. Can J Res 3:234–250

Stenvall N, Haapala T, Pulkkinen P (2004) Effect of genotype, age and treatment of stock plants on propagation of hybrid aspen (*Populus tremula* x *Populus tremuloides*) by root cuttings. Scand J For Res 19:303–311. doi:10.1080/02827580410024115

Stenvall N, Haapala T, Pulkkinen P (2006) The role of a root cutting's diameter and location on the regeneration ability of hybrid aspen. For Ecol Manage 237:150–155

Vinocur B, Carmi T, Altman A et al (2000) Enhanced bud regeneration in aspen (*Populus tremula* L.) roots cultured in liquid media. Plant Cell Rep 19:1146–1154. doi:10.1007/s002990000243

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

Wang YF, Zwiazek JJ (1999) Effects of storage temperature on physiological characteristics of fall-lifted white spruce (*Picea glauca*) bareroot seedlings. Can J Res 29:679–686. doi:10.1139/cjfr-29-6-679

Yu QB, Mantyla N, Salonen M (2001) Rooting of hybrid clones of *Populus tremula* L. x *P. tremuloides Michx*. by stem cuttings derived from micropropagated plants. Scand J For Res 16:238–245. doi:10.1080/02827581750285875

Zasada JC, Tappeiner JC, Maxwell BD, Radwan MA (1994) Seasonal changes in shoot and root production and in carbohydrate content of Salmonberry (*Rubus spectabilis*) rhizome segments from the Central Oregon Coast Ranges. Can J For Res 24:272–277

Zufa L (1971) Rapid method for vegetative propagation of aspens and their hybrids. For Chron 47:36–39