Depth of root placement, root size and carbon reserves determine reproduction success of aspen root fragments

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Abstract

The vegetative recovery of species capable of resprouting is strongly driven by the disturbance type, the resprouting traits of the species, and the resource availability in the surviving tissues. Since aspen (*Populus tremuloides*) commonly regenerates from root suckers after disturbance; we can take advantage of its ability to sprout by applying salvaged surface soils containing aspen root fragments to reclaim heavily disturbed forest sites where soil surfaces have been displaced by resource extraction. In two studies we investigated the role of root size, root carbohydrate reserves, and the presence of fine roots on the ability of root fragments to initiate and grow suckers from different soil depths. Roots of different diameters were collected from natural aspen stands and buried at three different soil depths (5, 20 and 40 cm). Non-structural carbohydrate (NSC) reserves were determined initially and after the experimental period at the end of August. The initiation of suckers was not affected by soil depth, root size, and the presence of fine roots. Suckers, however, did not emerge from the soil if root fragments were buried at a depth of 40 cm. The largest suckers were found on root fragments that were 2.1 to 3 cm in diameter. Sucker performance and root fragment survival increased with the initial NSC reserves stored in the root fragments. Insufficient initial NSC supply for suckering resulted in the death of root fragments indicating that there might be a lower threshold of NSC root reserves. The presence of fine roots appear to be a liability as overall sucker numbers were three times higher in root fragments, that had all their fine roots removed compared to root fragments with fine roots attached.

Keywords: vegetative regeneration, *Populus tremuloides*, leaf area, soil temperature, sucker initiation, forest reclamation

1. Introduction

The rapid recovery of photosynthetic potential from protected buds (budbank) is a common strategy in plants living in disturbance prone environments. Species capable of resprouting have strategies that allow them to translocate resources from surviving organs to the regenerating tissues and organs. The vegetative recovery of these species after disturbance is strongly driven by the disturbance type, the resprouting traits of the species involved, and the associated resource availability in the surviving tissues (Chapin et al. 1990; Poorter et al. 2012; Clark et al. 2013). Biomass allocation patterns and the related storage of non-structural carbohydrates (NSC) reserves fundamentally drive the resource availability for resprouting after a severe disturbance, where reserves need to be allocated to the maintenance of the surviving tissues (respiration) and the regeneration and maintenance of new stem, leaf, and root tissues (Lambers et al. 2002). These allocation patterns are known to be under the control of environmental variables such as light, temperature and moisture (Poorter et al. 2012).

Trembling aspen (*Populus tremuloides* Michx.) regenerates through suckering from its root system after disturbances such as fire or logging disturb the above-ground portion of the clone (DesRochers and Lieffers 2001; Farmer 1962; Frey et al. 2003). Vegetative regeneration is dense and vigorous when these disturbances create little damage to the root system (Frey et al. 2003; Renkema et al. 2009). In contrast heavy mechanical soil disturbances from trafficking of the forest floor during conventional forest harvesting (e.g. log decking areas) or after complete removal of roots during road construction or mineral resource extraction can cause severe reduction in the suckering potential from the fragmented root system (Renkema et al. 2009). In the latter case the surface soils are often salvaged and stored for several months in stockpiles and then placed back onto the disturbed area after operations have ceased (“roll back”), where the root fragments in the roll back material could be available for sprouting and aspen regeneration. The success of sucker initiation after this roll back of soil and root fragments is likely influenced by factors such as the depth a root fragment is placed, the physiological state of the fragment, and its ability to interact with the surrounding soil. Under natural undisturbed soil conditions most suckers originate from smaller diameter roots (0.5 – 2.5 cm) (DesRochers and Lieffers 2001; Kemperman 1978; Schier and Campbell 1978) and from roots that are located at depths of 4 to 15 cm; this is likely a result of the more favourable conditions in these upper soil layers (e.g. warm soil temperature, aeration, and high concentration of nutrients) (Brown and DeByle 1987;

Horton and Maini 1964; Kemperman 1978; Navratil 1991; Schier 1973; Schier and Campbell 1978). There is relatively little information on the vertical distribution of aspen roots in soil (Strong and LaRoi 1983), but aspen roots can also be found in deeper parts of the soil profile, as a result of root competition with other woody species (Mundell et al. 2007) or moisture limitation (Snedden unpublished). However there is little knowledge of the suckering ability of these deeper roots.

Available non-structural carbohydrate (NSC) reserves influence suckering performance of aspen roots (Landhäusser and Lieffers 2002; Schier and Zasada 1973). Although NSC concentrations appear not to influence the initiation of suckers, they are important for early sucker shoot expansion and growth (Frey et al. 2003; Landhäusser and Lieffers 2002; Schier and Zasada 1973). As a result root NSC concentrations might be pivotal to success of suckers when they originate from greater soil depths or are slowed by physical barriers (Landhäusser et al. 2007a, 2007b; Renkema et al. 2009). The role of the content of NSC reserves in aspen roots, however, is less clear. If present, plant NSC reserves can readily be mobilized and translocated within the plant to sinks where NSC are needed; however in root fragments the NSC reserves are limited by the size of the of the root fragment and as a result pool size of the fragments might play a larger role. Plant NSC reserves can be expressed as a concentration (tissue status) or content (pool size - root size and concentration) (Dirr and Heuser 1987; Ede et al. 1997; Nguyen et al. 1990).

Another factor possibly influencing the suckering ability of root fragments in rolled back materials could be related to the ability of the root fragments to connect with the surrounding soil and its ability to take up water and nutrients. Nutrient and water uptake occurs largely through fine roots and it is not clear if fine roots associated with fragments are a net benefit to the suckering ability of root fragments.

In two controlled field studies we explored the regeneration potential of aspen from root fragments in relation to (i) soil depth (e.g. depth of root burial), (ii) root tissue non-structural carbohydrate reserve concentration and content, and (iii) the importance of attached fine roots on root fragments for sucker initiation, emergence, and growth performance. We hypothesized that root fragment buried at a deeper soil depth will require more reserves to reach the soil surface and might face greater reserve exhaustion. We further hypothesized that root fragments with attached fine roots will have improve soil contact, allowing the regenerating root suckers to access water and nutrient resources more easily.

2. Methods

2.1 Research site

The experimental site was located at the Crop Diversification Centre North near Edmonton, Alberta (53°38'N, 113°21'W; 668 m a.s.l.). A level agricultural field was used for the experiment. The soil texture of the soil was a silty loam which was deep and well drained. Precipitation during the study between May 1 and August 30, 2010 amounted to 174.1 mm and no extended drought periods were observed. Mean air temperature over the four months was 14°C (Environment Canada 2010) and average soil temperatures were 16.9°C at 5 cm soil depth, 16.1°C at 20 cm, and 15.3°C at 40 cm.

2.2 Plant material

The root material used in this study was collected in February 2010 near Genesee, Alberta (53°19'N, 114°18'W) from an aspen stand (4 ha) that had been cut and naturally regenerated 9 years earlier. Trees were up to 7 m tall and stem density was 13000 stems/ha. The roots and soil materials were collected using large scale operational methods where the above-ground portion of the aspen stand was sheared off close to the soil surface under frozen conditions (>20 cm soil depth) using a straight blade mounted on a large bulldozer. Then the top 20 cm of the frozen forest floor and mineral soil containing the aspen roots were pushed into large windrows. On the same day, thirty root fragments (> 65 cm in length) were chosen for each of three diameter size classes (Class 1: 1-2 cm, Class 2: 2.1-3 cm; Class 3: 3.1-4 cm) for a total of 90 roots. All selected fragments were straight, had no major lateral roots, and were visibly undamaged. To test the influence of the presence of fine roots attached to fragments an additional 20 undamaged root fragments (0.6 – 1.5 cm in diameter, 18 – 60 cm long) were collected. All of those selected fragments had fine roots (>1 mm diameter) still attached and were collected at the same time. All collected roots were wrapped in plastic, and stored at -5°C until the end of April 2010.

2.3 Treatments

In late April, fragments were slowly thawed over two days and both ends were re-cut to remove potential pathogens that may have attached during storage. A sample of each root fragment (1 cm in length) was also taken to determine root non-structural carbohydrate (NSC) reserve concentrations (see below) prior to planting. To explore the impact of fragment size and burial depth, all root fragments were trimmed to a total length of 50 cm. To estimate root fragment volume, root diameter was measured at both ends of each fragment. Root fragments were planted (buried) horizontally at 3 different soil depths (5, 20 and 40 cm) at the research site. The experiment was designed as a complete block design with 10 blocks (2 × 3 m), each consisting of three plots (1 × 1 m), which were randomly assigned to one of the three soil depths and each containing one root fragment of each of the three different diameter size classes (class 1: 1-2 cm; class 2: 2.1-3 cm; class 3: 3.1-4 cm). HOBO soil temperature data loggers (Onset Computer Corporation, Bourne, Mass.) were also placed at the three soil depths (n = 3 at each depth) to record soil temperatures over the four summer months.

To test the impact of the presence of fine roots, collected root fragments were slowly thawed under moist conditions. The experiment was a paired design with 10 pairs of root fragments of similar diameter and length, as well as similar in the number and length of attached fine roots. Among the pairs, the number of attached fine roots per root fragment ranged from 8 to 19 fine roots. One of the root fragments in each pair had all fine roots removed prior to planting and a sample of each root fragment (1 cm) was taken to determine initial NSC reserves of each of the 20 root fragments. Each pair was buried horizontally at a depth of 10 cm. A soil temperature data logger was also placed at the same location.

Over the course of the growing season (May 1, 2010 to August 24, 2010) the plots were visited several times in order to remove weeds and to monitor sucker emergence.

2.4 Measurements

At the end of August, root fragments (and their suckers) were then carefully excavated and the entire clone was kept on ice until brought back to the lab. In the lab all roots and suckers were carefully washed and then separated into dead and live root fragments. Live root fragments were distinguished from dead root fragments by their yellow bark and white phloem, while dead fragments had dark brown bark with black phloem.

For each clone (root fragment), the total number of suckers initiated were counted and divided into emerged suckers (suckers that had made it above the soil surface) and non-emerged suckers. The total extension length of emerged and non-emerged suckers (below-ground plus above-ground length) and the above-ground height were recorded. A root tissue sample (1 cm long) was taken from the center of each root fragment to determine non-structural carbohydrate (NSC) reserve concentrations at the end of the study. As there were no new roots initiated directly on the root fragments, only the number and mass of new lateral roots initiated on the below-ground portion of the suckers (adventitious roots) were measured. All roots and suckers, as well as the root fragment NSC samples were oven dried at 68°C until constant weight. Dry mass of sucker stems and leaves were determined.

For NSC analyses, root fragment tissue samples were ground to pass 40-mesh (0.4 mm) using a Wiley mill (Thomas Scientific, Swedesboro, New Jersey). Soluble sugars were extracted from ground tissue by boiling samples three times in 80% ethanol at 95°C. Phenol-sulfuric acid assay was used to determine colourimetrically total soluble sugar concentrations. The residue was analyzed for starch by enzymatic digestion with a mixture of α-amylase and amyloglucosidase for 20 h, followed by the colourimetric measurement of glucose hydrolyzate with a peroxidase–glucose oxidase-o-dianisidine reagent (Chow and Landhäusser 2004).

Growing season degree-days were calculated by taking the average daily soil temperature minus the base temperature of 8°C and summing the number of day above the threshold during the experimental period. A base temperature of 8°C was chosen based upon Fraser (2002), who found that 8°C is the a soil temperature at which aspen starts to initiate suckers.

2.5 Data analysis

The root diameter and soil depth experiment was a complete block design with ten blocks consisting of three plots each, which were randomly assigned to one of the three depths and containing one root fragment each of the three different diameter size classes. Data of the non-emerged sucker variables were analyzed as a randomized 3 × 3 factorial design with three soil depths and three size classes as non-emerged suckers were present at all three depths and root diameter size classes.

The design of the fine root study was a paired design with ten replicate pairs of root fragments with and without attached fine roots. This study was analyzed as a paired one-way ANOVA. Tested variables for both studies included number of emerged suckers, number and length of fine roots associated with new suckers, leaf dry mass, total sucker dry mass and the number of non-emerged suckers, their heights and dry mass.

For both studies, the number of new roots, length of new roots, and number of non-emerged suckers did not meet the assumption of homogeneity of variances and therefore were log transformed. However, no response variables related to the whole root data set met the assumption of normality (using the Shapiro-Wilk test), as a result the variables were analyzed using both the non-parametric Kruskal-Wallis k-sample test and ANOVA. Since the interpretations were the same between both tests, only the results of the ANOVAs are presented. Emerged sucker numbers and their height growth over the course of the growing season (3 measurements) were analyzed using the repeated measures ANOVA. For this analysis only the data from root fragments buried at 5 and 20 cm depth were used, as suckers emerged only at these two depths. A reduced data set, which included only those root fragments that produced emerged suckers, was used in the analyses of differences in emerged sucker numbers per root fragment between treatments and of relationships between (1) sucker leaf dry mass and the number of new fine roots associated with emerged suckers, (2) the leaf mass and NSC content in the root fragment, and (3) initial May starch content and total sucker dry mass.

To explore the impact of initial and final NSC content and concentration of root fragments on sucker production and root fragment mortality, data were analyzed as a one-way ANOVA. Relationships between total sucker length and initial starch concentration of root fragments and between sucker leaf dry mass and root fragment starch concentration at the end of the experiment were analyzed using simple linear regression. The NSC data set included the root fragments of all three diameter size classes, soil depth 5 and 20 cm, and root fragments without fine roots attached of the fine root study. Since root mortality data were categorical, the influence of soil depth, root diameter size, roots with fine roots, roots without fine roots and presence of suckers on root fragment mortality was analyzed using the proc catmod procedure in SAS. A significance level of $\alpha = 0.05$ was used for all analyses.

3. Results

Soil depth

Average daily growing season soil temperatures (May to August 2010) decreased with soil depth from 16.8°C at 5 cm, 16.1°C at 20 cm to 15.3°C at 40 cm depth with the soil temperature at 5 cm depth being higher than at 40 cm depth ($p = 0.034$). Earlier research indicated that 60 degree-days (at a base temperature of 8°C) were generally needed for the sucker initiation on root fragments (Fraser et al. 2002). Our root fragments reached these heat sums on May 19th at a soil depth of 5 cm, on May 20th at a soil depth of 20 cm, and on May 29th, 2010 at a soil depth of 40 cm.

There was no significant interaction between soil depth and root diameter size for any of the measured response variables, as a result only main effects are presented. Mortality of fragments was 80% at 5 and 40 cm soil depth compared to 50% at 20 cm ($p = 0.045$; Table 1). All live root fragments had produced suckers; however, three of the root fragments that were dead at the end of the growing season had produced live suckers. These live suckers were all supported by new lateral roots originating from the below-ground portion of the sucker.

Of the 90 planted root fragments, 27 (30%) had produced suckers (emerged and/or non-emerged suckers (NES)). The total number of suckers initiated averaged 1.7 suckers root^{-1} and was not affected by soil depth ($p = 0.407$). Although root fragments buried at 40 cm depth had a similar total number of suckers per fragment than fragments buried at 5 and 20 cm, none of these suckers had emerged above the soil surface ($p = 0.011$). However, in fragments buried at 5 and 20 cm, the number of emerged suckers was not impacted by soil depth (average 0.55 emerged suckers root^{-1}; Table 1). As can be expected, non-emerged sucker height was impacted by soil depth with the taller NES at the deeper soil depths ($p = 0.005$; Table 1).

Of the 27 root fragments that had suckered, 18 root fragments produced emerged suckers (only at depths 5 and 20 cm), with more emerged suckers at a soil depth of 5 cm (2.7 emerged suckers root^{-1}) compared with 20 cm (1.3 emerged suckers root^{-1}) ($p = 0.026$). At 5 cm depth, suckers reached the soil surface 10 days earlier than at 20 cm depth ($p = 0.008$; Fig. 1a). The total length of emerged suckers (i.e. the length below-ground plus above-ground) was also affected by soil depth ($p = 0.029$); however, once the suckers were above-ground, growth and leaf dry mass was not different between the two soil depths (both $p > 0.471$; Table 1; Fig. 1b).

While the buried root fragments themselves did not produce any new roots, emerged suckers produced similar numbers of new adventitious roots on the below-ground portions of the sucker (3.1 new roots per root fragment; $p = 0.646$); however, the total length of those roots was higher at suckers from fragments buried at a soil depth of 5 cm ($p = 0.003$) (Table 1).

Importance of root fragment diameter

Root fragment diameter had no impact on fragment mortality ($p = 0.608$). The number of non-emerged suckers increased with root fragment diameter ($p = 0.011$); however, it did not affect the total number of suckers (sum of emerged and NES) produced by a fragment ($p = 0.378$; Table 2). Although fragment diameter did not affect the number of emerged suckers ($p = 0.629$), diameter class 2 root fragments had the greatest total sucker length (sum of below- and above-ground) ($p = 0.001$) and above-ground height ($p = 0.002$; Table 2). Accordingly, emerged suckers from class 2 root fragments had more leaf and total sucker dry mass per root fragment (Table 2). Suckers emerging from the two larger fragment diameters had on average 3 new adventitious roots sucker^{-1} compared to only 0.3 new roots sucker^{-1} when originating from the class 1 root fragment diameter ($p < 0.005$; Table 2). There was also an increase in growth of these adventitious roots with an increase in fragment diameter ($p < 0.005$); however, we could not detect a relationship between leaf dry mass and the number and length of new fine roots ($p = 0.982$).

Initial non-structural carbohydrate (NSC) content and concentrations in the root fragments varied with fragment diameter (Table 2). While NSC content increased with increasing diameter, NSC concentrations decreased; however, these relationships did not persist after the growing season, where root fragment death and/or suckering success likely impacted the reserves status of the root fragments. At the end of the experiment, live root fragments that had produced emerged suckers had on average 8.5 g NSC content root^{-1} (8.7% NSC of dry mass) while live root fragments with no emerged suckers had 5.4 g root^{-1} (4.5%) NSC and dead root fragments had an average NSC content of only 2.9 g root^{-1} (2.4%) (both content and concentration $p < 0.005$; Fig. 2). Dead root fragments had very little starch reserves (at the detection limit), while fragments with emerged suckers had higher tissue starch concentration (1.7%) compared to the initial measurement (0.5%) ($p = 0.003$) (Fig. 2).

Root fragments that died during the experiment started with lower initial NSC content (approx.. 40%) and concentrations (approx. 25% lower) compared to root fragments that stayed alive (both $p < 0.01$; Fig. 3). Initial starch content and concentration were also lower in root fragments that were dead at the end of the experiment than in live fragments (both $p < 0.001$; Fig. 3).

There was a positive linear relationship between the initial NSC and starch concentration of root fragments and the above-ground height of suckers ($p=0.033$; $R^2=0.348$; Fig 4A; NSC not shown), while initial NSC and starch content of root fragments was not related to sucker growth (p=0.913). Further, starch concentration in root fragments at the end of the study was positively related to sucker leaf dry mass (p = 0.008; R^2=0.517; Fig. 4B).

Importance of fine roots

Whether or not fine roots were attached to root fragments had no impact on fragment mortality (55%) (p = 0.185). However, there were more suckers (emerged and NES) initiated on fragments without fine roots (1.8 suckers $root^{-1}$) than on fragments with fine roots attached (0.6 suckers $root^{-1}$; p = 0.032; Table 3), but this effect was not detectable for emerged suckers only (p = 0.545; Table 3). However, there were more and longer new adventitious roots attached to emerged suckers when associated with root fragments without fine roots attached compared to emerged suckers on fragments with fine roots attached (both p < 0.005, Table 3).

4. Discussion

Soil depth had a considerable impact on the ability of suckers to emerge above the soil surface. Suckers originating from root fragments that were buried at a depth of 40 cm were unsuccessful in reaching the soil surface in one growing season, indicating that the required resources contained in the root fragments (fragment size and initial NSC concentration) were not sufficient to allow suckers to emerge above the soil surface from a depth of 40 cm. This is a plausible explanation as soil conditions (deep agricultural soil with silty loam soil texture) had no obvious restrictions to sucker expansion. There is strong evidence that sucker growth was dependent on the reserve status (starch concentration) of the fragment (Figure 4A). Suckers in our study emerged above-ground only successfully from fragments at a soil depth of 20 cm or less. Interestingly the failure to reach the surface was not influenced by fragment diameter, which indicates that fragment reserve content did not play a critical role in the suckering success of fragments.

Fragment survival was highest at the 20 cm depth, perhaps because at the 5 cm depth there may have been intermittent periods of water stress, while at the 40 cm depth no suckers made it to the surface not allowing a replenishment of root reserves. However at a depth of 5 cm, root fragments produced more emerged suckers than root fragments at a depth of 20 cm. We believe that the shorter distance to the soil surface enabled more suckers at the shallower depths to reach the surface before apical dominance started to suppress sucker initiation and growth (Eliasson

1971; Farmer 1962; Schier 1972; Steneker 1974; Wan et al. 2006). In turn, limited root reserves and the longer distance needed to reach the surface may have been the reason why root fragments buried at a depth of 20 cm were able to support only very few dominant suckers until they started photosynthesizing. This assumption is also supported by the fact that depth had a significant effect on emerged total sucker height as root fragments buried at depth 20 had to produce the tallest suckers to reach the surface, but their subsequent height growth above the surface was not different between soil depth treatments.

The initiation of suckers (total number of suckers) on root fragments was not affected by soil depth, root diameter size (within our tested range), and the presence of fine roots in our study. This is not too surprising, as it is known that the initiation of suckers is mainly driven by the absence of apical dominance, which is primarily mediated by growth regulators such as auxin and cytokinin (Eliasson 1971; Farmer 1962; Schier 1972; Steneker 1974). However, suckers initiated on fragments that were buried at 40 cm did not grow taller than 20 cm below-ground, and many of the root fragments were dead after the growing season, indicating that there were restrictions in resource availability from the fragment, as soil temperatures during the growing season were only marginally lower at 40 cm than at 5 or 20 cm depth and were well above the threshold for suckering at 8 ºC. This might suggest that independent of soil temperature, soil depth also plays a role in the emergence of suckers from aspen root systems. In natural conditions suckers typically emerge from roots located within 8 cm of the surface (Brown and DeByle 1987; Navratil 1991; Schier and Campbell 1978) but in natural conditions this is also where most roots are found. Root size, as controlled by diameter, did not influence emerged sucker numbers, which conforms to the results of several other studies which report that suckers mostly sprout from lateral roots with diameters between 0.5 – 2.5 cm (DesRochers and Lieffers 2001; Peterson and Peterson 1992; Schier 1973). Further, the diameter of small root cuttings (2 – 10 mm) of hybrid aspen (*P. tremula* L. × *P. tremuloides* Michx.) (Stenvall et al. 2006) and aspen (Schier 1978; Starr 1971) did not affect their suckering efficiency.

The presence of fine roots on root fragments was negatively related to the number of suckers that were initiated; indeed, when fine roots were attached to the fragment there were only 60% fewer suckers as when fine roots were removed. The mechanism for this inhibition are not clear but fine roots have much higher respiration costs which can be more than 10-times greater in fine roots than in coarse roots for a the root fragment (DesRochers et al. 2002) and given that fine roots are more likely to be injured during extraction, significant C resources may have also gone into the repair of these fine roots. Further, growth regulators (hormones) produced from wounded fine roots may also have inhibited sucker initiation (Frey et al. 2003). However, emerged suckers grew just as well whether the root fragments had fine roots attached or not. The shallow burial depth of these fragments in this study at only 10 cm may have also allowed suckers to quickly reach the soil surface. Also the similar growth of the suckers was not anticipated, as it is known that fine roots play an important role in the supply of trees with soil resources of water and nutrients (Charlton 1996).

Similar to natural conditions, where suckers will replenish carbohydrate reserves of the parent root system (DesRochers and Lieffers 2001, Landhäusser and Lieffers 2002), root fragments of our study were provided with NSC from the emerged suckers. The connection of the emerged suckers to the root fragment appear to be functional, as NSC reserves were higher in fragments with emerged suckers than in live fragments with no emerged suckers regardless of soil depth (Figure 2). This might indicate that root fragments might still be used as a reserve storage organ (we had only 3 root dead fragments with suckers that were alive after the growing season). This is further supported by our observation that an increase in sucker leaf dry mass also resulted in higher starch reserve levels in the root fragments at the end of the growing season (Figure 4B).

The initiation and growth of new adventitious fine roots on the belowground portion of the suckers was only associated with emerged suckers. The initiation of new roots is thought to be controlled by the successful emergence of suckers, where the supply of newly fixed carbohydrates from the leaves promotes root initiation (Eliasson 1968). This is further supported by work from Stenvall et al. (2006) who found that the initiation of new roots on root fragments took twice as long as the initiation of suckers and that root fragments with the best suckering produced also the highest number of new roots.

In summary, while soil depth did not influence root sucker initiation, it strongly affected the ability of suckers to reach the soil surface; therefore, suckers originating from root fragments buried deeper than 20 cm are unlikely to emerge above the soil. The amount of initial carbohydrate reserves stored in root fragments rather than the presence of fine roots played an essential role in sucker emergence and performance and root fragment survival. In this study the NSC reserves of roots were higher than what has been previously observed in healthy but mature aspen stands (Landhäusser and Lieffers 2003); this is likely the result of our donor roots originating from a 9-year-old stand with a stem density of 13000 stems per hectare. The importance of sufficient carbohydrate supply for sucker growth was underlined by the fact that root fragments, that were dead by the end of the experiment started out with lower initial reserves of NSC indicating that there might be a lower threshold of NSC root reserves (but above the complete exhaustion of root NSC reserves), below which aspen roots have difficulty to regenerate vegetatively. Regardless, these results highlight the importance of minimizing the handling and storage of roots to prevent excessive loss of NSC reserves prior to roll back. Therefore prolonged storage of roots during warm soil conditions should be avoided. Minimizing the loss of fine roots associated with fragments is likely not a priority in salvage as the benefit of not having to produce new adventitious roots on emerged suckers was countered by the reduction of suckers on fragments that had fine roots attached.

Acknowledgements

The authors would like to thank Eckehart Marenholtz, Stefan Stängle, Ryan Sherritt, and Jordana Fair for their field and laboratory assistance as well as Pak Chow for his help in the carbohydrate analysis. Further, we wish to acknowledge Cam Stevenson of the Crop Diversification Centre North. Funding was generously provided by the Natural Sciences and Engineering Research Council of Canada (NSERC) and the Environmental Reclamation Research Group (ERRG) of the Canadian Oil Sands Network for Research and Development (CONRAD), with sponsorship from Capital Power, Shell Canada, Suncor Energy Inc. and Syncrude Canada.

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Tables

Table 1

Impact of soil depth on suckering response and mortality after one growing season (mean ± SE; n = 10). Means sharing the same letter are not significantly different (α = 0.05; LSD means comparison test) across treatments.

Response variable	Soil depth (cm)		
	5	20	40
Total number of suckers per root fragment	1.2 ± 0.5 a	2.5 ± 0.8 a	1.3 ± 0.9 a
Number of emerged suckers per root fragment	0.6 ± 0.2 a	0.5 ± 0.1 a	0 b
Emerged sucker total length (below- + above-ground) (cm)	23.0 ± 1.7 b	39.8 ± 5.4 a	N/A
Emerged sucker height above-ground (cm)	18.0 ± 1.7 a	19.8 ± 5.4 a	N/A
Leaf dry mass per root fragment (g)	0.5 ± 0.2 a	0.8 ± 0.3 a	N/A
Emerged sucker dry mass per root fragment (g)	0.4 ± 0.1 a	0.8 ± 0.3 a	N/A
Number of new roots per emerged sucker	5.0 ± 3.3 a	1.2 ± 0.7 a	N/A
Length of new roots (cm)	58.7 ± 23.4 a	12.2 ± 3.2 b	N/A
Number of non-emerged suckers per root fragment	0.6 ± 0.2 b	2.0 ± 0.7 a	1.3 ± 0.9 ab
Non-emerged sucker length (cm)	2.2 ± 0.3 b	5.3 ± 0.6 a	8.5 ± 1.4 a
Non-emerged sucker dry mass per root fragment (g)	0.06 ± 0.03 b	0.4 ± 0.1 a	0.2 ± 0.1 a
Root fragment mortality (%)	80 ± 7.0 a	50 ± 7.0 b	80 ± 7.0 a

Table 2

Impact of root fragment diameter (class 1: 1-2 cm; class 2: 2.1-3 cm; class 3: 3.1-4 cm) on suckering response and mortality after one growing season (mean ± SE; n = 10). Means sharing the same letter are not significantly different (α = 0.05; LSD means comparison test) across treatments.

Response variable	**Root diameter class (cm)**		
	1 - 2	**2.1 - 3**	**3.1 - 4**
Total number of suckers per root fragment	0.9 ± 0.4 a	1.7 ± 0.6 a	2.4 ± 1.1 a
Number of emerged suckers per root fragment	0.3 ± 0.1 a	0.4 ± 0.2 a	0.3 ± 0.1 a
Emerged sucker total length (below- + above-ground) (cm)	24.2 ± 1.1 b	42.3 ± 3.4 a	26.9 ± 2.7 b
Emerged sucker height above-ground (cm)	9.1 ± 1.7 b	28.3 ± 2.9 a	12.5 ± 2.1 b
Leaf dry mass per root fragment (g)	0.2 ± 0.01 b	1.4 ± 0.2 a	0.3 ± 0.04 b
Emerged sucker dry mass per root fragment (g)	0.2 ± 0.1 b	0.7 ± 0.3 a	0.2 ± 0.1 b
Number of new roots per emerged sucker	0.3 ± 0.2 b	3.8 ± 2.5 a	2.1 ± 1.5 a
Length of new roots (cm)	4.7 ± 1.0 c	28.3 ± 9.1 b	147.1 ± 54.9 a
Number of non-emerged suckers per root fragment	0.6 ± 0.3 b	1.3 ± 0.4 ab	2.1 ± 1.0 a
Non-emerged sucker height (cm)	5.1 ± 0.9 a	4.4 ± 0.5 a	6.9 ± 1.4 a
Non-emerged sucker dry mass per root fragment (g)	0.1 ± 0.03 a	0.2 ± 0.07 a	0.3 ± 0.1 a
Root fragment mortality (%)	70 ± 9.0 a	70 ± 10.0 a	80 ± 7.0 a
Initial non-structural carbohydrate content per root fragment (g)	9.4 ± 0.7 c	16.1 ± 1.1 b	20.4 ± 1.3 a
Initial non-structural carbohydrate concentration per root fragment (%)	15 ± 0.8 a	12.9 ± 0.9 b	10.7 ± 0.4 c

Table 3

Role of fine roots in the suckering response and mortality of root fragments after one growing season (mean ±SE; n = 10). Means sharing the same letter are not significantly different (α = 0.05; LSD means comparison test) across treatments.

Response variables	**Treatment**	
	With fine roots	**No fine roots**
Total number of suckers per root fragment	0.6 ± 0.3 b	1.8 ± 0.6 a
Number of emerged suckers per root fragment	0.6 ± 0.3 a	0.9 ± 0.3 a
Emerged sucker total length (below- + above-ground) (cm)	41.1 ± 9.4 a	37.1 ± 5.7 a
Emerged sucker height above-ground (cm)	31.1 ± 9.4 a	27.1 ± 5.7 a
Leaf dry mass per root fragment (g)	2.0 ± 0.6 a	1.6 ± 0.4 a
Emerged sucker dry mass per root fragment (g)	4.2 ± 1.4 a	3.3 ± 0.8 a
Number of new roots per emerged sucker	1.5 ± 1.5 b	9.6 ± 4.2 a
Length of new roots (cm)	9.6 ± 5.3 b	108.3 ± 44.8 a
Number of non-emerged suckers per root fragment	0 b	0.9 ± 0.5 a
Non-emerged sucker height (cm)	N/A	3.6 ± 0.5 a
Non-emerged sucker dry mass per root fragment (g)	N/A	0.6 ± 0.02 a
Root fragment mortality (%)	70 ± 15.0 a	40 ± 16.0 a

Figures

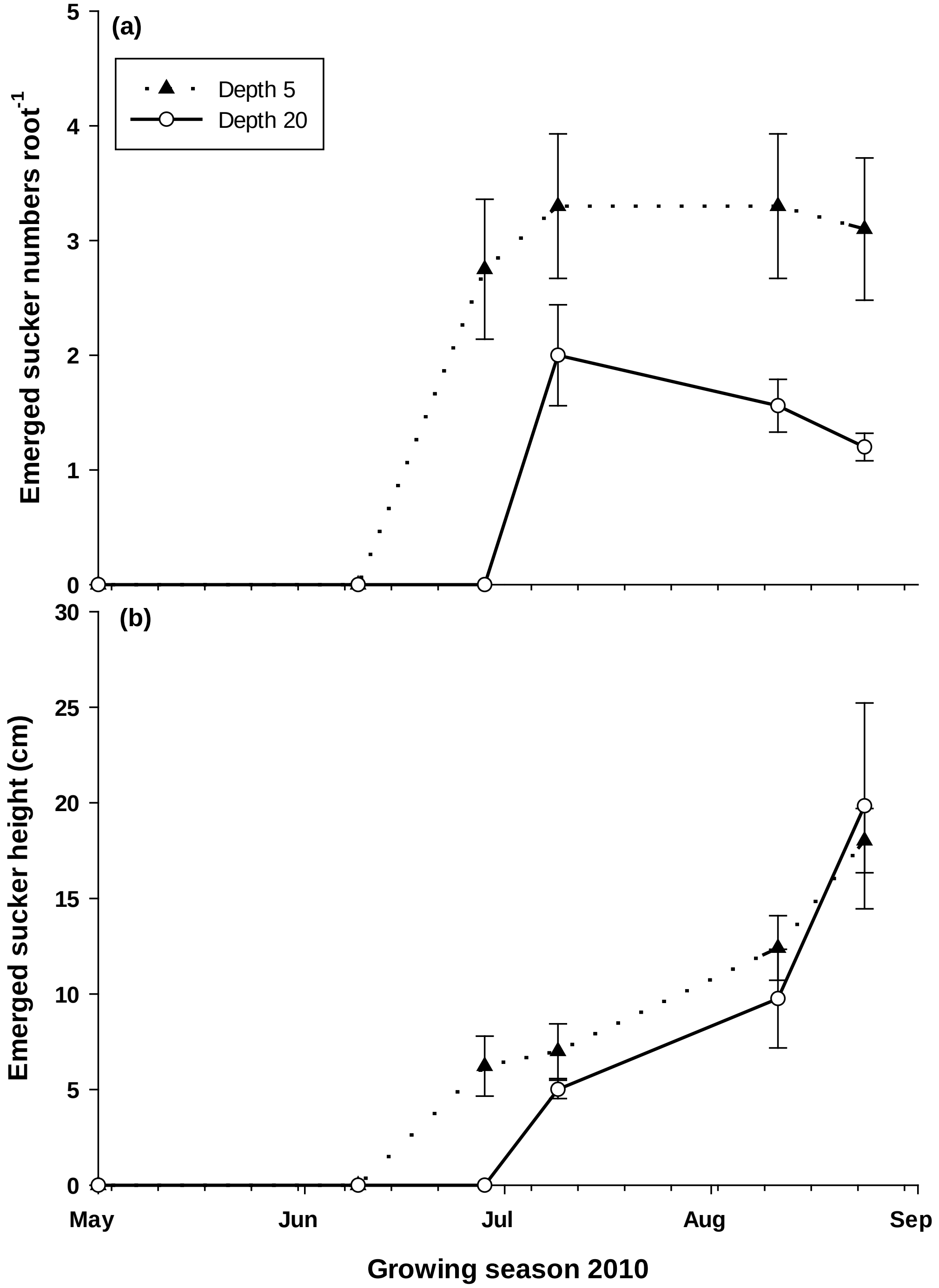

Fig. 1. Emerged sucker numbers (a) and height of suckers above the soil surface (b) sprouting from root fragments buried at 5 and 20 cm, over the 2010 growing season. Error bars indicate one standard error of the mean (n = 10).

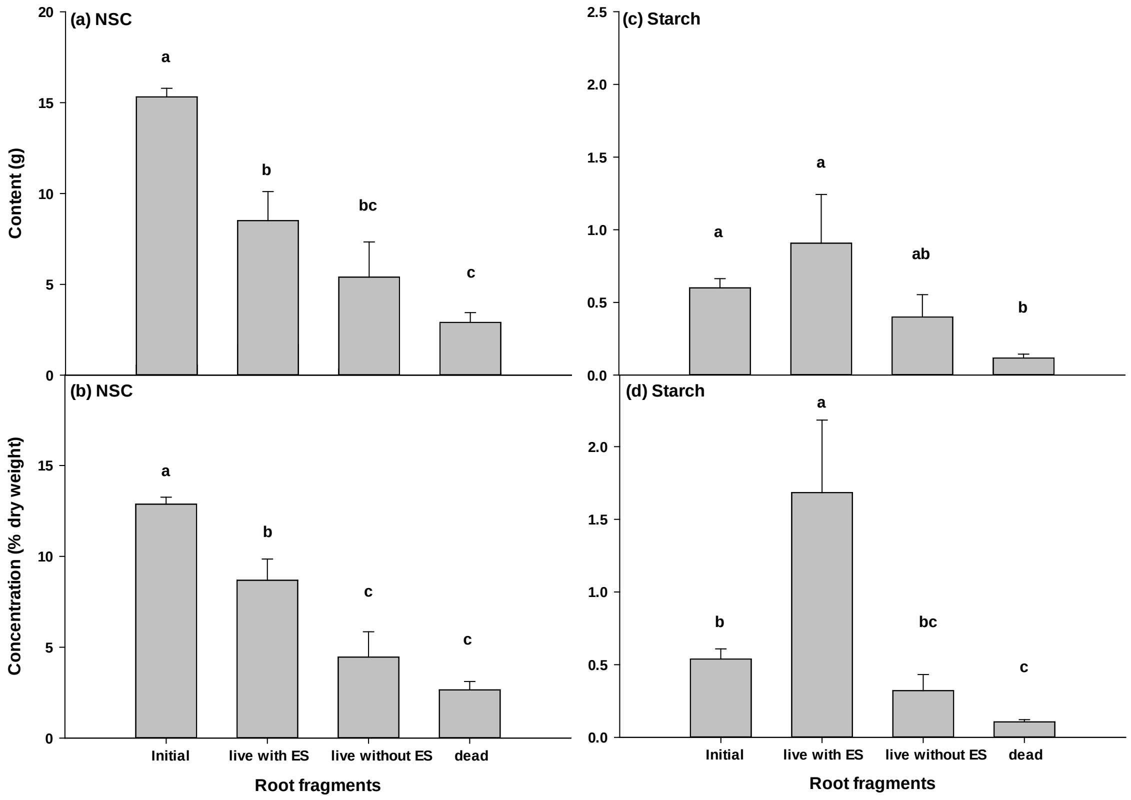

Fig. 2. NSC content (a) and concentration (b) and starch content (c) and concentration (d) of root fragments prior to burial (initial) and at the end of the first growing season (root fragments that produced emerged suckers (live with ES), live root fragments that did not produce emerged suckers (live without ES), and dead root fragments without suckers (dead) (n = 10). Data set for initial measurements includes all root fragments used in the diameter and depth study and fine root study. Data set of post experiment measurements only includes root fragments from both experiments buried at a depth ≤ 20 cm. Error bars indicate one standard error of the mean.

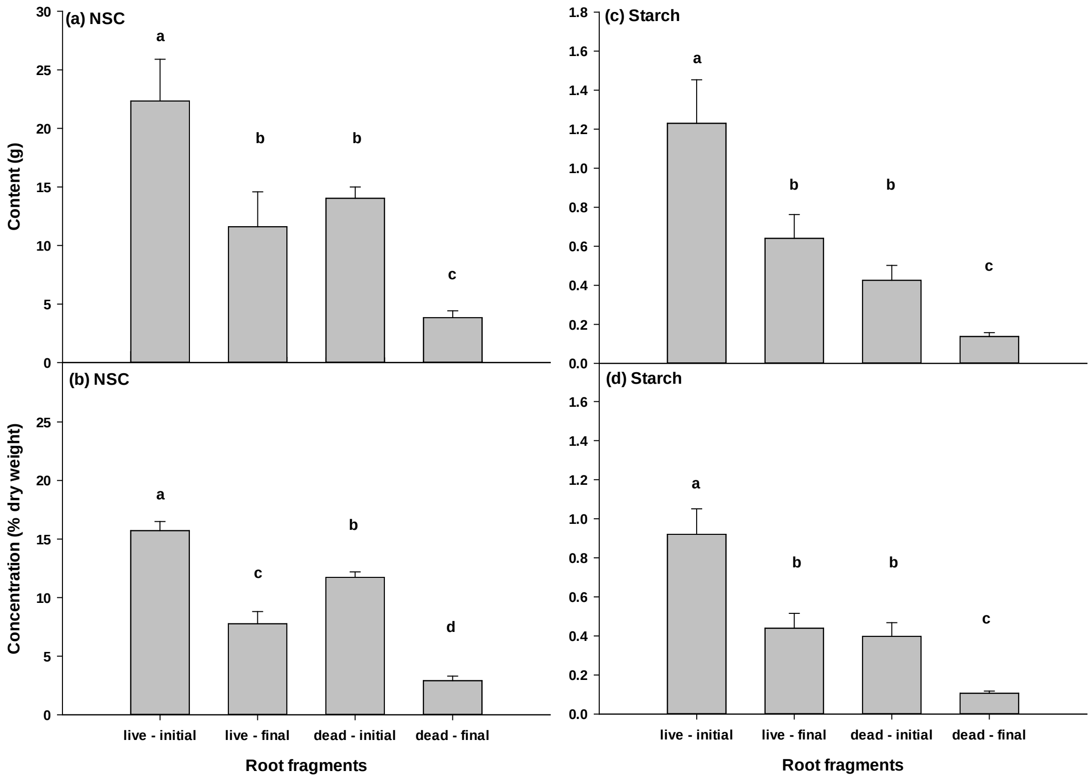

Fig. 3. NSC content (a) and concentration (b) and starch content (c) and concentration (d) of alive root fragments at the beginning of the experiment (live-initial) and at the end of the experiment (live-final) and of dead root fragments at the beginning (dead-initial) and at the end of the experiment (dead-final) (n = 10). Initial measurements were taken in late April and final measurements in late August. Data set for initial measurements includes all root fragments of the diameter and depth study and fine root study, regardless of emerged, non-emerged or no suckers. Data set of post experiment measurements only includes root fragments from both experiments buried at a depth ≤ 20 cm. Error bars indicate one standard error of the mean.

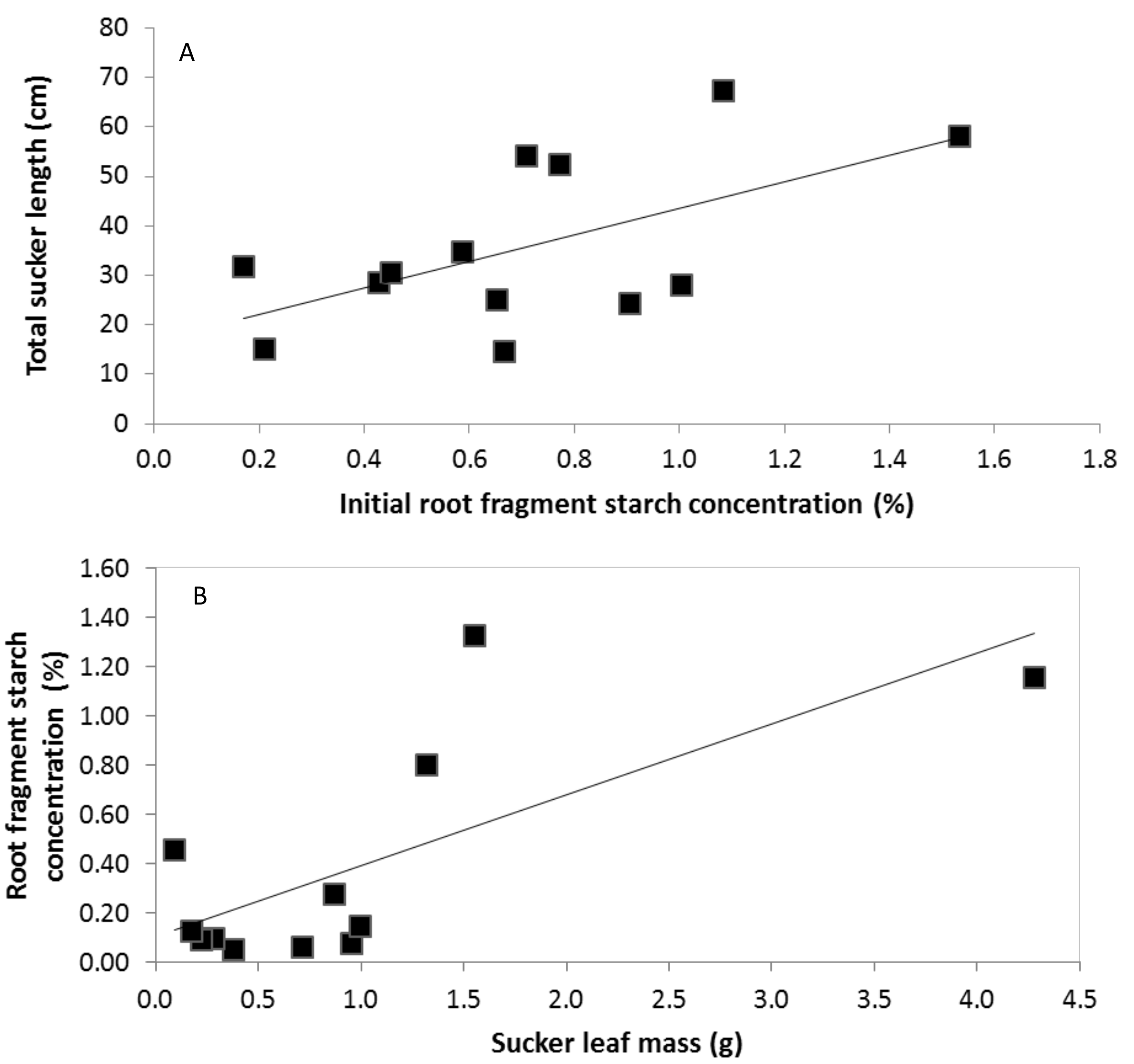

Fig. 4. Relationship between total sucker length and initial starch concentration of root fragments (A) and between sucker leaf dry mass and root fragment starch concentration at the end of the experiment (B) in aspen root fragments that had produced emerged suckers and were buried at 5 and 20 cm depth (n=12).