



## Research paper

# Root carbon reserve dynamics in aspen seedlings: does simulated drought induce reserve limitation?

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In a greenhouse study we quantified the gradual change of gas exchange, water relations and root reserves of aspen (*Populus tremuloides* Michx.) seedlings growing over a 3-month period of severe water stress. The aim of the study was to quantify the complex interrelationship between growth, water and gas exchange, and root carbon (C) dynamics. Various growth, gas exchange and water relations variables in combination with root reserves were measured periodically on seedlings that had been exposed to a continuous drought treatment over a 12-week period and compared with well-watered seedlings. Although gas exchange and water relations parameters significantly decreased over the drought period in aspen seedlings, root reserves did not mirror this trend. During the course of the experiment roots of aspen seedlings growing under severe water stress showed a two orders of magnitude increase in sugar and starch content, and roots of these seedlings contained more starch relative to sugar than those in non-droughted seedlings. Drought resulted in a switch from growth to root reserves storage which indicates a close interrelationship between growth and physiological variables and the accumulation of root carbohydrate reserves. Although a severe 3-month drought period created physiological symptoms of C limitation, there was no indication of a depletion of root C reserve in aspen seedlings.

**Keywords:** carbon limitation, native percentage loss of conductivity, non-structural carbohydrates, *Populus tremuloides*, starch, xylem cavitation.

## Introduction

Changes in the intensity, length and frequency of drought events have recently been associated with forest and tree mortality at the global level (Allen et al. 2010). In boreal forests, these changes have been characterized as rapid non-linear events occurring at a faster rate than previously predicted (Soja et al. 2007) and have been associated with drought-induced mortality of trembling aspen (*Populus tremuloides* Michx.) observed across a million hectares in Saskatchewan and Alberta (Hogg et al. 2008). Currently the processes leading to this mortality are widely discussed and are thought to be related to mechanisms of water transport and/or carbon (C) limitation (McDowell et al. 2008).

The dynamics of stem water potential, stomatal conductance and photosynthesis are dramatically affected during drought

and are proposed to lead to C depletion and C limitation in plants (McDowell et al. 2008, Sala et al. 2010). This becomes especially important for isohydric species which close their stomata early to maintain leaf evapotranspiration ( $E$ ) below a critical value (Tardieu and Simonneau 1998) to avoid catastrophic xylem failure (Tyree and Zimmermann 2002). This physiological response should be closely linked to C reserves (McDowell et al. 2008), particularly in the root system, which is a large sink for non-structural carbohydrates (NSC), as it is entirely dependent on the autotrophic parts of the plant and might require up to 50% of the produced photosynthates (Lambers et al. 2008).

Drought modulates C dynamics through a complex cascade of events: (i) as soil dries and stomata close, photosynthesis is reduced; (ii) if a plant's C demand is larger than the C supply

(via photosynthesis), overall C balance becomes negative, (iii) leading to a consumption of stored C reserves (C depletion) in order to maintain metabolic processes, some of which are critical under water stress (e.g., osmotic regulation); (iv) if drought conditions continue, C reserves become limiting to physiological processes (C limitation) (e.g., Sala et al. 2010).

Simultaneous changes in multiple physiological domains (i.e., water relations, gas exchange and root carbohydrate reserves) in response to drought are scarcely documented in the literature, normally including measurements of only one or two of these domains (e.g., Guehl et al. 1993, Carpenter et al. 2008), and are mostly presented comparing initial and final values (e.g., Runion et al. 1999), missing the dynamic and interdependent changes of these proxies over time. As a result, specific information on how drought events affect the dynamics of carbohydrate reserve accumulation and consumption in time and linking them to other physiological variables in tree species is limited (see McDowell and Sevanto 2010, Sala et al. 2010 and references therein).

Aspen and other members of the genus *Populus* have become key model tree species that are comprehensively used for research in plant molecular biology, ecology and physiology as they are economically important, easy to propagate from seeds or cuttings, and grow quickly. Specific mechanisms for drought tolerance have also been studied in *Populus* species; however, mainly focusing on gas exchange and water relations (Bassman and Zwier 1991, Silim et al. 2009), genetics (Street et al. 2006, Bonhomme et al. 2009) and growth (Strong and Hansen 1991, Huang et al. 2008) and not on the interaction between photosynthesis and water relations and C accumulation under drought conditions.

Here we describe the short-term dynamics and the interrelationships among physiological variables and root carbohydrate reserves in aspen seedlings, an isohydric species, in response to a severe 3-month drought period. We aim to answer the following two questions: (i) how are water relations, gas exchange and root carbohydrate reserves influenced by a seasonal drought event in aspen seedlings? (ii) is a 3-month drought, the average length of a natural drought in the prairie area of Western Canada, long enough to significantly reduce root C reserves of an aspen seedling?

## Materials and methods

### Plant material

Ninety *P. tremuloides* seedlings were established from seed under well-watered conditions in a greenhouse at the University of Alberta, Canada in April of 2009. After 4 weeks, seedlings were transplanted into individual 4-l plastic pots, one seedling per pot, filled with Metromix media (Metro Mix 290, Terra Lite 2000; W. R. Grace of Canada, Ajax, ON, Canada). Pots had four equidistant perforations at the base to allow excess water to

drain. After growing for 10 weeks under an 18-h photoperiod at 21 °C and watered daily to field capacity, 72 seedlings were randomly selected and assigned to two groups of 36 plants each. A one-way analysis of variance (ANOVA) was performed to check whether there were significant differences in initial plant height or stem basal diameter between the groups. Plants were randomly reassigned in each group until no significant differences were detected ( $P=0.203$ ). These groups were then designated as Drought and Control groups.

### Application of the drought treatment

At the beginning of the experiment (referred hereafter as Week 0), pots assigned to the Drought treatment were weighed daily using an Adam Equipment digital balance model PGW 4502e (Danbury, CT, USA). After each pot weight was recorded, plants were re-watered by adding the equivalent of half the weight lost from the day before. This water regime was maintained for 10 days until the midday stem water potential ( $\Psi_{md}$ ) was approximately  $-2.25$  MPa (for details, see below). This desiccation protocol was implemented to simulate a gradual soil drying process, more similar to a natural drought event. Midday stem water potential was determined daily in 10 randomly selected plants from each treatment (data not shown). Measurements were performed on one leaf per plant. Leaves were kept inside an aluminum foil envelope for 2 h prior to the measurement to allow leaf and stem water potentials to equalize so that leaf water potential can be used as a proxy for stem water potential. Measurements were performed using a Compact Water Status Console (i.e., a portable Scholander-type pressure chamber) model 3115P40G4 (Soilmoisture Equipment Corp., Goleta, CA, USA) and repeated until midday stem water potential ( $\Psi_{md}$ ) in the Drought group was  $-2.15 \pm 0.0428$  (MPa; mean  $\pm$  SE,  $n = 10$ ). This  $\Psi_{md}$  value was chosen as a target for being slightly less negative than  $\Psi_{md}$  associated with 50% loss of conductivity in aspen seedlings growing under similar environmental conditions ( $P_{50} = -2.25$  MPa; Cai and Tyree 2010). After the  $\Psi_{md}$  target value in Drought plants was reached, Drought pots were watered daily by adding the full amount of weight lost from the day before for the rest of the experiment. Plants in the Control group were weighed daily for the first 10 days and re-watered daily to field capacity for 12 weeks.

### Gas exchange and percentage loss of conductivity measurements

At Week 0,  $CO_2$  assimilation rate ( $A$ ) and leaf stomatal conductance ( $g_s$ ) were measured in 10 randomly selected plants of each group using a LI-6400 portable photosynthesis system (Li-Cor, Lincoln, NE, USA). Measurements were performed between 09:00 and 11:00 h on the youngest fully expanded leaf. The chamber's reference  $CO_2$  concentration was set to 385 p.p.m. using a 12-g Li-Cor  $CO_2$  cartridge as  $CO_2$  source. The light

environment in the chamber was set to 2000  $\mu\text{mol m}^{-2} \text{s}^{-1}$  after a 10-min induction period at 500  $\mu\text{mol m}^{-2} \text{s}^{-1}$  using the 6400-2B red/blue LED light source of the LI-6400's chamber. The induction period was implemented to stabilize air humidity, flow and temperature prior to exposing the measured leaf to the light-saturating photon flux density level. Similar changes in photon flux density levels are common in natural environments where *P. tremuloides*, a fast-growing pioneer trees species, germinates and grows. Measurements were logged after 3 min at 2000  $\mu\text{mol m}^{-2} \text{s}^{-1}$  when  $A$  and  $g_s$  values were stable. The cuvette conditions were based on three light response curves that were determined on individual plants in which the optimum induction time and the photon flux density to archive maximum  $A$  were determined. Measurements were taken on six randomly selected plants in each treatment after 4, 6, 8, 10 and 12 weeks (no repeated measures). All measurements in the rest of this section were performed using the same number of samples and time schedule described above.

Percentage loss of hydraulic conductivity (PLC) was measured using a conductivity apparatus (Sperry et al. 1988) following a standardized protocol. Seedlings were cut at the stem base in the greenhouse and transported to the lab (~200 m) inside black plastic bags to minimize stem dehydration. Stems cut from the pot were re-cut under water, discarding the 15-cm stem section proximal to the original cutting site in order to remove embolisms induced by cutting in air. Keeping the re-cut stem under water, five consecutive 2-cm stem segments from each stem were cut using a razor blade. Segments from each stem were mounted and measured at the same time in the conductivity apparatus. The apparatus's reservoir tank was filled with filtered (0.2  $\mu\text{m}$ ) 100 mM KCl solution prepared in deionized water. After the initial hydraulic conductivity (i.e., the initial value of  $k_i$ , expressed as  $k_i$  in Eq. (1)) of each stem segment had been measured, the native embolism was displaced by flushing KCl solution from the reservoir under constant pressure (120 kPa) for 2 min. After being flushed, the segment's measured final hydraulic conductivity was taken as  $k_{\text{max}}$ . Preliminary tests were performed to ensure that  $k_{\text{max}}$  values did not change after repeated flushing. Percentage loss of hydraulic conductivity was calculated from Eq. (1):

$$\text{PLC} = \left[ (k_{\text{max}} - k_i) / k_{\text{max}} \right] \times 100 \quad (1)$$

$$\text{PLC} = \frac{k_{\text{max}} - k_i}{k_{\text{max}}} \times 100$$

$k_s$  was calculated from  $k_i/A_w$ , where  $A_w$  is stem cross-sectional area.

### Seedling and NSC measurements

At each collection, the seedling heights were recorded and plants were individually bagged in paper bags, separating leaf and washed root material (stems were used to perform the PLC

measurements described above). Total leaf area per plant was measured the same day using a LI-3000 leaf area meter (Li-Cor, Lincoln, NE, USA). Leaf and root material was oven dried at 70 °C for 72 h and weighed. All dried root samples were ground in a Wiley Mill to pass a 40 mesh screen and water-soluble sugar and starch concentrations were determined for the root tissues. Soluble sugars were extracted three times with hot 80% ethanol, followed by a reaction between the extract and phenol–sulfuric acid which allowed sugars to be measured colorimetrically (Chow and Landhäusser 2004). To measure starch concentrations, the tissue remaining after the ethanol extraction was digested with the enzymes  $\alpha$ -amylase and amyloglucosidase followed by a colorimetrically measurable reaction with peroxidase–glucose oxidase-*o*-dianisidine (Chow and Landhäusser 2004). Since the leaf area of seedlings changed over the 12-week experimental period, soluble sugar and starch root content was scaled by leaf area to account for these changes in leaf area. Therefore, we present root sugar and starch as content per seedling leaf area ( $\text{mg cm}^{-2}$ ), referred to hereafter as specific sugar (SSUC) and specific starch content (SSTC), respectively. To illustrate the conversion dynamics between water-soluble sugars and starch during the experimental period, the ratio of sugar to starch concentrations (SSTR) was calculated.

The experimental design was analyzed as a  $2 \times 6$  factorial design with two drought treatments (Drought and Control) and five collection times (4, 6, 7, 10 and 12 weeks). All response variables of seedlings growing in Drought and Control treatments were contrasted at Week 0 (starting point) using a *t*-test to identify potential differences between them before the drought treatment was applied. *t*-tests were performed with statistical software package SigmaStat 4 (Systat Software Inc., Chicago, IL, USA). Values of all response variables at Weeks 4, 6, 8, 10 and 12 were fitted using a linear mixed-effects model using the functions *lme* and *varIdent* from the *nlme* R package (Pinheiro et al. 2010). The *lme* function allowed the use of time as random variable while *varIdent* allowed for a different variance structure for each level of the random variable (time). Once the data were fitted, they were analyzed using ANOVA procedures with statistical package R (R-CRAN) (R Development Core Team 2008). Differences between means were considered significant at  $\alpha = 0.05$ .

## Results

### Effects of drought on seedling growth

At the beginning of the experiment (Week 0), the height and total leaf area of seedlings in the Drought and Control groups were not significantly different (Figure 1a and b;  $P > 0.05$ ). Height in Control seedlings gradually increased over the entire duration of the experiment from  $43.1 \pm 2.0$  cm at Week 0 to  $98.3 \pm 1.9$  cm at Week 12 (mean  $\pm$  SE;  $n = 6$ ), while height in Drought seedlings increased by 14% over the first 4 weeks

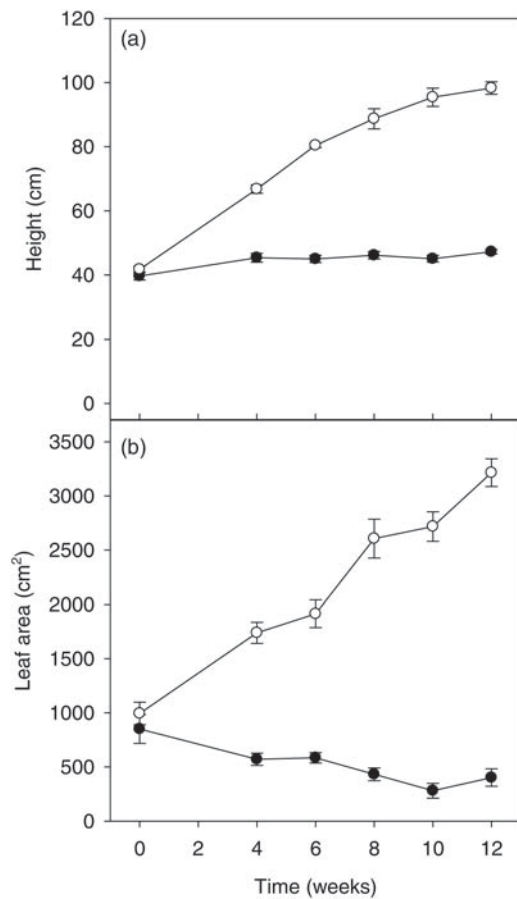


Figure 1. Plant height (a) and total leaf area (b) of aspen (*P. tremuloides*) seedlings growing under drought (closed circles) and well-irrigated (open circles) conditions for 12 weeks. Data are means  $\pm$  SE;  $n = 6$ .

but then remained the same until the end of the experiment (Figure 1a;  $P > 0.05$ ).

Total leaf area in Control seedlings increased threefold during the length of the experiment from  $994 \pm 103$  cm<sup>2</sup> at Week 0 to  $3215 \pm 128$  cm<sup>2</sup> at Week 12 (mean  $\pm$  SE;  $n = 6$ ) (Figure 1b). In contrast, total leaf area in Drought seedlings decreased over time. At Week 4, average leaf area was 32.8% less than at Week 0. During Week 2, all Drought seedlings had shed approximately the bottom third of their leaves, and they gradually shed more leaves during the rest of the experiment. Total leaf area in Drought seedlings decreased by 52.7% over the whole experiment, from  $850 \pm 132$  cm<sup>2</sup> at Week 0 to  $403 \pm 80$  cm<sup>2</sup> at Week 12 (mean  $\pm$  SE;  $n = 6$ ) (Figure 1b).

#### Gas exchange and water relation response to drought

Leaf stomatal conductance ( $g_s$ ) of Drought seedlings decreased 86.6% during the first 6 weeks from  $0.15 \pm 0.008$  to  $0.02 \pm 0.001$  mol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup> (mean  $\pm$  SE;  $n = 6$ ) remaining without significant change during the rest of the experiment (Figure 2a;  $P > 0.05$   $n = 6$ ). The relatively abrupt stomatal closure in Drought seedlings during the first weeks of the

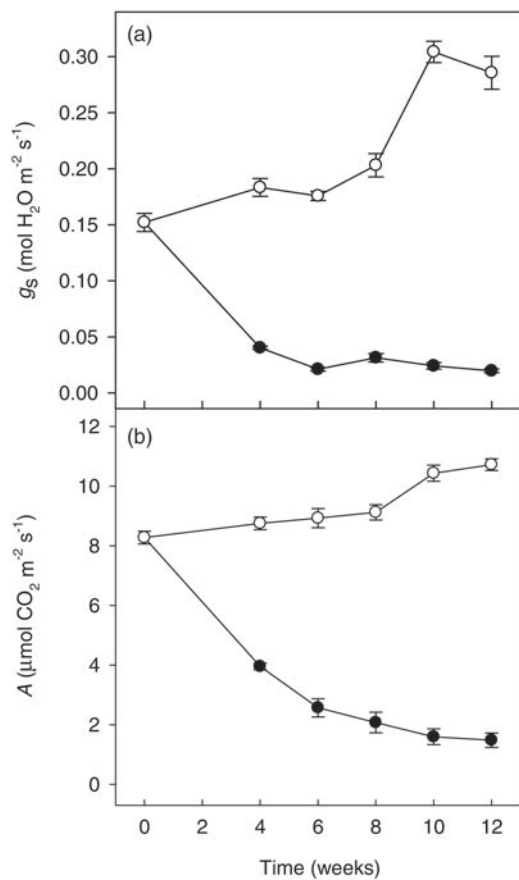


Figure 2. Leaf stomatal conductance (a) and assimilation rate (b) of aspen seedlings growing under drought (closed circles) and well-irrigated (open circles) conditions for 12 weeks. Data are means  $\pm$  SE;  $n = 6$ .

experiment concatenated with a more gradual reduction in CO<sub>2</sub> assimilation rate ( $A$ ), which did not plateau until Week 12, showing an 82% reduction by the end of the experiment (Figure 2b).  $A$  and  $g_s$  values in Control seedlings increased by 29.7 and 90.1%, respectively, during the course of the experiment, relative to values recorded at Week 0 (Figure 2a and b).

Values of midday stem water potential ( $\Psi_{md}$ ), a good integrator of soil and plant water stress, were not significantly different between Control and Drought seedlings at Week 0 ( $P > 0.05$   $n = 6$ ). Seedlings under the Drought treatment showed a significant and abrupt reduction in  $\Psi_{md}$  closely following the reduction in leaf stomatal conductance reported above, from  $-0.54 \pm 0.05$  MPa in Week 0 to  $-2.15 \pm 0.04$  MPa by Week 4 followed by a more gradual reduction to  $-2.43 \pm 0.06$  MPa (mean  $\pm$  SE;  $n = 6$ ) at the end of the experiment. After 4 weeks, aspen seedlings in the Drought treated lost 72.4% of stem conductivity. This value of PLC remained relatively constant during the rest of the experiment (Figure 3b). There were no significant changes in stem water potential and PLC values in Control seedlings over the whole experiment (Figure 3a and b;  $P > 0.05$   $n = 6$ ).



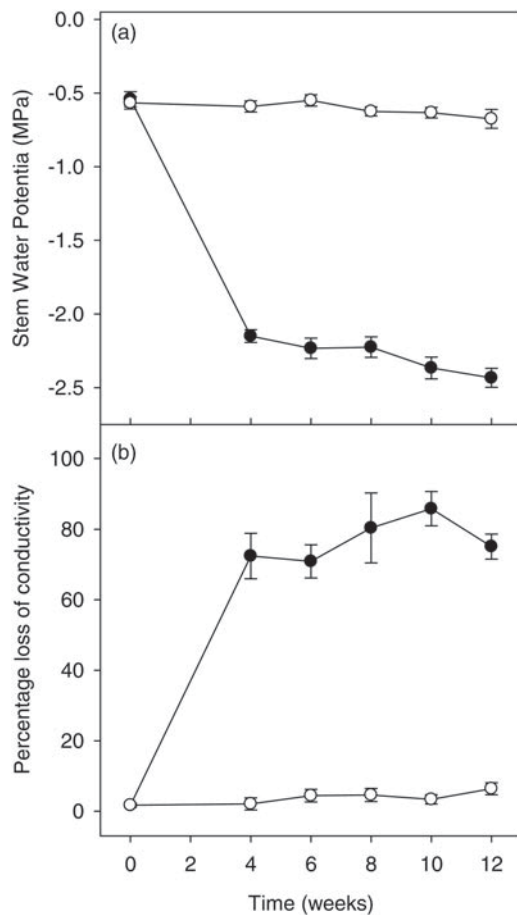


Figure 3. Stem water potential (a) and PLC (b) in aspen seedlings growing under drought (closed circles) and well-irrigated (open circles) conditions for 12 weeks. Data are means  $\pm$  SE;  $n = 6$ .

### Concentration and content of soluble sugars and starch in roots

At Week 0 soluble sugar and starch concentrations of Control and Drought seedlings were not different ( $P > 0.05$ ), with soluble sugar concentrations being much higher than starch concentrations. During the experimental period, sugar and starch concentrations in Control seedlings increased from 3.17 and 0.29% at Week 0 to 5.57 and 9.64%, respectively, at Week 12. In the roots of Control seedlings, sugar concentration only increased between Weeks 4 and 6 and remained relatively constant until Week 12 (Figure 4a). In contrast, starch concentrations increased slowly between Weeks 4 and 8 and more rapidly between Weeks 10 and 12 (Figure 4b). In Drought seedlings, root sugar concentration increased from 4.13% at Week 0 to a maximum of 10.2% at Week 6 followed by a reduction at Week 8 with a slight recovery at Week 12 (Figure 4a). Starch concentration increased rapidly from 0.14% at Week 0 to 17.7% at Week 6 and then appeared to fluctuate somewhat between Weeks 8 and 12 (Figure 4b).

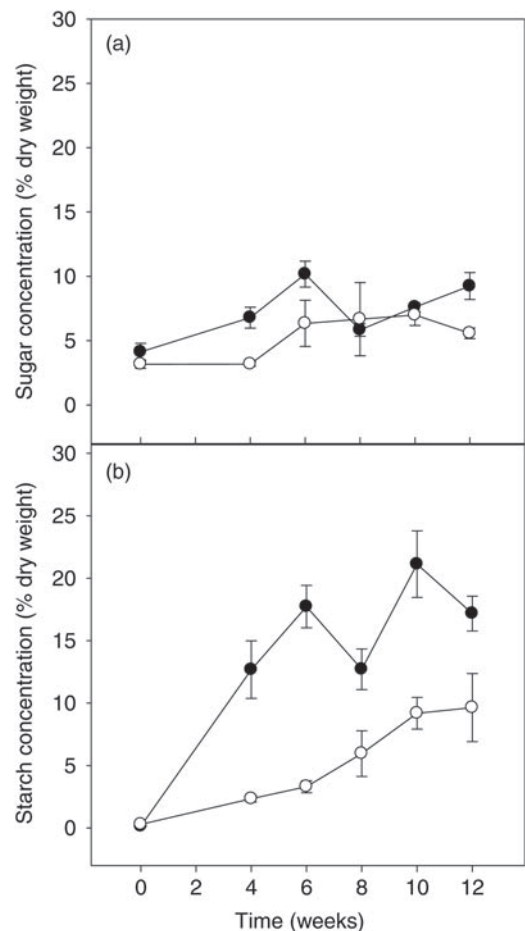


Figure 4. Sugar (a) and starch (b) concentration (% dry weight) in roots of aspen seedlings growing under drought (closed circles) and well-irrigated (open circles) conditions for 12 weeks. Data are means  $\pm$  SE;  $n = 6$ .

Overall, total non-structural carbohydrate concentrations (sum of soluble sugars and starch) in root tissues was 73.7% higher in Drought than in Control seedlings.

At Week 0, neither SSUC nor SSTC in roots of aspen seedlings was significantly different between Control and Drought seedlings (Figure 5a and b). Specific sugar and starch content of Control seedlings increased only slightly from 0.004 to 0.099  $\text{mg cm}^{-2}$  and from 0.037 to 0.061  $\text{mg cm}^{-2}$ , respectively (Figure 5a and b), whereas SSUC and SSTC of Drought seedlings increased by two orders of magnitude from 0.005 to 0.815  $\text{mg cm}^{-2}$  and from 0.061 to 0.416  $\text{mg cm}^{-2}$ , respectively (Figure 5a and b).

At Week 0, SSTR in roots of aspen seedlings was not significantly different between the Control and Drought treatments (Figure 5c) and in both treatments SSTR had declined steeply by Week 4. After Week 4, SSTR remained relatively constant for the rest of the experimental period but SSTR in Control seedlings was on average 2.7 times higher in Drought seedlings.

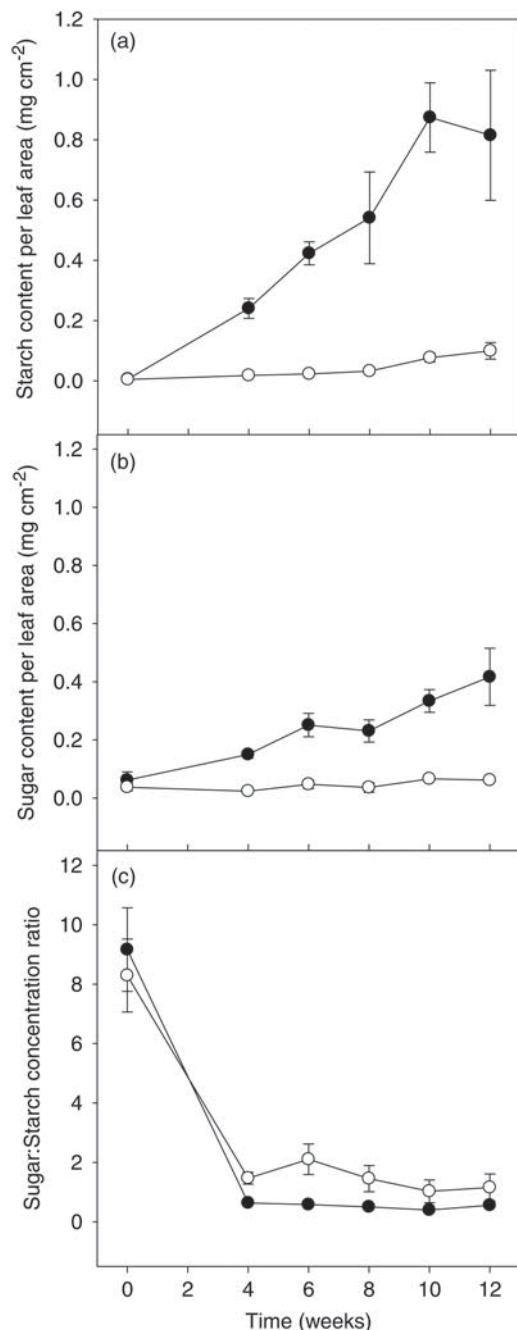


Figure 5. Starch (a) and sugar (b) content per unit leaf area and SSTR (c) in roots of aspen seedlings growing under drought (closed circles) and well-irrigated (open circles) conditions for 12 weeks. Data are means  $\pm$  SE;  $n = 6$ .

## Discussion

Contrary to what might be expected from an isohydric species, reduced C assimilation as a result of severe drought stress did not result in a significant reduction in root C reserves in aspen seedlings. The results from our experiment quantified the complex interrelationship between water relations, gas exchange and root C dynamics, showing that roots of aspen seedlings growing under severe water stress increased soluble sugar

and starch reserves in the root system. This suggests a different use of the limited photoassimilates produced under drought conditions, which were likely used for additional height growth and leaf area in the non-stressed seedlings. Drought seedlings had proportionally more C stored in the form of starch than Control seedlings. Accumulation of starch during the initial stages of the drought period can be seen as a critical process because once a certain threshold of water deficit is reached, starch can be utilized to maintain a necessary concentration of soluble sugars needed for osmoregulation and osmoprotection (Chaves 1991). The higher SSTR in Drought seedlings could indicate more efficient C production but could also suggest a prioritization of starch accumulation for osmoregulatory processes over growth under drought conditions, as has been suggested for other plants (Chaves et al. 2003).

Overall, this prioritization scheme may also constitute a strategy for aspen to prioritize root system survival, which can actively resprout (sucker) from its root system after a disturbance or stress such as fire, defoliation or drought has killed the aboveground portions (Bailey and Whitham 2002, Wan et al. 2006, Worrall et al. 2010). This idea concurs with a hypothesis presented by Cowan and Farquhar (1977) and Raven (2002) proposing that the optimization of C uptake versus water loss plays an important role in plant evolution. Additional support for evolutionary pressure favoring C optimization versus water loss can be found in the strong relationship between maximum stomatal conductance and leaf nitrogen concentration, and hence photosynthetic capacity, as reported by Schulze et al. (1994) over a range of vegetation types. The increase in sugar and starch reserves that we observed in the roots of Drought seedlings indicates that a >50% loss of hydraulic conductance in the xylem caused by water stress was not sufficient to limit C translocation, although reduced conductance has been reported as a possible mechanism regulating reserves and root mortality (Sung and Krieg 1979, Marshall 1986).

The duration of our stress period (3 months) is also ecologically significant for boreal forests because it spans the normal frost-free growth period in Alberta. Our results showed that even under this severe water stress (i.e., significantly reduced leaf stomatal conductance and CO<sub>2</sub> assimilation, stem water potential near the species' specific  $P_{50}$  value and terminated growth) reserves in the roots of young aspen seedlings increased over the 3-month drought period.

This study highlights some of the intricacies of C dynamics in plants under stress: while some symptoms of C limitation in sink tissues were observed in Drought seedlings (e.g., terminated height growth and reduction in leaf area, and stomatal conductance), there was no indication of C depletion in the root system. The increase in soluble sugar concentrations in Drought seedlings may also suggest the potential onset of osmotic adjustment, a well-documented response to drought in *Populus* species (Gebre et al. 1998, Tschaplinski et al. 1998)

in order to maintain higher (less negative) values of leaf water potential. However, we caution against overextending the significance of our results based on the first-year growth of seedlings. Both adult trees and seedlings facing several consecutive seasons of drought are likely to behave very differently. We recognize that there may be additional ecological and environmental factors modulating the conditions that potentially lead to C limitation and C depletion.

Aspen seedlings in the Drought treatment started to show indications of water stress 1 week after the drought treatment was initiated (e.g., reduction of leaf blade angle relative to seedling stem, reduction of leaf blade apparent turgor). Two weeks later all seedlings in this treatment shed approximately the basal third of their leaves. Shedding of basal leaves during periods of drought stress is a well-documented mechanism in poplars that simultaneously reduces transpiring surface area while making the limited water supply available to growing leaves and meristems in the seedling's distal section (Chen et al. 1997, Rood et al. 2000, Giovannelli et al. 2007). Leaf shedding may also play several additional roles besides reducing transpirational surface such as remobilization of nutrients during stress (Munné-Bosch and Alegre 2004), prevention of run-away embolisms by maintaining a favorable water balance at the whole-plant level and leaf temperature control (see Chaves et al. (2003) for a review on the subject). By Week 4, drought-stressed seedlings had terminated height growth (i.e., terminal bud set) and with that the growth of new leaf area, which is a well-documented response of aspen to drought (Hogg and Hurdle 1995). Reduced leaf area in combination with stomatal closure also triggers a series of physiological responses such as changes in ion uptake and transport, osmotic adjustment, nitrogen metabolism, starch reallocation from leaves (Iljin 1957, Hsiao 1973) and reduction of leaf water potential (Farquhar and Sharkey 1982). As leaf water potential decreases, stem water potential also decreases, reaching values near 50% loss of conductivity ( $P_{50}$ ). Traditionally, conductivity values near  $P_{50}$  have been utilized as a proxy for severe whole-plant stress (Hacke et al. 2006) because compromising hydraulic conductivity may limit C gain, growth and productivity (Tyree 2003). Drought seedlings reached an average PLC value of 80% (Figure 3b) after Week 8; however, this level of PLC did not decrease the reserves in roots and is unlikely to affect short-term survival of aspen seedlings. Tyree (unpublished) has shown that aspen seedlings of the same age could be droughted to  $\geq 90\%$  PLC and survive after re-watering; hence we tentatively reject the possible notion that hydraulic failure could confound our results.

Canadian boreal forests have suffered record periods of drought and corresponding tree die-offs during the past decade and this trend is expected to continue dramatically impacting forest composition in Western Canada (Hogg et al. 2008, Mbogga et al. 2009, Allen et al. 2010). More information is

needed about the dynamics of C accumulation and depletion before, during and after multiple annual cycles of drought and dormancy, especially in the case of aspen, a clonal species with remarkable capacity to regenerate via root sprouts. Also we need to gain a better understanding of the role of physiological (e.g., isohydric and anisohydric stomatal behavior), anatomical (e.g., pit membrane size and distribution) and environmental (e.g., length and intensity of seasonally repeated drought periods) factors modulating the overall impact of drought on C reserves.

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