Impact of warming and drought on carbon balance related to wood formation in black spruce

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Background and Aims Wood formation in trees represents a carbon sink that can be modified in the case of stress. The way carbon metabolism constrains growth during stress periods (high temperature and water deficit) is now under debate. In this study, the amounts of non-structural carbohydrates (NSCs) for xylogenesis in black spruce, Picea mariana, saplings were assessed under high temperature and drought in order to determine the role of sugar mobilization for osmotic purposes and its consequences for secondary growth.

Methods Four-year-old saplings of black spruce in a greenhouse were subjected to different thermal conditions with respect to the outside air temperature (T0) in 2010 (2°C warmer than T0 during the day or night) with a dry period of about 1 month in June of each year. Wood formation together with starch, NSCs and leaf parameters (water potential and photosynthesis) were monitored from May to September.

Key Results With the exception of raffinose, the amounts of soluble sugars were not modified in the cambium even if gas exchange and photosynthesis were greatly reduced during drought. Raffinose increased more than pinitol under a pre-dawn water potential of less than –1 Mpa, presumably because this compound is better suited than polyol for replacing water and capturing free radicals, and its degradation into simple sugar is easier. Warming decreased the starch storage in the xylem as well the available hexose pool in the cambium and the xylem, probably because of an increase in respiration.

Conclusions Radial stem growth was reduced during drought due to the mobilization of NSCs for osmotic purposes and due to the lack of cell turgor. Thus plant water status during wood formation can influence the NSCs available for growth in the cambium and xylem.

Key words: Cambium, black spruce, Picea mariana, drought, non-structural carbohydrate, soluble sugars, raffinose, starch, global warming, climate change, wood formation, xylogenesis.

INTRODUCTION

Climatic models predict increases in temperature in boreal forests of up to 3°C over the next 50 years, with the greatest increases occurring in winter and spring, at resumption of plant growth (Plummer et al., 2006). Changes in the precipitation regime are also predicted, with more extreme events, especially during winter (increase in precipitation) and summer (drought). However, temperatures are not expected to change linearly during the day: between 1950 and 1998, unlike the daily maximum, the daily minimum increased significantly, indicating that the nights were warmer (Bonsal et al., 2001). These modifications could affect gas exchanges (Way and Sage, 2008b) in the plant and consequently the production of photosynthates (i.e. soluble sugars) as well as degradation of starch which are necessary during the growth process.

Within the stem of conifers, the formation of wood represents a powerful carbon sink that is linked with the non-structural carbohydrate (NSC) in cambium and xylem (Deslauriers et al., 2009; Simard et al., 2013). As reviewed by Pantin et al. (2012), cell growth involves the movement of water and solute into the cell, generating sufficient turgor pressure for irreversible growth as well as an accumulation of biomass into new structures. Under drought, growth can be inhibited before photosynthesis, which can temporarily increase NSCs (McDowell, 2011; Muller et al., 2011) or not (Gruber et al., 2012; Duan et al., 2013). Thus, growth constraints during drought are related to turgor but unrelated to carbon availability (Woodruff and Meinzer, 2011).

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include solute accumulation, such as inorganic ions (K⁺, Cl⁻ and Na⁺), organic components (proline, serine, malate, etc.) and other soluble sugars (raffinose, sucrose and pinitol) that play an important role in active osmotic adjustments (Liu et al., 2008; Aranjuelo et al., 2011). Among the soluble sugars, the role of raffinose in plant cell protection as an osmoprotectant or antioxidant is very well known (Nishizawa-Yokoi et al., 2008; dos Santos et al., 2011). In many herbaceous (Ford, 1984; McManus et al., 2000; Streeter et al., 2001) and tree species (Ericsson, 1979; Streit et al., 2013), pinitol has been described as an important polyol, especially under stress conditions such as drought, salinity or low temperature (Orthen et al., 1994), acting as an osmolyte (Reddy et al., 2004). The variation of cyclitols, such as ϑ-pinitol, and soluble sugars has recently been assessed in conifers (Gruber et al., 2011; Simard et al., 2013; Streit et al., 2013) and related to secondary growth of the stem as well as cell protection. Consequently, the main challenge could be to understand how plant growth may be influenced by changes in concentration of each single sugar in response to drought and warming.

The aim of this study was to determine how an increase in temperature and drought may modify the amount of soluble sugars available for xylogenesis in the stem of black spruce (Picea mariana). We tested the hypothesis that during a period of water deficit, the pool of available sugars in cambium and xylem will be directed towards cell osmoregulation more than water deficit, the pool of available sugars in cambium and xylem will be directed towards cell osmoregulation more than cell protection. Consequently, the main challenge could be to understand how plant growth may be influenced by changes in concentration of each single sugar in response to drought and warming.

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MATERIALS AND METHODS

Study area and experimental design

The study took place in a greenhouse complex located at the University of Quebec in Chicoutimi (48°25′N, 71°04′W, 150 m a.s.l., QC, Canada). The mean temperatures in 2010 and 2011 were 5.2 and 2.2 °C, respectively. The higher mean in 2010 was caused by a particularly mild winter and spring with a higher mean January–May temperature of 0.2 °C compared with 4.5 °C in 2011. The average temperatures in summer 2010 and 2011 were 18.1 and 17.6 °C, respectively.

Two independent experiments were performed in a greenhouse divided into three independent sections and automatically controlled with misting and window-opening systems for cooling. Approximately 300 saplings of black spruce, Picea mariana, were installed in every section in both years. Plants were 4-year-old saplings transplanted in 4.5 L plastic pots with a peat moss, perlite and vermiculite mix, and left in an open field during the entire previous growing season and winter. In April of each year, the saplings were taken inside the greenhouse for the experiment and fertilized with 1 g L⁻¹ of NPK (20:20:20) fertilizer dissolved in 500 mL of water. Only the vigorous trees were selected for the experiment, while the others were used in the buffer zone at the borders. On average, the saplings were 48.9 ± 4.7 cm in height, with a diameter of 8.0 ± 2.0 mm at the collar. Each sapling was equipped with drip trickles to perform the irrigation. Different irrigation and temperature regimes were applied in each section. The control (named T0) corresponded to outside temperature, while the other two sections were subjected to specific thermal regimes. In 2010, T2 and T5 experienced a temperature 2 °C higher than T0, respectively (Balducci et al., 2013). In 2011, day-time temperature (TD) and night-time temperature (TN) were 6°C warmer than T0 during the day (TD, from 0700 to 1859 h) or during the night (TN, from 1900 to 0659 h), respectively (Fig. 1). For irrigation, the soil water content was maintained at >80 % of field capacity in the control, while the other saplings were submitted to a water deficit from about mid-May to mid-June in 2010 and from June to the beginning of July in 2011, when cambium is vigorously differentiating (Rossi et al., 2006b). The water deficit period corresponded to DOY (day of the year) 142–173 in 2010 and DOY 158–182 in 2011. At the end of the water deficit period, the soil water content of non-irrigated saplings was <10% while irrigated saplings had a soil water content varying between 40 and 50%.

Xylem growth

Each week from May to September, stem discs were collected 2 cm above the root collar from 36 randomly selected saplings (6 saplings × 3 thermal conditions × 2 water regimes (Balducci et al., 2013)). The samples were dehydrated with successive immersions in ethanol and p-limonene, embedded in paraffin and transverse sections of 8–10 μm thickness were cut with a rotary microtome (Rossi et al., 2006a). The sections were stained with cresyl violet acetate (0.16 % in water) and examined within 10–25 min with visible and polarized light at magnifications of ×400–500 to distinguish the developing xylem cells. For each section, the total radial number of cells including (1) cambial, (2) enlarging, (3) cell wall thickening and (4) mature cells were counted along three radial files and averaged according to Rossi et al. (2006a).

Water relations, gas exchange and CO₂ assimilation

Plant water status was followed by measuring pre-dawn leaf water potential (Ψpd) from May to August on branches of the first whorl of three saplings per treatment (3 thermal conditions × 2 irrigation regimes) with a pressure chamber (PMS Instruments, Corvalis, OR, USA). Stomatal conductance (gs, mol m⁻² s⁻¹) and maximum photosynthesis rate (Amax, μmol m⁻² s⁻¹) were measured from 1000 to 1300 h under saturating irradiance conditions [1000 μmol m⁻² s⁻¹ (Bigras and Bertrand, 2006)] using a portable photosynthesis system (Fig. 1) [Li-6400; LI-COR Inc., Lincoln, NE, USA] and processed according to Balducci et al. (2013). In the greenhouse, the saplings were grown at 400 μmol m⁻² s⁻¹. In order to
avoid light stress, the saplings were acclimated for 15–20 min at 1000 μmol m$^{-2}$ s$^{-1}$ before the measurements.

**NSC extraction and assessment**

Each 2 weeks, 18 of the 36 saplings used for xylem analysis were selected for sugar extraction. The branches were removed and the bark separated from the wood to expose the cambial zone of the stem. The two parts (bark and wood) were plunged into liquid nitrogen, stored at –20°C and placed for lyophilization for a period of 5 d.

The cambium zone, probably including some cells undergoing enlargement, was manually separated by scraping the inner part of the bark and the outer surface of the wood with a surgical scalpel (Giovannelli et al., 2011). After having removed the cambium, the wood was milled to obtain a fine powder.

The extraction of soluble carbohydrates followed the protocol proposed by Giovannelli et al. (2011). For the cambium, only 1–30 mg of powder was available and used for the sugar extraction, while 30–600 mg of powder was available for wood. Samples with <1 mg of cambium powder were not considered, this quantity being lower than the HPLC (high-performance liquid chromatography) detection limit. Soluble carbohydrates were extracted three times at room temperature with 5 mL of 75% ethanol added to the powder. A 100 μL volume of sorbitol solution (0.01 g mL$^{-1}$) was also added as an internal standard at the first extraction. In each extraction, the homogenates were gently vortexed for 30 min and centrifuged at 10 000 rpm for 8 min. The three resulting supernatants were evaporated and recuperated with 12 mL of nano-filtered water. This solution was then filtered by the solid phase extraction (SPE) method using a suction chamber with one column of N+ quaternary amino (200 mg per 3 mL) and one of CH cyclohexyl (200 mg per 3 mL). The solution was evaporated to 1.5 mL and filtered through a 0.45 μm syringe filter to a 2 mL amber vial.

An Agilent 1200 series HPLC with an RID and a Shodex SC1011 column and guard column, equipped with an Agilent Chemstation for LC systems program, was used for assessment of soluble carbohydrates. Calculations were made following the internal standard method (Harris, 1997). A calibration curve was created for each carbohydrate using pure sucrose, raffinose, glucose, fructose (Canadian Life Science) and D-pinitol (Sigma-Aldrich). All fitting curves had $R^2$ values of 0.99 and an $F$-value near 1, indicating that each sugar had a 1:1 ratio with sorbitol.

The sugar loss during extraction was calculated by comparing the concentrations of sorbitol added to the sample at the beginning of the extraction with those of free sorbitol. The percentage loss was then calculated and added to the final results.

Xylem powder was used for starch extraction, performed according to Chow and Landhäusser (2004). The extraction consisted of adding 5 mL of 80% ethanol to 50 mg of powder at 95°C. The solution was vortexed for 30 min and centrifuged, and the supernatant was removed. This step was repeated twice.
The starch was solubilized with 0·1 M NaOH and 0·1 M acetic acid, and digested with an α-amylase solution at 2000 U mL⁻¹ and amylglucosidase at 10 U mL⁻¹. PGO (peroxidase–glucose oxidase) colour reagent and 75 % H₂SO₄ were added to the solution 24 h later. Starch was assessed using a spectrophotometer at 533 nm (Chow and Landhäusser, 2004).

**Statistical analysis**

Because of asymmetric distributions in the water potential data (few points with a Ψ₁d less than −1 MPa) across treatments, Spearman’s rank correlations were used to assess the monotonic relationship between the Ψ₁d and sugar concentrations of sucrose, pinitol and raffinose [water deficit (W), temperature (T), and DOY] (Quinn and Keough, 2002). For each sugar and starch, the effect of temperature and water deficit was tested by general linear models (GLM procedure in SAS) with a factorial model with three (d.f. = 3) as the error term for testing the treatment effects (W, T and DOY) (Quinn and Keough, 2002). Differences between treatments were found with the Tukey test. Starch data were transformed into their log in order to respect the homogeneity of variance.

To verify the effect of treatment on radial growth response, comparisons of fitted curves were performed. The Gompertz logistic function (NonLINear regression, SAS) was fitted to the total number of cells for the six combinations of water and temperature treatments for each year and compared (Potvin et al., 2003). The Gompertz function was defined as:

\[ y = A \exp\left[-e^{\beta t}\right] \]

where \( y \) is weekly cumulative sum of cells, \( t \) the time computed in DOY, \( A \) the upper asymptote (maximum of growth expressed as cell number or tree-ring width), \( \beta \) the \( x \)-axis placement parameter and \( k \) the rate of change of the shape (Deslauriers et al., 2003). In the Gompertz function, the inflection point \( (t_p) \) corresponds to the culmination of growth rate. The placement of the inflection point on the horizontal axis \( (t_p) \) occurs where the second derivative is equal to 0, i.e. when \( t_p = \beta/k \) (Rossi et al., 2006b). A weighted mean absolute cell formation rate \( (r, \text{ cells d}^{-1}) \) was also calculated as (Deslauriers et al., 2003):

\[ r = A k/4 \]

**RESULTS**

*Xylem cell production*

 Sapling radial growth was characterized by a sharp increase starting around DOY 120–130, followed by a plateau indicating the end of radial growth and resulting in a typical S-shaped curve (Fig. 2). Significant differences were found between the radial growth curves in 2010 and 2011 (group effect, \( P < 0.0001 \); Supplementary Data Table S1). Successive pairwise comparisons revealed a significant difference between the water treatments for each year (\( P < 0.0001 \)), thus reducing the rate \( (r) \) and total number of formed cells \( (A) \) in the non-irrigated saplings. Temperature treatment in 2010 and 2011 led to different results. Although the number of cells decreased with increasing temperature \( (T_2 \text{ and } T_5) \) for both irrigated and non-irrigated saplings in 2010, the effect was not significant (\( P = 0.59 \)). A temperature effect was found in 2011 (\( P = 0.025 \)), but with contradictory results between the irrigation treatments: TD and TN treatments increase the total number of cells \( (A) \) in the irrigated saplings whereas both decrease \( A \) in the non-irrigated saplings.

*Leaf water relations, gas exchanges and photosynthesis*

During 2010, the leaf Ψ₁d of non-irrigated saplings dropped dramatically in response to the decrease of soil water availability, reaching the lowest values on DOY 172 (−2.7 MPa) without evident differences between thermal regimes (Supplementary Data Table S2). In 2011, leaf Ψ₁d of non-irrigated saplings was at −0.5 MPa at T0 and ranged from −1.09 to −2.28 MPa for TN. One week after the resumption of irrigation, the leaf Ψ₁d values of non-irrigated saplings were similar to those observed in irrigated saplings, showing that the saplings were able to recover an optimal water status after a period of water deficit. These conditions persisted for the rest of the summer. At the end of the water deficit period in 2010, \( A_{\text{max}} \) of irrigated saplings was 10-fold higher than that of non-irrigated samples \( (2.57 \text{ and } 0.27 \mu \text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}) \), for irrigated and non-irrigated saplings, respectively. The differences in \( A_{\text{max}} \) were more pronounced under warmer temperature than at T0. Average values of stomatal conductance \( (g_s) \) ranged from 0.06 to 0.00 mol m⁻² s⁻¹ for irrigated and non-irrigated saplings, respectively. Similar patterns were observed in 2011; at the end of water deficit period, \( A_{\text{max}} \) of irrigated saplings was also 10-fold

![Fig. 2](https://academic.oup.com/aob/article-abstract/114/2/335/2769086)  
**Fig. 2.** Effect of temperature and water deficit treatments on tree-ring formation, expressed as the number of cells formed each week in 2010 and 2011. After the water deficit period, the growth values are those of the surviving plants. Temperature treatments are control (T0), +2 °C (T2), +5 °C (T5), +6 °C during the day (TD) and +6 °C during the night (TN). Open circles represent the control, and filled circles represent water deficit plants. The grey bands represent the water deficit period.
higher than that of non-irrigated saplings (5.37 and 0.51 μmol CO₂ m⁻² s⁻¹, for irrigated and non-irrigated saplings, respectively). Average values of gs ranged from 0.13 to 0.03 mol m⁻² s⁻¹, for irrigated and non-irrigated saplings, respectively.

Variation of carbohydrates in cambium and xylem during the growing season

Cambium. Each soluble sugar varied in a similar way with respect to the temperature and irrigation regime in cambium (Fig. 3). Sucrose was 2–30 times more abundant than the other sugars, followed by 6-pinitol (Table 1, Fig. 3). In both years, the amount of sucrose in the cambium was high at the beginning of stem growth, with a concentration of about 100 mg g⁻¹ d. wt, and then showed several decreases and increases in concentration. In 2010, the variation of fructose and glucose showed a similar pattern, while in 2011, these sugars increased at the beginning of tree-ring formation and gradually decreased towards the end of the growing season (Fig. 3). The 6-pinitol concentration followed the seasonal trend of sucrose, but did not drop to almost zero like sucrose (Fig. 3). The concentration of raffinose was always very low in cambium during the growing season, with the exception of high values recorded in non-irrigated saplings between DOY 160 and 180 in both years, as well as in TN saplings at the end of tree-ring formation (Fig. 3).

Xylem. In xylem, fructose was the most abundant soluble carbohydrate, followed by sucrose and 6-pinitol, with an amount lower than 3 mg g⁻¹ d. wt (Table 1). Concentrations of sucrose in the xylem were generally high at the beginning and end of the growing season (Fig. 4). As in cambium, sucrose almost disappeared in July (DOY 160–170), reaching concentrations close to zero. Variations of 6-pinitol, fructose and glucose showed no specific seasonal trend. The concentration of raffinose was always near 0 mg g⁻¹ d. wt throughout the growing season, except for the high values observed mainly in non-irrigated saplings during and after water deficit.

Starch in xylem did not follow the same pattern as the other sugars and showed a pronounced seasonal trend. It was more abundant at the beginning of the growing season and dropped to almost zero on DOY 180, then stayed low until the end of summer when starch reserves started to build up again (Fig. 4).

Effects of plant water status, temperature and water deficit on soluble sugars

The concentration of sucrose, 6-pinitol and raffinose in cambium was influenced by the plant water status (Fig. 5, Table 2). Sucrose and 6-pinitol concentrations changed according to leaf water potential. For irrigated saplings at T0, sucrose and 6-pinitol (Fig. 5) increased with decreasing leaf ψpd, with significant regression (except for sucrose in 2010) (Table 2). However, under water deficit (leaf ψpd less than –1 MPa), sucrose and 6-pinitol did not increase proportionally. Contradictory results were observed for the temperature treatments. In 2011, increasing daily temperature did not affect the relationship between leaf ψpd and the measured quantities of sucrose and 6-pinitol, whilst no relationships were found at increasing night temperature in 2011. In 2010, no significant correlation was observed at T2 and T5 (Fig. 5, Table 2) and the signs of the correlation were mostly positive, as for the night temperature in 2011.

Only raffinose showed an increase in concentration with a decrease of ψpd under water deficit. With leaf ψpd values higher than –1 MPa, no clear relationships were observed for any
Table 1. Soluble sugars (mg g⁻¹ d. wt) found in cambium and xylem for the different water and temperature treatments

<table>
<thead>
<tr>
<th></th>
<th>Irrigated</th>
<th>Water deficit</th>
<th>Effect (P)</th>
<th>Irrigated</th>
<th>Water deficit</th>
<th>Effect (P)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>T0</td>
<td>T2</td>
<td>T5</td>
<td>W</td>
<td>T</td>
<td>W × T</td>
</tr>
<tr>
<td>Cambium</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Suc</td>
<td>65.46</td>
<td>66.63</td>
<td>56.36</td>
<td>61.89</td>
<td>60.02</td>
<td>56.95</td>
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<tr>
<td>Pin</td>
<td>23.02</td>
<td>27.01</td>
<td>27.15</td>
<td>21.94</td>
<td>27.58</td>
<td>24.69</td>
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<tr>
<td>Fru</td>
<td>15.37</td>
<td>13.33</td>
<td>13.00</td>
<td>14.63</td>
<td>11.51</td>
<td>11.11</td>
</tr>
<tr>
<td>Glu</td>
<td>10.98</td>
<td>8.41</td>
<td>8.84</td>
<td>11.28</td>
<td>8.17</td>
<td>7.93</td>
</tr>
<tr>
<td>Raff</td>
<td>1.78</td>
<td>1.34</td>
<td>1.52</td>
<td>2.72</td>
<td>2.25</td>
<td>1.97</td>
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<tr>
<td>Xylem</td>
<td></td>
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<tr>
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<tr>
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<td>1.51</td>
<td>1.78</td>
<td>1.62</td>
<td>1.49</td>
</tr>
<tr>
<td>Raff</td>
<td>0.069</td>
<td>0.068</td>
<td>0.111</td>
<td>0.143</td>
<td>0.150</td>
<td>0.225</td>
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<tr>
<td>Starch</td>
<td>2.59</td>
<td>1.93</td>
<td>1.53</td>
<td>2.63</td>
<td>1.60</td>
<td>2.06</td>
</tr>
</tbody>
</table>

In 2010, T2 and T5 experienced a temperature 2 and 5 °C higher than T0, respectively. In 2011, TD and TN were 6 °C warmer than T0 during the day (D) or night (N), respectively. The effect in bold represents the significant probability (P = 0.05) between treatment [water deficit (W), temperature (T), day of the year (DOY)]. Results were all significant (P < 0.001) for DOY (not shown).
Under water stress, the behaviour of black spruce was typical of an isohydric species, with early stomatal closure that prevented desiccation while photosynthesis was shut down. Despite this, similar patterns were observed in the concentration of sugars within the stem under water deficit and warming, except for raffinose. According to Sala et al. (2012), time (short vs. long stress period) and scale (specific tissues vs. whole plant) have to be taken into account when interpreting carbon dynamics of trees under stress. In the case of fast-acting drought, carbon reserves are relatively untouched and carbohydrate availability depends more on water potential and phloem functioning than on photosynthetic production (Sevanto et al., 2014) since water molecules are essential in many reactions of starch degradation (i.e. hydrolysis of maltose) and sucrose hydrolysis for the production of hexoses. On a short time scale, radial growth slowed down or even stopped for about 2 weeks during water deficit, meaning that the population of cells undergoing differentiation was lower, which in turn decreased the need for carbohydrate. A decrease in respiration during drought could also decrease the carbon consumption, leading to a surplus of total carbon (Duan et al., 2013). As hypothesized, during a fast-acting drought, osmoregulation was far more important for survival than wood formation. However, the osmoregulatory response was directly dependent on the raffinose concentration. In both cambium and xylem, raffinose was the key sugar for osmoregulation until a leaf $W_{pd}$ of −3.6 (the minimum we measured on a living sapling). Beyond that value, carbohydrate unavailability could compromise both osmoregulation and hydraulic conductivity, leading to plant death (Sevanto et al., 2014). Contrary to our hypothesis, osmoregulation was not affected by increased temperature as the raffinose concentration was essentially driven by the leaf water potential (i.e. global plant water status) while ambiguous patterns were observed for sucrose and pinitol. At a longer time scale (i.e. over the whole wood formation period), warming affects the hexose pool and starch recovery after the summer minimum, which could eventually compromise the growth and metabolism of the sapling in the following year.

**Seasonal trend**

The observed intra-annual trends of increase and decrease in soluble sugars during wood formation were probably caused by carbon partitioning to sustain growth in different parts of the trees and starch to sugar conversion. Similar patterns of sucrose were found over the 2 years of the experiment, with an alternation of low and high quantities in both cambium and xylem. Fructose and glucose were strongly correlated and both followed the same pattern over the growing season in both cambium and xylem. Seasonal low (sucrose) and high values (glucose, fructose and pinitol) were found on around 20 July (DOY 200) in all treatments and years. The increase in the hexose pool and decrease in sucrose could correspond to the beginning of starch mobilization in mid-summer in order to refill the reserves within the storage compartment. According to Witt and Sauter (1994), the concentration of glucose and fructose...
in ray cells showed peaks in certain periods of the year, such as during starch mobilization in April and in the phase of rapid starch deposition during the summer. Although not reported in the literature, the relatively low quantities of sucrose found in June and July could also be linked to growth activities of primary meristems and roots. The maximum period of needle growth corresponded to the decrease of sucrose in June (data not shown). An accumulation of NSC was observed in coarse roots of *Pinus sylvestris* at the end of July (Gruber et al., 2012) and used for root growth after the end of the above-ground growth period (Hansen and Beck, 1994). In older trees, a parallel change between the dynamics of wood formation and the available pool of sugar in cambium was reported in larch and spruce (Simard et al., 2013). In the xylem, however, difficulties in observing a clear pattern in the stem were found in other species such as red spruce (*Picea rubens* Sarg.) (Schaberg et al., 2000) and white spruce [*Picea glauca* (Moench) Voss] (Hoch et al., 2003).

**Sugar variations under water deficit and warming**

Under mild water deficit, a proportional increase in pinitol and sucrose concentration was observed with a decreasing leaf $\Psi_{pd}$, but this relationship was not maintained with leaf $\Psi_{pd}$ lower than $-0.8$ MPa. The osmoregulatory roles of pinitol and sucrose thus seem to be limited to a definite range of water potential. This pattern was not followed by raffinose. The concentration of this sugar, a member of the raffinose family oligosaccharides

![Figure 5](https://academic.oup.com/aob/article-abstract/114/2/335/2769086)

**Table 2.** Spearman correlations coefficients between the non-structural soluble sugars (mg g$^{-1}$ d. wt) and the pre-dawn leaf water potential ($\Psi_{pd}$ MPa)

<table>
<thead>
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<th>2010</th>
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<td>Water deficit</td>
</tr>
<tr>
<td></td>
<td>T0</td>
<td>T2</td>
<td>T5</td>
<td>T0</td>
</tr>
<tr>
<td>Pin</td>
<td>$-0.52^*$</td>
<td>0.05</td>
<td>0.05</td>
<td>$-0.45^*$</td>
</tr>
<tr>
<td>Suc</td>
<td>$-0.35$</td>
<td>0.06</td>
<td>0.17</td>
<td>0.19</td>
</tr>
<tr>
<td>Raf</td>
<td>0.00</td>
<td>$-0.09$</td>
<td>$-0.39$</td>
<td>$-0.29$</td>
</tr>
</tbody>
</table>

* Asterisks represent significance, with $^*P < 0.05$, $^{**}P < 0.01$. 

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(RFOs), increased proportionally with decreasing $\Psi_{pd}$ potential. We postulate that in stem of black spruce, living cells first accumulated pinitol and hexose in order to regulate cell osmosis and they only began to produce complex sugars (oligosaccharides) when the level of stress increased ($\Psi_{pd}$ less than −0.8 MPa), which directly prevented cell oxidation caused by stress. In this study, raffinose was the only sugar affected by water stress (Table 1, Fig. 5). In 2011, high night temperature also led to an increase in raffinose with respect to T0, but this was caused by the lower water potential reached during the TN temperature treatment.

According to Ford (1984), tropical legumes accumulated pinitol with decreasing leaf water potential. In our experiment, however, the pinitol concentration did not continue to rise with a more negative water potential (Fig. 5), showing a substantial independence from water stress (Table 1). Pinitol concentration increased in Maritime pine seedlings with decreasing osmotic water potential ($\Psi_s$) in roots, with the minimum value of $\Psi_s$ reaching −0.8 MPa (Nguyen and Lamant, 1988). During water stress, pinitol could replace water molecules, because of its alcohol function (Nguyen and Lamant, 1988), and also act as a hydroxyl radical scavenger as drought favours the development of oxygen free radicals (Orthen et al., 1994). In black spruce, a leaf water potential of −2.5 MPa can severely injure black spruce because of it having only a limited osmotic adjustment capacity (Johnsen and Major, 1999; Marshall et al., 2000). The response of sucrose, being similar to that of pinitol, demonstrated that the effect of this sugar was also $\Psi_{pd}$ limited. Nishizawa-Yokoi et al. (2008) found in Arabidopsis thaliana leaves that the increase in intracellular levels of galactinol and raffinose had no effect on level of glucose, fructose and sucrose.

Raffinose has important roles such as osmoprotection and ROS scavenging (Nishizawa-Yokoi et al., 2008; dos Santos et al., 2011) associated with several types of stress responses (i.e., drought, cold, salinity and warming). Galactinol synthase is the enzyme catalysing the first step of RFOs by forming galactinol from UDP-galactose and myo-inositol. Raffinose is then formed by the addition of a galactinol unit to sucrose, which liberates a myo-inositol molecule, a reaction catalysed by raffinose synthase (Castillo et al., 1990). As sucrose is an essential sugar for the biosynthesis of raffinose, sucrose could be directed through this pathway and thus its concentration fails to increase at low $\Psi_{pd}$. Raffinose molecules are more effective at capturing free radicals than the myo-inositol, which have high reducing power. In comparison, pinitol molecules have fewer OH functions. Cyclitols, such as sorbitol (Ahmad et al., 1979) and pinitol, do not easily diffuse through cell membranes and thus accumulate in the cells, causing an osmotic pressure change. Sugars are better molecules for replacing water in membranes to maintain the space between the phospholipid molecules, thus avoiding membrane fusion. Finally, for plant metabolism, raffinose degradation into simple sugars (glucose, fructose and galactose) may be faster, more useful and less harmful than pinitol degradation such as for trehalose (Wingler, 2002). Thus, the fact that pinitol changed in a definite range of $\Psi_{pd}$ could be caused by its own molecular structure and eventual degradation, despite having a similar role to raffinose.

In this study, confusing results were found for sucrose depending on the temperature treatment and type of tissue (xylem vs. cambium), which make interpretation more difficult. Despite this, results for cambium in 2010 were in agreement with literature reports for plants: an increase of pinitol and decrease of sucrose with increasing temperature (Guo and Oosterhuis, 1995; Liu et al., 2008). In 2011, sucrose slightly decreased at high night temperature but increased at TD in the non-irrigated plants.

At higher temperature, the decrease in the hexose pool was probably caused by an increase in respiration, with glucose and fructose more involved through glycolysis and the pentose phosphate pathway. According to Anthor (2000), with increasing temperature, maintenance respiration increased more than respiration due to growth. In arabidopsis cell culture, increasing temperature induced a change in the proportion of both ATP and NADPH that were used for maintenance (Cheung et al., 2013). The hypothesis that hexose was used for metabolic needs was also verified over the growing season in both 2010 and 2011: mean values of glucose and fructose were lower at higher temperature with respect to T0 (Table 1). Both glucose and fructose could be transformed to hexose-phosphate before entering glycolysis. These results are in agreement with Way and Sage (2008a), who found that glucose and fructose concentrations (% of dry mass in needles) were lower for black spruce growing at higher (30 °C/24 °C day/night) compared with lower temperature (22 °C/16 °C day/night), suggesting a rise in respiration.

**Starch variations under water deficit and warming**

Starch tended to decrease as the temperature rose in both 2010 and 2011. Diminution of the amount of starch in ray cells during the warmer night can be explained by a higher respiration rate induced in plants growing under high temperature. Thus, a high respiration rate could require an elevated amount of glucose to use in glycolysis, which in turn could derive from the starch accumulated during the day (Turnbull et al., 2002, 2004). Higher temperature during the day enhances the export rate and utilization of sucrose in the plant, lowering sucrose allocation for starch production (Hussain et al., 1999). Contemporaneously, the impact of severe drought on carbon reserves was confirmed in young Norway spruce trees. Severe events induced a use of the above-ground starch reserves as starch was only completely depleted in roots when the trees were dead (Hartmann et al., 2013).

**Consequence for availability of NSCs during xylogenesis**

We found that water availability (i.e., water potential) during the growing season has an effect on the availability of NSCs in both cambium and xylem. Under limited water availability, even if carbon was not depleted, the availability of NSCs for wood formation in stem was significantly reduced due to their mobilization for osmotic purposes (Pantin et al., 2013): growth differences between the irrigated and water deficit saplings were most probably caused by (1) hydromechanical limitations due to lack of cell turgor for growth and (2) the mobilization of NSCs for osmotic adjustment in order to protect the living cells. However, more studies are needed to link the available NSCs in cambium and xylem parenchyma with the phases of wood formation and to determine the effect of water deficit on this link.
In eucalyptus, total NSCs also remained unchanged under high temperature (Duan et al., 2013). In this study, ambiguous results were obtained with an increase in temperature. The number of woody cells produced decreased slightly with a temperature increase in 2010 and 2011, except for the irrigated trees in 2011. In the long term, however, increased temperature could impair the carbon reserves in the stem, which are fundamental in the case of stresses such as drought, herbivore damage or heating. Although small plants may need less carbon to cope with stresses because of their lower biomass (Sala et al., 2012), the decrease in starch refilling and the use of more hexose in both cambium and xylem at higher temperature could, in the long term, affect the growth and survival of young plants.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxfordjournals.org and consist of the following. Table S1: summary of fitting of growth curves during 2010 and 2011. Table S2: leaf parameters of black spruce saplings before, during and after the water deficit period under three sets of thermal conditions in 2010 and 2011.

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LITERATURE CITED


