Xylogenesis in black spruce: does soil temperature matter?

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In boreal ecosystems, an increase in soil temperature can stimulate plant growth. However, cambium phenology in trees was better explained by air than soil temperature, which suggested that soil temperature is not the main limiting factor affecting xylogenesis. Since soil temperature and snowmelt are correlated to air temperature, the question whether soil temperature directly limits xylogenesis in the stem will remain unresolved without experiments disentangling air and soil temperatures. This study investigated the effects of an increase of 4 °C in soil temperature and a consequent 1-week earlier snowmelt on growth of black spruce [Picea mariana (Mill.) BSP] in the boreal forest of Quebec, Canada. The soil of two natural stands at different altitudes was warmed up with heating cables during 2008–2010 and cambial phenology and xylem production were monitored weekly from April to October. The results showed no significant effect of the treatment on the phenological phases of cell enlargement and wall thickening and lignification. The number of cells produced in the xylem also did not differ between control and heated trees. These findings allowed the hypothesis of a direct influence of soil temperature on stem growth to be rejected and supported the evidence that, in the short term, air temperature is the main limiting factor for xylogenesis in trees of these environments.

Keywords: boreal forest, cambium, climate change, intra-annual growth, microcores, soil temperature, soil warming, wood production, xylem phenology

Introduction

The boreal forest is the biome with a greater expected increase in surface temperature than that of any other forest ecosystem in the world (Strömgren and Linder 2002, Bronson and Gower 2010). Recent forecasts for eastern Canada estimate increases of 3 °C in mean annual temperature and of 5–20% in annual rainfall for the year 2050 (Plummer et al. 2006). In this biome, plant growth is limited by low temperatures and, indirectly, by availability of nutrients, especially nitrogen, which is expected to increase with climate warming according to the change in the rate of mineralization of the soil (Rustad et al. 2001, Strömgren and Linder 2002, Campbell et al. 2009). Some experiments conducted in boreal ecosystems have observed that soil temperature can affect plant growth (Van Cleve et al. 1990, Strömgren and Linder 2002, Jyske et al. 2011). Needles of black spruce growing on heated soils contained greater amounts of nitrogen, phosphorus and potassium and showed 20% higher photosynthetic rates (Van Cleve et al. 1990). Strömgren and Linder (2002) reported increases in stem production attaining 115% in irrigated and heated plots of Norway spruce. The understanding of current and future growth dynamics of forest ecosystems requires a clear assessment of the mechanism of action of climate on growth, including cambial activity and phenology. However, the direct and indirect role of temperature in growth still remains uncertain (Vaganov et al. 1999, Jarvis and Linder 2000, Rossi et al. 2011).

The key role of temperature in cambial reactivation and cell production has recently been demonstrated (Oribe et al. 2001, Begum et al. 2007, Deslauriers et al. 2008, Rossi et al. 2008) and may be explained by the evidence that a soil temperature <6 °C strongly inhibits root activity and water uptake in various conifers (Alvarez-Uria and Körner 2007). Also, observations carried out at the northern treeline showed no cambial activity when soil temperature was <3–5 °C (Körner 2003). Since
 cambium seems to have synchronous activity in the stem and roots (Thibeault-Martel et al. 2008), and root growth is limited by low temperatures (Alvarez-Uria and Körner 2007), xylogenesis in the stem could be assumed to begin after soil starts to warm up (Rossi et al. 2007, Turcotte et al. 2009, Lupi et al. 2010). Nevertheless, Rossi et al. (2007) and Swidrak et al. (2011) found that the onset of wood formation could be better explained by air than soil temperature and concluded that soil temperature was not the main limiting factor affecting xylogenesis in the stem. When air temperature increases, soil temperature increases accordingly. Thus, only manipulative experiments addressing separately the effect of air and soil temperatures can resolve the question of whether soil temperature directly limits xylogenesis in the stem (Vogel et al. 2008).

Because of the well-known insulating effect of snow, soil maintains a temperature close to 0 °C until complete snowmelt, which is below the minimum threshold for growth (Decker et al. 2003, Körner 2003). Vaganov et al. (1999) suggested that the later snowmelt occurring as a result of the increased snowfalls of the last century in subarctic Eurasia delayed the onset of xylogenesis and reduced tree growth. Indeed, it has been observed that the resumption of radial growth follows, not precedes, soil thaw and snowmelt (Graumlich and Brubaker 1986, Cairns and Malanson 1998, Turcotte et al. 2009, Rossi et al. 2011). According to Jarvis and Linder (2000), neither cambial division nor uptake of nutrients and CO₂ can occur while the soil is frozen; thus the length of the snow-free period should influence tree growth and C assimilation by affecting photosynthesis in spring and nutrient cycling and availability in summer.

Even if the effect of soil temperatures and timings of snowmelt on tree growth is considered obvious, there is a need to ultimately rule out whether the relationship is direct or derives from the fact that soil temperatures and snowmelt are correlated to air temperature. This study investigated the effects of an increase in soil temperature and the consequent earlier snowmelt on cambial phenology by warming the soil of natural stands with heating cables during 2008–2010 in two sites of the boreal forest of Quebec, Canada. The treatment produced earlier snowmelt and longer snow-free periods in accordance with the increase in winter temperature predicted for eastern Canada (Plummer et al. 2006). Many studies underline the importance of soil temperature for cambial reactivation and wood production (Vaganov et al. 1999, Körner 2003, Alvarez-Uria and Körner 2007); thus the hypothesis was tested that increased soil temperatures and earlier snowmelts produce earlier onsets of growth and greater amounts of xylem.

Materials and methods

Study sites and tree selection

The study took place in the boreal forest of Quebec, Canada, in two mature and even-aged black spruce [Picea mariana (Mill.) BSP] stands at different altitudes. The first site (BER) was located near Lake Bernatchez, in the Monts-Valin (48°51'N, 70°20'W, 611 m a.s.l.). The second site (SIM) was at a lower altitude, in the Laurentides Wildlife Reserve, within the Simoncouche research station (48°13'N, 71°15'W, 350 m a.s.l.). The density of the two stands was similar, ~3000 trees ha⁻¹, though slightly smaller trees were present at BER. The region is included in the balsam fir–white birch ecological domain (Saucier et al. 1998), with an understorey vegetation mainly composed of Kalmia angustifolia, Ledum groenlandicum, Cornus canadensis and Vaccinium myrtillus, and soil vegetation of Sphagnum spp. and mosses (Hylocomium splendens, Pleurozium schreberi, Ptilium crista-castrensis) (Marie-Victorin 1995). The average May–September rainfall is 401.8 and 425.4 mm, at SIM and BER, respectively. SIM derived from a forest fire in 1922, while in BER, a forest fire at the origin of the stand was estimated to have occurred between 1865 and 1870. The stands are growing on gentle slopes (8–17%) and drain glacial tills.

In each site, six co-dominant trees were chosen with upright stem, healthy overall appearance and similar growth patterns. The homogeneity in growth rates was assessed during a preliminary investigation by extracting wood cores and counting the number of tracheids along three previous tree rings (Rossi et al. 2007). The average diameter at breast height and the average height of sampled trees were 17 ± 2 and 21 ± 4 cm and 15 ± 2 and 14 ± 2 m, at BER and SIM, respectively.

Experimental design

During autumn 2007, heating cables were installed in the soil at 20–30 cm depth, where the majority of the root system of black spruce is localized (Ruess et al. 2003), between the organic and mineral layers, following a spiral pattern at a distance of 90–200 cm from the stem collar, leaving 30 cm between coils of the cables. The experimental design included a control and a heated treatment, each applied to three trees per site. Non-heating cables were similarly installed around the control trees to account for root damage at the moment of cable laying. In the treated trees, soil temperature was increased by 4 °C according to the forecasts for 2050 proposed by the FORESTEM climatic model developed for the boreal forest of eastern Canada (Houle et al. 2002). Heating started on different dates according to year and site, usually with 2 weeks delay between SIM and BER, to reflect the difference in temperature between the two altitudes (Lupi et al. 2010). In SIM, heating usually started at the end of March except in 2008 when it started in mid-April. Soil temperature was measured, at ~1–2 m from the stem, between the coils of the cables in three heated and three non-heated points per site. A diesel generator was used to maintain the temperature differential between control and treated trees during April–July, the period in which most cambial division takes place (Rossi}
et al. 2006a, Thibeault-Martel et al. 2008). Measurements were taken every 15 min and data were stored as hourly averages in CR1000 dataloggers (Campbell Scientific Corporation, Edmonton, Canada). Soil volumetric water content of heated and non-heated plots was measured during June–July 2009 but no difference was found between treatments.

Standard weather stations were installed in a forest gap close to the experimental plots to measure air temperature. Measurements were taken at the same time interval as that of soil temperature and stored in CR10X dataloggers.

Sample collection and preparation
During 2008–2010, wood microcores (2.5 mm in diameter and 25 mm long) were collected from the stem weekly from April to October with a Trephor (Rossi et al. 2006b) following a counter-clockwise rising spiral centered at breast height. Microcores usually contained the previous five tree rings and the developing annual layer with the cambial zone and adjacent phloem tissues. Wood samples were always taken at 5–10 cm intervals to avoid the formation of traumatic resin ducts (Deslauriers et al. 2003). The microcores were stored in Eppendorf microtubes containing a water:ethanol solution (1:1). In the lab, the microcores were dehydrated through successive immersions in ethanol and Histosol™ and embedded in paraffin (Thibeault-Martel et al. 2008). Transverse sections 6–10 µm in thickness were cut with a rotary microtome, stained with cresyl violet acetate (0.16% in water) after the paraffin was removed and observed within 20–30 min under visible and polarized light at a magnification of ×400–500 to differentiate cambium and developing xylem cells.

The cambial zone and cells in radial enlargement showed only a primary wall, which, unlike the secondary wall, did not shine under polarized light (Gričar et al. 2006). Cambial cells were characterized by thin cell walls and small radial diameters, while enlarging cells had a radial diameter at least twice that of a cambial cell. Cells in wall thickening shone under polarized light and, during the maturation process, showed a coloration varying from light to deep violet. As lignification advanced, a blue coloration starting from the cell corners spread into the secondary walls. Since lignin deposition may persist after the end of cell wall thickening (Gindl et al. 2000), cells were considered lignified and mature when the violet was completely replaced by the blue coloration (Thibeault-Martel et al. 2008). The number of cells in enlargement and in wall thickening and lignification and mature cells was counted along three radial rows. The number of total cells was calculated as the sum of cells in enlargement, wall thickening and lignification, and mature cells. In spring, xylem formation was considered to have begun when the average of number of cells in the enlarging phase was greater than one. In late summer, when the average of number of cells undergoing wall thickening and lignification was less than one, xylem formation was considered complete. The phenology of xylem development was assessed for each tree. Four phenophases, computed in days of the year (DOY), were considered, including onset and ending of (i) cell enlargement and (ii) wall thickening and lignification. Duration of xylem formation was calculated as the difference between the onset of cell enlargement and the ending of cell wall thickening and lignification.

Four microcores were also taken around the stem of each tree after the end of the experiment, in October 2010. The number of xylem cells produced during 2005–2010 was counted on each sample along two radial rows per tree ring.

Statistical analyses
Analysis of variance with repeated measurements for mixed linear models was used to analyze the statistical differences in onset and ending of each differentiation phase and in duration of xylogenesis. Analyses were conducted using PROC MIXED of the SAS statistical package (SAS version 9.2, SAS Institute, Cary, NC, USA) considering site, temperature treatment, year and all interactions as fixed factors. The denominator degrees of freedom for testing the fixed effects were calculated using the Satterthwaite method of approximation, and different variance–covariance structures were tested to select the best model (SAS version 9.2). Akaike’s information criterion was used to evaluate the models and to find a suitable covariance structure (Quinn and Keough 2002, Kilpeläinen et al. 2007). When significant effects of a factor or interactions between factors were found, multiple comparisons of least squares (LS) means were performed using the method of Scheffe for adjusting the $P$ values.

Specifically for cell production, the tests were conducted on the treatment period (2008–2010) by removing the initial differences between trees and treatments using as covariate the average number of cells of the three tree rings formed prior to the treatment (i.e., 2005–2007) (Jyske et al. 2009). The relation between the covariate and dependent variable was linear. Results were reported as LS means calculated by the mixed models.

Results
Air and soil temperatures
The climate of the sites is continental with long, cold winters and warm summers. During 2008–2010, the mean annual temperature was 0.2 °C in BER and 2.2 °C in SIM (Figure 1). May–September average temperature varied between 11.2 and 14.2 °C, with the highest values recorded in 2010, 12.5 and 14.2 °C in BER and SIM, respectively. Mean monthly temperatures during winter were close to or below zero for a period ranging between the beginning of November and the end of March and dropping in January 2009 to −21.2 and −19.1 °C in BER and SIM, respectively (Figure 1). The coldest
months showed absolute minimum temperatures reaching −39.8 °C in BER in 2009 and −32.0 °C in SIM in 2010. Summers were generally short with absolute maximum temperatures attaining 31 °C in 2010 (Figure 1).

Soil temperatures were close to 0 °C from December to April. The 4 °C differential between treatments was achieved in ~3 days after heating started and was maintained up to the end of the heating period (Figure 1). Heating took between 2 and 3 weeks before the snow completely melted, ~1 week before that around the control trees (Figure 2). Complete snowmelt was generally 2 weeks later in BER, the site at the higher altitude. Treatment stopped at the beginning of July in SIM and 2 weeks later in BER (Figure 1). The warmest soil temperatures were measured around the heated trees, with 19.1 °C in BER and 21.6 °C in SIM, both occurring in 2010.

**Cambial activity and cell differentiation**

In both treatments, during the inactive period, the cambium was constituted by 4–6 cells (Figure 3). In May, the number of cells in the cambial region rapidly increased, reaching its maximum in June when up to 14 cells were counted, with higher values observed in 2009 and 2010. The number of cells in the cambial zone decreased to minimum values at the end of August (Figure 3). The number of cells in enlargement increased rapidly at the beginning of xylogenesis, reached its maximum between the end of June and mid-July, and then decreased to zero, following the decrease in cambial divisions. No clear differences between treatments, except a higher maximum number of cells in enlargement in the heated trees at BER, were observed (Figure 3). The number of cells in wall thickening and lignification followed a similar pattern, but usually decreased to zero 2–4 weeks later than the number of cells in enlargement (Figure 3). At both sites, 1–2 more cells in wall thickening and lignification were often observed in heated trees than in control trees (Figure 3).

The maximum number of cambial cells was similar between the two sites and varied between 9 and 14, depending on year. More differences were observed for cells in enlargement and
in wall thickening and lignification, especially during the period of maximum cell production. Up to 8 and 15 enlarging cells were counted in BER and SIM, respectively. Similarly, up to 12 and 24 cells in wall thickening and lignification were observed in BER and SIM, respectively. However, in SIM the maximum number of cells in wall thickening and lignification was more frequently 10–12, while 7–9 was more common in BER (Figure 3). Higher total number of cells was generally observed in SIM than BER (Figure 3).

The number of cambial cells was similar between the two treatments both before and during the period of cambial activity (Figure 3). Heated trees occasionally showed more enlarging cells in BER, and heated trees had more cells in wall thickening and lignification than control trees in both sites (Figure 3). The total number of cells was usually higher in the heated trees (usually 5–7 more cells), especially in BER.

**Xylem phenology and cell production**

The onset of xylogenesis was statistically different between sites and years (Table 1). On average, xylogenesis started on DOY 156 in BER, 9 days later than in SIM (Scheffe’s test, \( t = 2.77, P < 0.05 \)). The year with the earliest onset was 2010 (DOY 145), while that with the latest was 2009 (DOY 156). The onset of wall thickening and lignification differed between years, with the earliest and latest onset observed in 2010 (DOY 150) and 2009 (DOY 171), respectively (Table 1, Figure 4). No effect of treatment was observed on the phenological phases of xylem (Figure 4), (Table 1).

The ending of cell enlargement differed between years (Table 1), with 2008 being that with the earliest ending (DOY 204). The ending of xylogenesis was not affected by treatment, site or year (Table 1). The duration of xylogenesis varied between years and showed a significant interaction between treatment and year (Table 1). Overall, during 2009 and 2010 the duration of xylogenesis was longer in the heated trees of both sites, lasting on average 18 days more than in control trees. However, no significant difference was detected by the multiple comparisons performed for each year.

The overall number of cells decreased up to 2009, while a higher amount of cells was produced in 2010 (Figure 5). Between the three study years, cell production differed significantly, but no significant results were detected between treatments and sites (Table 1). Before the treatment began, more cells were counted in heated than control trees. This difference was maintained during 2008–2009, the first 2 years of the experiment. In 2010, the gap between treatments increased substantially in both sites, but no statistically significant difference was observed between heated and control trees (Scheffe’s test, \( t = 0.95, P > 0.05 \)).

**Discussion**

In this study, the hypothesis that cambial phenology and cell production are directly affected by soil temperature and snowmelt was explicitly and experimentally tested in the field through a manipulative approach that disentangled air and soil temperature.
The treatment consisted of an increase of 4 °C in soil temperature and a consequent 1-week earlier snowmelt. After 3 years of experiment, no difference in xylem phenology or cell production was observed. As a result, the initial hypothesis was rejected, allowing the existence of a direct relation between soil temperature and tree growth in the short term to be ruled out.

During late winter, localized heating of the stem could induce localized reactivation of the cambium, which however ceased its activity soon after a few cells had been produced, suggesting that meristems require additional conditions to maintain cell division (Oribe et al. 2001, 2003, Gricar et al. 2006). Depending on the species, cambial reactivation may be limited by factors other than temperature (Oribe and Kubo 1997). For example, in the deciduous Japanese larch cambium was not responsive to heating before bud break (Oribe and Kubo 1997). Moreover, certain studies suggest that, after cambial reactivation, a continuous downward flow of auxin through the phloem may be needed for the maintenance of cambial divisions and cell differentiation (Oribe and Kubo 1997, Oribe et al. 2003). According to some authors, air temperature should affect the transport of auxin inside the stem, and thus an insufficient supply of auxin may inhibit the reactivation of cambial tissues in early spring (Oribe et al. 2003, Fonti et al. 2007). A hypothesis is that the responsiveness of cambium to auxin may indeed be modulated by temperature and the presence of cytokinins supplied by the root apices (Aloni et al. 2006, Fonti et al. 2007). The production of cytokinins followed by the synthesis of auxin in the apical region of active shoots seems to be promoted by warm temperatures (Aloni et al. 2003, Friml 2003). Thus, we could assume that although the raised soil temperature in our experiment may have increased the production of cytokinins, the absence of a favorable air temperature prevented the production and transport of auxin, denying one of the conditions required for cambial activity along the stem.

Although no significant increase in soil CO₂ efflux was observed in an experiment of soil heating (Vogel et al. 2008), extreme soil heating of 8 °C increased the aboveground productivity and

![Figure 4. Phenological phases of xylem differentiation and duration of xylogenesis in control and heated trees in BER and SIM during 2008–2010. Values are reported as LS means ± standard error.](image)

Table 1. F-values of the mixed models for the type III test of fixed effects applied to the phenological phases and cell production during the 3 years of treatment (2008–2010). The average number of cells of the 3 years preceding the treatment (2005–2007) was used for cell production as covariate to remove the initial differences in the number of cells between trees, treatments and sites.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Cell enlargement</th>
<th>Wall thickening and lignification</th>
<th>Duration of xylogenesis</th>
<th>Number of cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Onset  Ending</td>
<td>Onset  Ending</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Covariate</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>598.20**</td>
</tr>
<tr>
<td>Treatment (Tt)</td>
<td>3.37  1.32</td>
<td>1.70  2.24</td>
<td>2.09</td>
<td>0.09</td>
</tr>
<tr>
<td>Site</td>
<td>7.68*  2.15</td>
<td>4.88  1.35</td>
<td>2.69</td>
<td>0.03</td>
</tr>
<tr>
<td>Year</td>
<td>16.43** 14.82**</td>
<td>159.79** 0.76</td>
<td>7.64**</td>
<td>27.78**</td>
</tr>
<tr>
<td>Site x year</td>
<td>0.54  0.10</td>
<td>1.17  0.36</td>
<td>0.82</td>
<td>1.09</td>
</tr>
<tr>
<td>Site x Tt</td>
<td>0.69  0.01</td>
<td>0.01  1.16</td>
<td>0.05</td>
<td>0.81</td>
</tr>
<tr>
<td>Tt x year</td>
<td>2.05  1.87</td>
<td>0.62  1.10</td>
<td>4.73*</td>
<td>1.23</td>
</tr>
<tr>
<td>Site x Tt x year</td>
<td>1.05  0.38</td>
<td>1.50  0.66</td>
<td>1.95</td>
<td>0.75</td>
</tr>
</tbody>
</table>

*P < 0.05, **P < 0.01.
In experiments with localized heating of the stem, cambial activity and cell differentiation were influenced only within the treated area, which pointed out the non-transference of the effect of temperature from the site of its application to other parts of the tree (Gričar et al. 2006, 2007). This may explain the absence of any effect on wood production and phenology of xylogenesis in our experiment. Several studies have found changes in C allocation between above-ground and below-ground pools (Strömgren and Linder 2002, Majdi and Ohrvik 2004, Vogel et al. 2008, Kane and Vogel 2009). Nevertheless, although synchronous xylogenesis in stem and roots has been observed in natural stands (Thibeault-Martel et al. 2008), the question whether the manipulative treatment of soil heating affects xylem phenology and production in the roots of black spruce still remains unanswered.

Conclusions

This paper describes a heating treatment of 4 °C applied to the root system of mature black spruce in the field over 3 years to simulate the warmer soil and earlier snowmelt predicted for 2050 by the climatic models for eastern Canada. The results showed no effect of the treatment on cambial phenology and xylem production, which allowed the hypothesis of a direct influence of soil temperature on tree growth in the stem to be rejected. However, the observed high variability among trees and the limited size of the sample could have affected the significance of the statistical analyses. Moreover, different results could be expected with a soil warming performed over larger areas or with heating cables penetrating to greater soil depths. Nonetheless, our findings support the evidence that, in the short term, air temperature is the main factor determining annual increment variation in the boreal forest. However, the observed trends in the amount of cells produced by cambium during 2008–2010 suggest that the future prolongation of the experiment might reveal long-term effects of soil temperature on nutrient cycles, especially nitrogen, and, indirectly, on tree growth. This may have important implications for the growth of boreal forest trees given the increase in soil and air temperature and the consequently increased availability of nutrients expected with climate change.

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