Original article

DURATION OF XYLOGENESIS IN BLACK SPRUCE LENGTHENED BETWEEN 1950 AND 2010

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Running title: trends in cambium phenology

ABSTRACT

Background and Aims Reconstructions have identified the 20th century as uniquely warm in the last 1000 years. Changes in phenology of the primary meristems converged toward increases in length of the growing season. Has the phenology of secondary meristem changed during the last century, and to what extent?

Methods Timings of wood formation in black spruce [*Picea mariana* (Mill.) B.S.P.] were monitored for nine years on a weekly time-scale at four sites in the boreal forest of Quebec, Canada. Models for assessing xylem phenology were defined and applied to reconstruct onset, ending and duration of xylogenesis between 1950 and 2010 using thermal thresholds on chronologies of maximum and minimum temperatures.

Key Results All sites exhibited increasing trends of both annual and May-September temperatures, with the greatest changes observed at the higher latitudes. Phenological events in spring were more affected than those occurring in autumn, with cambial resumptions occurring 0.5-0.8 days decade^{-1} earlier. Duration of xylogenesis has significantly lengthened since 1950, although the models supplied wide ranges of variations, between 0.07 and 1.5 days decade^{-1}, respectively.

Conclusions The estimated changes in past cambial phenology demonstrated the marked effects of the recent increase in temperature on the phenological traits of secondary meristems. In the long run, the advancement of cambial activity could modify the short time window for growth of the boreal species and dramatically affect the dynamics and productivity of trees in these temperature-limited ecosystems.

Keywords: boreal forest, cell differentiation, *Picea mariana*, threshold temperature, wood formation, xylogenesis

INTRODUCTION

Hemispheric-scale reconstructions of surface temperature fluctuations over the last millennium have identified the 20th century as uniquely warm (cf. Hughes, 2002). Although an increase in the global temperature of between 1.4 and 5.8 °C is expected during the period 1990-2100, the greater increases are predicted to occur at the higher latitudes of the northern hemisphere (IPCC, 2007). For the boreal forest of North-Eastern Canada, climatic models predict increases in temperature of up to 3 °C over the next 50 years, with the largest increases occurring in winter and spring, when plant growth resumes (Plummer *et al.*, 2006). In cold ecosystems, the growing season is strictly defined by the harsh winter temperatures (Rossi *et al.*, 2008b, 2011b). Such climate modifications could thus severely affect the growth timings and dynamics of the boreal species, with consequent changes in productivity of biotopes that have obvious ecological importance and economic potential.

The interest in the cold forests of high altitude and latitude is linked to various causes such as the short growing season and high sensitivity of the species to climate changes (Körner, 2003a). Plants of these ecosystems are assumed to be good indicators of changes in their environment (Pisaric *et al.*, 2003). With the current changes in temperature, possible variations in phenology – the study of the timings of recurring seasonal biological events – have acquired particular importance worldwide. According to Forrest and Miller-Rushing (2010), interest has been focused on documenting the recurrent appearance of the first flower blossoms or bud bursts in spring, the dates of animal migration, or timings of the first frost-damaged leaves in autumn. The longest monitoring periods in plants are concentrated in the botanical gardens of temperate Europe and concern the effects of such changes on the growth dynamics of the primary meristems (buds, leaves and flowers). There is no historical documentation on the phenology of the secondary meristem, the cambium, because it is not a macroscopically perceptible phenomenon like leaf development or flower maturation. Cambial activity occurs beneath the bark and, in high-latitude forests, produces a number of xylem cells that increases the stem diameter annually by one tree ring, which integrates the effects of climatic events occurring during the season when the cambium was active (Frankenstein *et al.,* 2005). Tree rings have thus been used as a tool to explore the long-term growth reactions to historical climate variations (Huang *et al.,* 2010). However, to our knowledge, retrospective studies concerning reconstructions of cambium phenology are still lacking due to the very recent identification and application of the standard procedures of analysis (Rossi *et al.,* 2006a, 2006b; Seo *et al.,* 2008; Gričar *et al.,* 2009).

Analyses on the variations in the concentrations of CO_2 in the atmosphere showed that the biospheric activity increased remarkably as a result of warming surface air (Myneni *et al.,* 1997). This implies that even small changes in global temperature may be reflected by disproportionate responses at regional level that can markedly influence all biological processes, in particular those concerning growth. Although several endogenous variables can influence the dynamics of xylem formation (Marion *et al.,* 2007; Rossi *et al.,* 2008a; Rathgeber *et al.,* 2011, Anfodillo *et al.,* 2012), temperature remains the main driving factor in cold environments. A local overheating in spring can reactivate cambium, inducing the conversion of starch reserves into sucrose for the activation of cell division and production of secondary xylem (Begum *et al.,* 2007; Gričar *et al.,* 2007). Deslauriers *et al.* (2008) observed that the higher temperatures occurring in spring 2003 led to earlier onsets of division and differentiation of xylem cells. The onset of xylogenesis influences the number of cells produced by the cambium which, in turn, influences the ending of cell differentiation (Lupi *et al.*, 2010; Rossi *et al.*, 2012). Several studies in cold environments demonstrated that a certain temperature, in the form of heat sum or thermal threshold, is necessary to enable the cambium to divide (Rossi *et al.*, 2008b; Seo *et al.*, 2008; Swidrak *et al.*, 2011). Rossi *et al.* (2011b) simulated several warming scenarios to predict changes in xylem phenology. The model predicted longer duration of xylem growth at higher temperatures, with increases of 8-11 days/°C because of an earlier onset and later ending of growth. Twenty-five percent longer durations of xylogenesis were predicted with an increase of 3 °C in the mean annual temperature (Rossi *et al.*, 2011b).

Although substantial increases in temperature were observed during the last 100 years, with the mean surface temperature rising by 0.7 °C at global scale and by 0.5-1.5 °C across North America since the late 19th century (Zhang *et al.*, 2000; IPCC, 2007), the rate of warming over the last 50 years (0.13 °C decade^{-1}) is almost double that over the last 100 years (IPCC 2007). For the northern regions of North America, McKenney *et al.* (2006) estimated increases attaining 0.26 °C decade^{-1} in the second half of the 20th century. The question is if and to what extent the modifications in air temperature during this period have affected cambial phenology. The model developed by Rossi *et al.* (2011b) simulated a potential xylem phenology under a possible and simplified climate warming, represented by a uniform increase in air temperature. However, no information was provided about changes in the timings of cambial growth occurring in the past. Moreover, there is evidence of divergent effects of climate change on the seasonal temperatures, with spring having the greater warming (Zhang *et al.*, 2000). Improvements in the precision of the models of cambial growth are thus expected to produce results consistent with the more realistic climatic scenarios.

This paper aimed to reconstruct timings of cambium phenology over the last 60 years in Quebec, Canada. This was done by (i) collecting and analyzing a dataset of cambium phenology and wood formation in black spruce [*Picea mariana* (Mill.) B.S.P.] based on a weekly monitoring for 9 years in four permanent sites at different latitudes and altitudes, (ii) defining and validating a phenological model of xylem based on the air temperature measured in the sites, and (iii) applying the phenological model on the chronologies of air temperature generated for the period 1950-2004 by the ANUSPLIN model (McKenney *et al.*, 2006). The effects of climate change on plants have mainly been demonstrated by changes in the phenology of primary meristems, which have revealed marked increases in length of the growing season (Menzel, 2000; Zhou *et al.*, 2001; Sparks and Menzel, 2002; Badeck *et al.*, 2004). Thus, according to the results provided on primary meristems, the hypothesis that duration of xylogenesis has lengthened since 1950 was tested by the model.

MATERIALS AND METHODS

Study area and xylem sampling

The study was conducted on black spruce in the Saguenay-Lac-Saint-Jean area, in the boreal forest of Quebec, Canada. Four sites [Simoncouche (abbreviated as SIM), Bernatchez (BER), Mistassibi (MIS) and Camp Daniel (DAN)] were identified in mature even-aged stands at different altitudes and latitudes to obtain as wide as possible a range in the dynamics of tree growth (Table 1). Details on site characteristics were provided by Rossi *et al.* (2011b).

In each site, tree-ring formation was studied from April to October during 2002-2010 in 5 (2002-2005) and 10 (2006-2010) trees. Wood microcores were collected weekly following a spiral trajectory on the stem from 30 cm below to 30 cm above breast height (1.3 m) using surgical bone sampling needles in 2002-2006 and Trephor in 2007-2010 (Rossi *et al.*, 2006a). Samples usually contained the previous 4-5 tree rings and the developing annual layer with the cambial zone and adjacent phloem. Samplings were always taken at least 5 cm apart to avoid getting resin ducts on adjacent cores.

The microcores were stored in ethanol solution (10% in water) at 5 °C to avoid tissue deterioration. Microcores were dehydrated with immersions in ethanol and D-limonene and embedded in paraffin (Rossi *et al.*, 2006a). Transverse sections of 6-10 µm thickness were cut from the samples with a rotary microtome, stained with cresyl violet acetate (0.16% in water) and examined within 10−25 minutes under visible and polarized light at magnifications of 400−500× to differentiate the developing and mature xylem cells. Occasionally, distorted rows of cells prevented a adequate analysis of the sample. In these cases, the sections were gently stressed on the slide with thin needles to better observe all cells of the developing tree ring.

Microscopic observations

In each sample, the radial number of cells in the cambial zone, radial enlargement phase, cell wall thickening phase, and mature cells were counted along three radial rows. In cross section, cambial cells were characterized by thin cell walls and small radial diameters (Rossi *et al.,* 2006b). The dormant cambium was composed of 3-5 closely-spaced cells. At the onset of cambial activity, the cambial zone began to widen rapidly as the number of cells increased, revealing that cell division had started. During cell enlargement, the tracheids were composed of a protoplast still enclosed in the thin primary wall but with radial diameter at least twice that of a cambial cell. Observations under polarized light discriminated between enlarging and cell wall thickening tracheids (Thibeault-Martel *et al.*, 2008). Because of the arrangement of cellulose microfibrils, the developing secondary walls were birefringent when observed under polarized light. Instead, no glistening was observed in enlargement zones, where the cells were still composed of just primary wall (Abe *et al.,* 1997). Lignification was detected with cresyl violet acetate by a color change from violet to blue. The color change over the whole cell wall revealed the end of lignification and the tracheid reaching maturity (Gričar *et al.,* 2005).

The cell number in the 3 rows was averaged for each tree and used to assess onset and ending of xylogenesis. In spring, when at least one tangential row of cells was observed in the enlargement, xylem formation was considered to have begun. In late summer, when no further cell was observed in wall thickening and lignification, xylem formation was considered complete. The duration of xylogenesis was assessed as the number of days occurring between onset and ending of xylogenesis and was calculated as the average among trees for each studied site and year.

Datasets of air temperature

Two datasets of air temperature were used in this study and consisted of time series (i) measured in the four sites and (ii) estimated by a climatic model. Measured and estimated temperatures were used for the definition and application of the phenological model, respectively. For the first dataset, a standard weather station was installed in 2001 in a forest gap on each site. Air temperature data were collected at 3 m above ground level every 15 minutes and recorded as averages every hour by means of CR10X dataloggers (Campbell Scientific Corporation, Canada). Maximum and minimum values were later calculated from the 24 measurements per day. The second dataset consisted of air temperatures generated at a daily resolution for the period 1950-2004 by the ANUSPLIN model of the Canadian Forestry Service. This model used a multi-variate non-parametric surface and point fitting approach to estimate the time series of maximum and minimum temperature corresponding to the location of each site according to the algorithm described by McKenney *et al.* (2006). To verify the consistency of the modeled time series, linear regressions were performed between the measured and estimated temperatures for the overlapping years (2002-2004).

Definition and validation of the phenological model

The model consisted in applying thermal thresholds for estimating xylem phenology using logistic regressions to calculate the probability of xylem growth being active at a given measured daily temperature. According to Rossi *et al.* (2011b), binary responses were coded as non-active (value zero) or active (value 1) growth, and temperature thresholds were calculated when the probability of xylem growth being active was 0.5. For each site and year, the model was fitted with minimum and maximum temperature series and results from each site were compared by analysis of variance (ANOVA) and Tukey's test. None of the 72 estimated functions was excluded because of lack of fit. Model validation was performed according to Legendre and Legendre (1998) by comparing the observations with the predicted values calculated using the estimated temperatures. A classification table was produced in the form of a contingency table, which for each day compared the observed active or non-active xylem growth to that predicted by the model.

Application of the phenological model

A quadratic logistic regression was applied on the time series of daily temperatures generated by the ANUSPLIN model, with binary responses coded as 0-1 if temperatures were lower or higher than the threshold, respectively. The two solutions of the quadratic regression corresponded to the days of the year when the probability of temperature being higher than the threshold was 0.5, and included the period of xylem growth (Rossi *et al.*, 2011b). The phenological model was iteratively applied to the temperature series of each site to estimate changes in the timings of xylem growth. The resulting time series, which consisted of the dates of onset and ending of xylogenesis from 1950 to 2010, were tested for the presence of autocorrelation until the fourth order (McKenney *et al.*, 2006). Since no autocorrelation was observed for the onset of xylogenesis and the errors were only occasionally serially correlated at the second and third order for ending and duration of xylogenesis, the long-term trends were analyzed by Analysis of Covariance (ANCOVA).

RESULTS

Observed and modeled temperatures

The region has a typical boreal climate with cold winters and cool summers (Table 1). The mean annual temperature in the four study sites varied between -0.9 and 2.0 °C while May-September temperature was 11.0-13.3 °C. The sites are characterized by long winters with temperatures close to or below zero, with the coldest generally being measured in January and reaching -47.1 °C in DAN in 2009. The summers are short with absolute temperatures exceeding 30 °C in all sites (Table 1). The warmest maximum temperatures were observed in 2002 in all sites. The temperature patterns were synchronous across the four study sites, with the coldest being DAN and BER, those located at the highest latitude and altitude, respectively. SIM was the warmest site both for annual and May-September temperatures (Supplementary data Fig. S1). The hottest year was 2010, which globally showed the highest annual temperatures, although high May-September maximum temperatures were also observed during 2005. The lowest maximum and minimum temperatures were detected in 2004.

The regressions indicated correlations between measured and modeled temperatures with R square varying between 0.56 and 0.94 (Supplementary data Table S1). The stronger relationships were found for the annual maximum temperature, which on average showed an R square of 0.93. SIM exhibited the lowest R square while DAN and MIS were the sites with the higher R square for both the annual and May-September temperature. Overall, statistics confirmed that modeled data could represent the temperatures occurring in the four study sites during 1950-2001.

The positive slopes of the regressions performed on the temperature series for the period 1950-2010 indicated an increasing trend of both annual and May-September temperatures, although all models showed $p>0.05$ (Fig. 1). The highest slopes were observed for maximum temperatures of May-September with increases of 0.04-0.28 °C decade^{-1}. Overall, lower slopes were estimated for annual temperature than for May-September temperature except for the minimum temperature in DAN and MIS. A clear pattern of change in the slopes with latitude was noticeable, with the greater increases in maximum temperature being observed at the higher latitudes, although this pattern was less obvious for minimum temperature.

The deviation from the 60-years average was calculated for the temperature series (Supplementary data Fig. S2). The 1960s were characterized by below-average values of both minimum and maximum temperatures of May-September. However, a similar pattern was not detected for the annual temperature. From 1970 to 1998, values were located around the average. After those years, both annual and May-September temperatures were clearly above the historical average, with the greater deviations for the maximum temperature of May-September.

Model definition and validation

At the four sites, xylem growth lasted between 80 and 133 days, with SIM having the longest duration (Fig. 2). Overall, the onset of xylem growth occurred from mid-May to mid-June (DOY 139-166), covering a range of c.a. one month. Later onsets of xylogenesis were detected in 2002 and 2009 and at the highest altitude and latitude, in BER and DAN, respectively. The ending of xylem growth differed by more than one month between the end of August in BER, MIS and DAN, and the beginning of October in SIM.

On average, the logistic regressions assessed the temperature thresholds at 4.4 and 15.5 °C for minimum and maximum temperature, respectively (Table 2). For a temperature above the calculated thresholds, xylem growth was more likely to be active than non-active. Although MIS had slightly higher values compared with the other sites, no significant difference was detected by ANOVA for either minimum or maximum temperature ($P>0.05$, Table 2).

The results generated by the logistic regressions were verified by forecasting the presence or absence of xylem growth in the study sites and comparing results by means of a contingency table (Table 3). Overall, observations showed that xylem growth was active during about one third of the year and not active during 72.3% of the year. For minimum and maximum temperatures, 94.9 and 95.0% of the predictions were confirmed by observations, respectively, confirming that the model with both temperatures produced reliable estimations of the thresholds and suitably predicted timings of the phenological phases of xylem. On average, not active and active xylem growth was correctly predicted for 69.0 and 25.9% of days, respectively, while the predictions were not confirmed for only 1.5–3.1% of days.

Model application

The model generated using the threshold temperatures was used for estimating chronologies of cambium phenology (onset, ending and duration of xylogenesis) for the period 1950-2010 (Fig. 3). Long-term trends of all series were tested using ANCOVA, which calculated models with R square ranging between 0.36 and 0.60 and significance $p<0.0001$ (Table 4). Predictions of the models using both minimum and maximum temperatures showed significant negative trends in all sites ($p<0.05$), revealing an earlier onset of xylogenesis that was estimated by the slope of the regression at 0.5-0.8 days decade^{-1}. Significant slopes for the ending of xylogenesis were detected only with the model using minimum temperatures, which indicated a delay of 0.7 days decade^{-1} (Table 4). Longer durations of xylogenesis were predicted for the period 1950-2010, with the higher (1.5 days decade^{-1}) and lower (0.07 days decade^{-1}) increases estimated by the model using minimum and maximum temperatures, respectively. Significant differences among sites were found for all phenological models ($P<0.0001$, Table 4), with SIM having earlier onsets, later endings and longer durations of xylogenesis over all chronologies (Fig. 3). Differences in cambial phenology among BER, MIS and DAN were less marked. No significant interaction year×site was observed by ANCOVA ($p>0.05$, Table 4).

DISCUSSION

Compared with the primary meristems such as leaves and buds, analyses on the phenology of the secondary meristem have only been emerging in the last decade. At the time of writing, the chronologies of cambial phenology deriving from direct observations are spatially and temporally fragmented (Rossi *et al.*, 2008b; Moser *et al.*, 2010; Rathgeber *et al.*, 2011; Swidrak *et al.*, 2011), which prevents the understanding of long-term responses of the cambium to environmental changes and any interpretation of the trends over time (Sparks and Menzel, 2002). This paper thus aimed to reconstruct timings of cambium phenology of black spruce over the last 60 years to test the hypothesis that duration of xylogenesis has lengthened since 1950. In the boreal forest, wood formation is restricted within 3-4 months by thermal limits that characterize the change between favourable and unfavourable periods for growth. In spring, cambial reactivation occurs from mid-May to mid-June, when the temperatures allow snow to melt and soil to warm up (Rossi *et al.*, 2011a). Mature xylem is observed in September, when all cells have concluded differentiation, which corresponds to nighttime temperatures of 4-5 °C, and maximum temperatures reaching 15-16 °C. For the first time, long-term chronologies of cambial phenology are provided, which allows investigation of the impact of past increases in temperature on wood formation. The dynamics and periods of xylem growth and their eventual changes over time are of particular interest for the global carbon budget as they define the period of main biomass accumulation in wood, during which trees act as an important sink of the carbon sequestrated from the atmosphere. An extending period of tree growth associated with warmer temperature could allow cambial cells to divide more vigorously and longer, thus producing wider tree rings and greater amounts of wood.

Trends of temperature and phenology

The chronologies generated by the ANUSPLIN model (McKenney *et al.*, 2006) for the four study sites exhibited increasing trends of both annual and May-September temperatures between 1950 and 2010. In some cases, the modeled climatic data were only partially correlated with measurements, and this may be explained by the remote location of the sites and by the scarcity of nearby weather stations used for the climatic modeling. The greatest changes were observed for maximum temperature at the higher latitudes, attaining increases of up to 0.28 °C decade^{-1} in DAN, the most northern site. For a similar period (1950-1998), Zhang *et al.* (2000) estimated that the higher increases in temperature for this region occurred in summer, with values ranging between 1.0 and 1.5 °C. Plummer *et al.* (2006) showed different trends for Quebec, with temperature rising by up to 6 °C in all seasons except in early spring, before the observed onset of xylogenesis. Our temperature chronologies exhibited a higher warming than the estimations of Zhang *et al.* (2000) and were more conservative than those of Plummer *et al.* (2006).

Changes in phenology of the primary meristems represents one of the best-documented effects of climate change on plants, with results converging toward increases in length of the growing season (Zhou *et al.*, 2001; Sparks and Menzel, 2002; Badeck *et al.*, 2004). In Canada, Beaubien and Freeland (2000) reported that the first flowering date of aspen poplar showed a marked trend of earlier flowering with an advance of 26 days over the period 1900-1997. For the northern hemisphere, Schwartz *et al.* (2006) estimated that during 1955-2002 the dates of first leaf and first bloom have been 1.2 and 1.0 days decade^{-1} earlier. Greater advances of 2.1 days decade^{-1} were calculated between 1951 and 1996 for leaf unfolding in Europe (Menzel, 2000). Despite similar lengths of the study periods, the results vary widely, which could be explained by the complex origin (observations or estimations) and nature (leaf or flower buds) of the datasets and species over the broad spatial scales of analysis. Moreover, a high heterogeneity in change of temperature has been observed across North America, with lower effects of warming occurring in the eastern part of the continent, where this work was carried out (Schwartz *et al.,* 2006). Overall, the hypothesis that duration of xylogenesis has lengthened over the last 60 years has been confirmed, although the estimated increasing trends of cambial phenology exhibited lower slopes than those observed in the primary meristems.

Does phenological cascade prevent estimating the end of xylogenesis?

In Europe, the beginning of the growing season has advanced by 2.7 days decade^{-1} in the last 30 years, while its ending showed smaller annual variations and has occurred just 1 day decade^{-1} later (Chmielewski and Rötzer, 2001). Sparks and Menzel (2002) definitely confirmed that earlier events were more variable and changed faster than later events. This was consistent with our results, which showed more marked changes in the spring onset than in late-summer ending of xylogenesis, and contrasting slopes and significances were observed over the study period from the trends of ending of xylogenesis. During development, the cambial derivatives (i.e. the cells produced by cambial division) alter both morphologically and physiologically, progressively assuming definite features. In other words, cells differentiate into the specific elements of the stem tissues, represented by the phases of enlargement, wall thickening and lignification. Investigations into xylem phenology and climate-growth relationships have focused mainly on the onset of the growth process, i.e. onset of xylem production or differentiation, while the end of growth still remains partly or completely unexplored (Gričar *et al.,* 2007; Rossi *et al.,* 2007; Seo *et al.,* 2008; Turcotte *et al.,* 2009). According to our findings, this could essentially be due to a greater number of significant responses being obtained between onset of growth and climate rather than a mere lack of interest in the final phases of the growth process (Hänninen and Tanino, 2011).

In cold environments, cell production is closely related to xylem phenology (Lupi *et al.*, 2010; Rossi *et al.*, 2012). The date of onset of xylogenesis affects the number of cells produced by the cambium which, in turn, influences the ending of cell differentiation. As a result, earlier cambial resumptions lengthen the period available for cell division in the secondary meristem, increasing the growth potential during the year (Gričar *et al.*, 2005; Deslauriers *et al.*, 2008). In conifers, wider tree rings (i.e. with higher amount of cells) require a longer period for differentiating and maturing the tracheids, which delays the ending of wood formation. Thus, any environmental factor affecting the resumption of growth in spring could indirectly influence the production and temporal dynamics of cell differentiation by affecting all successive phenological phases of xylem (Rossi *et al.*, 2006b). The hypothesis of an indirect effect of environment on the chain of phenological events in the xylem provides valuable cues for identifying the relative importance of the factors affecting timings and dynamics of xylem growth, and makes the relationships between the temperatures occurring in late-summer and the date of ending of xylem growth more complex.

Model and thermal predictors of xylogenesis

Several methods have been applied to investigate plant growth and its changes over time. Definitions of the growing season differ according to plant species, and are calculated in different ways, either directly (bud or cambial phenology), or indirectly (thermal sums, days with air and soil temperatures above certain thresholds, freezing days) (Nizinski and Saugier, 1988; Körner and Paulsen, 2004; Schwartz *et al.*, 2006; Seo *et al.*, 2008). In our reconstruction of past cambial phenology, the applied temperature thresholds were not defined a priori. They were instead statistically assessed on a wide dataset of observations collected weekly from four permanent plots over nine years, by defining a binary response of presence or absence of growth, and modeling the logistic response probability according to a vector of explanatory variable, either minimum or maximum temperature. The resulting temperature thresholds have the advantage of being objectively assessed and statistically validated, and are as close as possible to the biological limits of the growth process in the stem. However, the definition of the model assumed a linear response of cambial phenology to temperature, which is expected to occur only for narrow thermal ranges.

In this study, the minimum and maximum temperature thresholds allowing xylogenesis ranged between 4-5 °C and 15-16 °C, respectively, which confirm previous findings from other conifer species of cold climates (Rossi *et al.*, 2008b; Swidrak *et al.*, 2011) but contrast with the hypothesis of a cumulated effect of temperatures for cambial resumption (i.e. heat sums, Seo *et al.*, 2008; Swidrak *et al.*, 2011). Cambium is a sink for carbohydrates, and its activity requires a continuous supply of energy in the form of sucrose which, for the first cells to be formed, is extracted from the storage tissues or produced by photosynthesis (Oribe *et al.*, 2003; Deslauriers *et al.*, 2009). During cell maturation, trees assign a large amount of carbon obtained from photosynthesis to the deposition of cellulose microfibrils in order to provide the developing cells with secondary walls. The thresholds estimated in this paper could represent the critical temperatures limiting the demand for photo-assimilates by the metabolic processes involved in cell growth. Moreover, as xylogenesis is the most important net accumulation of biomass in forest ecosystems, knowledge about the climatic factors on the verges of the growing season is crucial to determine the time window during which the carbon sequestrated by the atmosphere is permanently stocked in trees.

The models using maximum and minimum temperature produced similar results in terms of onset of xylogenesis, but calculated different endings, and hence different durations of xylogenesis for the last 60 years (Table 4). Both models provided equally reliable estimations of xylem phenology, which prevented a definitive choice of the most suitable model. Unlike photosynthesis that is able to maintain high assimilation rates even at temperatures below 5 °C, xylem formation necessitates large amounts of available sucrose to be allocated in the growing tissues to complete growth, which is a temperature-limited process (Körner, 2003a; Deslauriers *et al.,* 2009). Cell doubling time remains quite constant at temperatures of 10−25 °C, but triples when temperatures fall from 10 to 5 °C, and cell division stops at 1−2 °C (Körner, 2003b). Moreover, comparing the daily growth responses of conifers to maximum and minimum temperatures, the better results were observed with the latter (Deslauriers and Morin, 2005; Wei *et al.,* 2007, Rossi *et al.,* 2008b). However, the effects of the two variables have still not been experimentally and definitively disentangled, and which temperature mostly controls the length of the growing period remains an unresolved question.

CONCLUSIONS

This paper used the phenological model developed by Rossi *et al.* (2011b) and weekly observations performed for 9 years in four permanent sites in Quebec, Canada to reconstruct the timings of cambium phenology over the last 60 years. All sites exhibited increasing trends of both annual and May-September temperatures, with the greatest changes occurring for maximum temperature at the higher latitudes. Accordingly, earlier cambial resumptions by 0.5-0.8 days decade^{-1} were estimated, while significant delays for the ending of xylogenesis were calculated only with the model using minimum temperatures. Phenological events in spring were confirmed to be more variable and changing faster than those occurring in autumn. Results confirmed the initial hypothesis that duration of xylogenesis has lengthened since 1950. However, contrasting extents were observed, ranging between 0.07 and 1.5 days decade^{-1}, calculated with the model based on maximum and minimum temperatures, respectively. To our knowledge, this is the first time that past cambial phenology has been modeled and reconstructed.

Consistent with the findings reported for leaf and flower buds, changes in cambial phenology showed increasing trends in length of the growing season, demonstrating the effects of the recent global warming on secondary meristems of trees. If the observed trend is maintained unaltered in the long run, the demonstrated advancement of cambial activity could dramatically modify the short time window for growth of the boreal species and markedly affect cell production of the secondary meristem. The findings of this work revealed that long-term increases in temperature can substantially extend wood formation and, consequently, the dynamics and productivity of cold ecosystems, by removing the thermal constraints to the activity of carbon sinks in trees.

However, evidence of these trends in forest ecosystem productivity requires to be confirmed by further specific investigations.

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FIGURE LEGENDS

Fig. 1 Temperatures during 1950-2010 in the four sampling sites (graphics with different scales). Gray lines correspond to linear regression analyses

Fig. 2: Cambial phenology in black spruce during 2002-2010 in the boreal forest of Quebec, Canada.

Fig. 3 Estimation of cambial phenology from period 1950-2010 (onset, ending and duration of xylogenesis). Lines correspond to regression analyses performed by ANCOVA

Table 1: Location of the four study sites listed in decreasing latitude and climatic characteristics measured during the period 2002-2010.

Site	Latitude	Longitude	Altitude (m a.s.l.)	Mean temperature		Absolute annual temperature	
				Annual (°C)	May-September (°C)	Maximum (°C)	Minimum (°C)
DAN	50°41’N	72°11’ W	487	-0.9	11.0	34.2	-47.1
MIS	49°43’ N	71°56’ W	342	1.0	12.7	35.1	-42.4
BER	48°51’ N	70°20’ W	611	0.3	11.4	33.1	-39.8
SIM	48°13’ N	71°15’ W	338	2.0	13.3	35.7	-39.7

Table 2: ANOVA comparisons performed among the threshold temperatures occurring when the probability of xylem growth in black spruce being active was 0.5. The thresholds were estimated on a dataset of cambial phenology collected during 2002-2010 in the boreal forest of Quebec, Canada.

Threshold temperature (°C)	SIM	BER	MIS	DAN	*F*-value	*P*
Minimum	4.2±1.0	4.0±1.9	5.4±1.3	4.0±1.3	2.68	0.06
Maximum	15.1±0.7	15.2±1.4	16.3±1.4	15.4±1.4	1.64	0.19

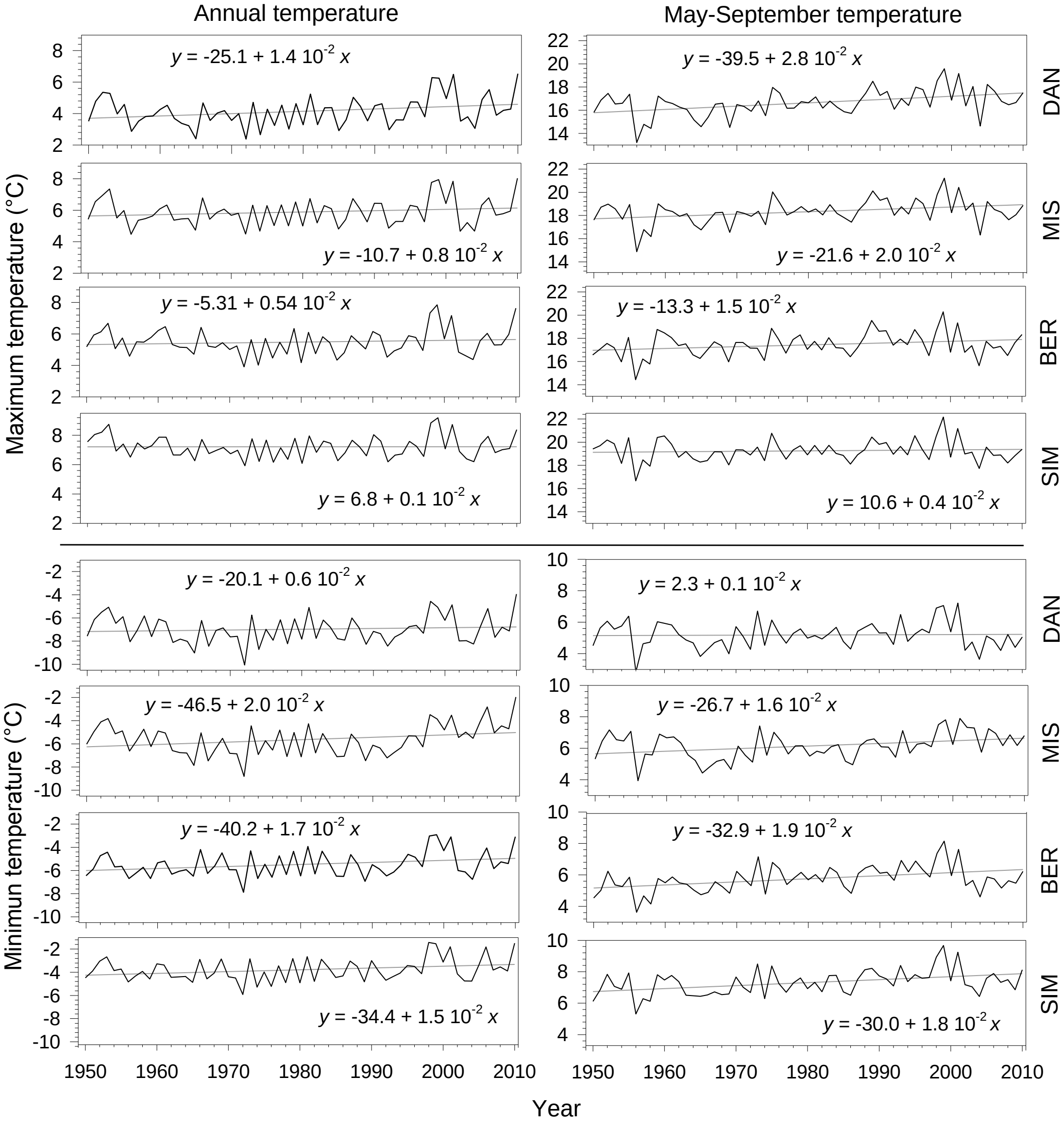
Table 3: Proportions of observed and predicted days with non-active (first and second row) or active (third and fourth row) xylem growth in black spruce. Predictions were obtained using the minimum and maximum temperatures estimated during 2002–2010 in the boreal forest of Quebec, Canada. The first and fourth rows correspond to the days correctly predicted by the model.

Observed xylogenesis	Predicted xylogenesis	Model using minimum temperatures (%)	Model using maximum temperatures (%)
No	No	69.2	68.8
No	Yes	3.1	3.5
Yes	No	2.0	1.5
Yes	Yes	25.7	26.2

Table 4: ANCOVA models comparing the chronologies of cambial phenology (onset, ending and duration of xylogenesis) of black spruce predicted for 1950-2010 using thresholds of minimum and maximum temperature. One, two and three asterisks indicate statistically significant differences at $p<0.05$, $p<0.001$ and $p<0.0001$, respectively.

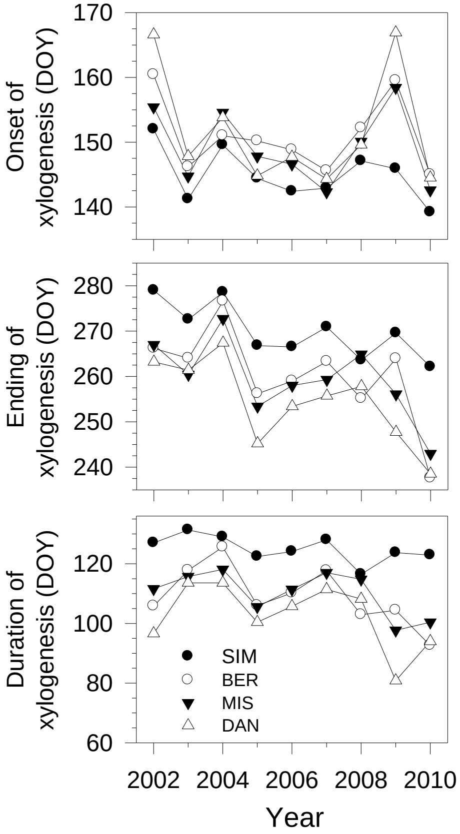
			Regressors		Statistics		Coefficients	
Xylogenesis	Temperature	Source	Type I SS	F-value	F-value	R square	Intercept	Slope (10^1)
Onset	Minimum	Year	219.9	5.6*	22.9***	0.40	307.9	-0.8
		Site	6000.0	51.1***				
		Year×site	64.2	0.5				
	Maximum	Year	713.5	14.3**	19.1***	0.36	237.5	-0.5
		Site	5852.0	39.1***				
		Year×site	100.8	0.6				
Ending	Minimum	Year	627.0	15.3**	23.6***	0.41	125.3	0.7
		Site	60303.2	49.0***				
		Year×site	125.8	1.0				
	Maximum	Year	36.8	0.9	32.3***	0.48	351.9	-0.4
		Site	8754.5	74.1***				
		Year×site	116.4	0.9				
Duration	Minimum	Year	1589.6	21.4***	49.3***	0.59	-182.6	1.5
		Site	23747.7	106.7***				
		Year×site	308.2	1.3				
	Maximum	Year	1074.9	13.0**	52.5***	0.60	114.4	0.07
		Site	28846.8	116.6***				
		Year×site	409.3	1.6				

Figure 1



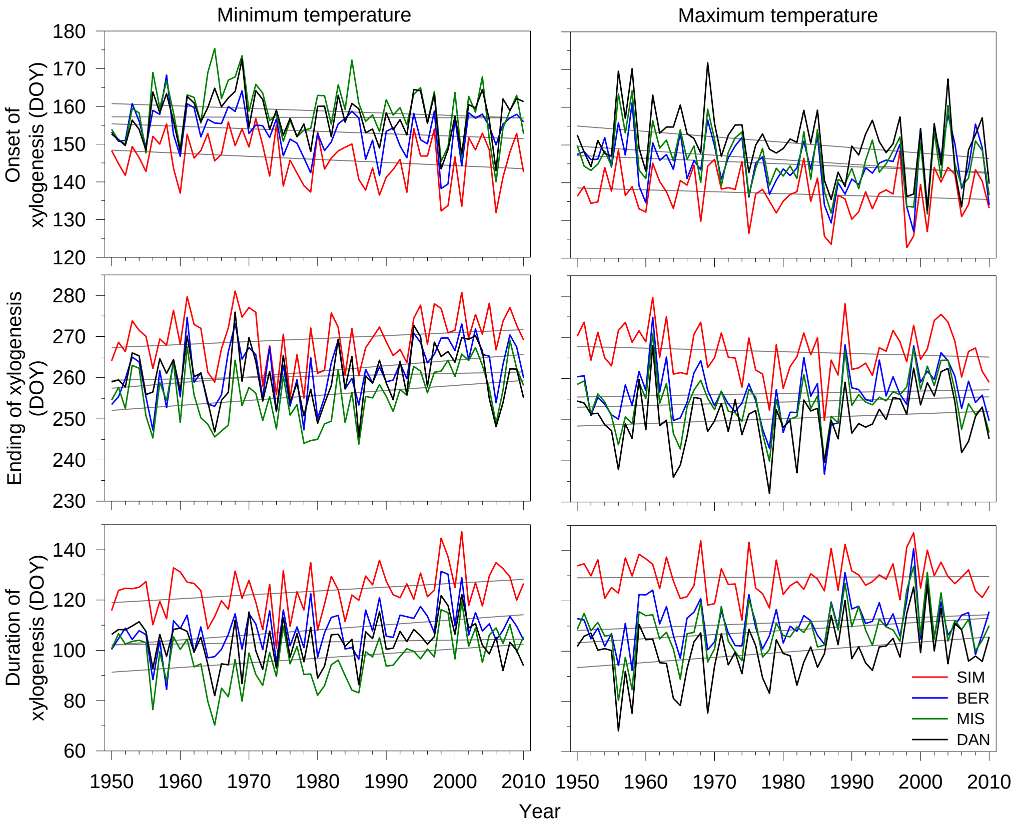
Annual temperature
May-September temperature
Maximum temperature (°C)
Minimun temperature (°C)
DAN
MIS
BER
SIM
DAN
MIS
BER
SIM
$y = -25.1 + 1.4\ 10^{-2}\ x$
$y = -39.5 + 2.8\ 10^{-2}\ x$
$y = -10.7 + 0.8\ 10^{-2}\ x$
$y = -21.6 + 2.0\ 10^{-2}\ x$
$y = -5.31 + 0.54\ 10^{-2}\ x$
$y = -13.3 + 1.5\ 10^{-2}\ x$
$y = 6.8 + 0.1\ 10^{-2}\ x$
$y = 10.6 + 0.4\ 10^{-2}\ x$
$y = -20.1 + 0.6\ 10^{-2}\ x$
$y = 2.3 + 0.1\ 10^{-2}\ x$
$y = -46.5 + 2.0\ 10^{-2}\ x$
$y = -26.7 + 1.6\ 10^{-2}\ x$
$y = -40.2 + 1.7\ 10^{-2}\ x$
$y = -32.9 + 1.9\ 10^{-2}\ x$
$y = -34.4 + 1.5\ 10^{-2}\ x$
$y = -30.0 + 1.8\ 10^{-2}\ x$
2
4
6
8
14
16
18
20
22
-10
-8
-6
-4
-2
4
6
8
10
1950
1960
1970
1980
1990
2000
2010
Year

Figure 2



170
160
150
140
Onset of xylogenesis (DOY)
280
270
260
250
240
Ending of xylogenesis (DOY)
120
100
80
60
Duration of xylogenesis (DOY)
● SIM
○ BER
▼ MIS
△ DAN
2002
2004
2006
2008
2010
Year

Figure 3


Minimum temperature
Maximum temperature
Onset of xylogenesis (DOY)
Ending of xylogenesis (DOY)
Duration of xylogenesis (DOY)
180
170
160
150
140
130
120
280
270
260
250
240
230
140
120
100
80
60
1950
1960
1970
1980
1990
2000
2010
Year
SIM
BER
MIS
DAN

SUPPLEMENTARY DATA

Fig. S1: Variation of the maximum and minimum temperature expressed as annual means and as the May-September period in the four sampling site (graphics with different scale).

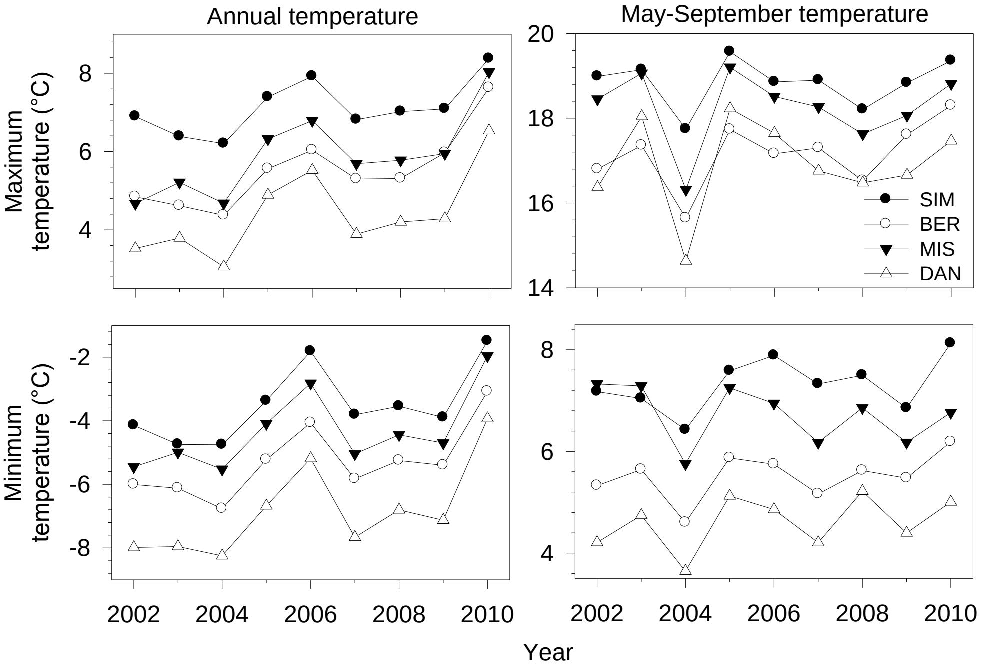


Table S1: Relationships in the form of linear regressions between measured and estimated temperatures in the four study sites for the overlapping years 2002-2004. All models were significant at p<0.0001.

Temperature	Site	Annual			May-September		
		Intercept	Slope	R square	Intercept	Slope	R square
Minimum	DAN	0.80	0.95	0.87	2.22	0.82	0.62
	MIS	-0.35	0.96	0.87	1.66	0.76	0.57
	BER	0.93	0.90	0.86	2.37	0.77	0.60
	SIM	0.82	0.90	0.86	2.95	0.73	0.56
Maximum	DAN	0.96	0.97	0.93	4.02	0.82	0.72
	MIS	0.87	0.99	0.94	3.76	0.85	0.71
	BER	0.81	0.97	0.93	3.82	0.81	0.69
	SIM	0.52	0.98	0.92	4.52	0.79	0.67

Fig. S2: Deviation from average of the period 1950-2010 in the form of difference between the actual temperature and historical mean.