UNIVERSITÉ DU QUÉBEC À CHICOUTIMI

ÉTUDE DE LA XYLOGÉNÈSE DE L'ÉPINETTE NOIRE (*picea mariana*) LE LONG D'UN GRADIENT LATITUDINAL

MÉMOIRE
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PAR
MARIE-JOSÉE GIRARD **B.SC.**

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INTRODUCTION GÉNÉRALE

La Terre a souvent été le siège de changement climatique cyclique, où se succèdent alternativement des périodes de réchauffement et refroidissement. Le renversement se produisant actuellement est différent des précédents, étant accéléré par les activités anthropiques. Différentes conséquences sont déjà observées telles qu'une augmentation de température, une hausse ou une baisse des précipitations selon les régions et une amplification des évènements météorologiques extrêmes. Ces modifications atmosphériques auront une incidence sur de nombreuses formes de vies. Les arbres, immobiles, doivent subvenir à leurs besoins en exploitant les éléments disponibles dans leur environnement immédiat. Pourront-ils s'adapter à ces changements? Si oui, de quelle façon?

La phénologie est la science qui étudie les manifestations cycliques chez les êtres vivants. Pour les végétaux des milieux tempérés, chaque année est constituée d'une longue période froide d'inactivité et d'une courte période chaude de croissance. Ces alternances de température dirigent le bourgeonnement, l'apparition des feuilles et des fleurs, la perte des feuilles, la production de bois et plusieurs autres étapes du développement de l'arbre. La facilité d'observation de ces différents évènements en fait des éléments de choix pour l'observation des changements climatiques et de leur incidence sur les arbres. D'ailleurs, des études font déjà état d'un devancement de certains évènements cycliques, comme le début de la croissance au printemps, en comparaison avec les années précédentes. La xylogénèse correspond à la production de bois par les cellules cambiales et est intimement reliée aux conditions climatiques. Elle devrait donc être touchée par les changements futurs.

Il est important d'établir un modèle entre les deux vecteurs d'une relation pour comprendre comment un changement de l'un produira un changement chez l'autre. Le moment de l'apparition d'un évènement cyclique, par exemple le réveil au printemps chez un arbre, est relié à la température par une relation mathématique. La relation peut être linéaire, ce qui implique qu'une augmentation de température produira un changement proportionnel dans la date d'apparition de l'évènement. Cependant, rare sont les relations qui sont parfaitement linéaire, car les êtres vivants ont des limites au-delà desquelles ils ne peuvent s'adapter. La relation sera alors non linéaire, signifiant qu'un changement de la variable indépendante produira une réponse non proportionnelle de la variable qui en dépend. Étant moins documentés que la relation linéaire, ce dernier modèle pourrait cependant être plus commun en milieu naturel. La connaissance des coefficients des équations rendrait alors possible d'extrapoler les dates clés dans l'espace et/ou le temps.

C'est dans une perspective de compréhension et d'approfondissement des connaissances que le projet suivant a été lancé. Les études précédentes effectuées par Hubert Morin ainsi que par Sergio Rossi avaient nécessité l'aménagement de quatre sites de types observationnels le long d'un gradient latitudinal du 48^{ième} au 50^{ième} parallèle nord dans la forêt boréale de la province de Québec au Canada. Simoncouche étant le plus au sud et Bernatchez, Mistassibi et Camp Daniel répartis vers le nord. Ces sites avaient été choisis pour la similarité de leur paramètre biotique tel que l'âge des arbres, la composition de la forêt, l'éloignement des activités humaines, la pente et le type de sol. Certains sites étaient le lieu de microcarottage depuis une dizaine d'années et les données indiquaient une production similaire de cellule pour les trois sites les plus au nord. Désirant savoir si la production cellulaire serait diminuée de façon importante dans un individu plus au nord un nouveau site, Mirage, a été installé en 2011 près du 53^{e} parallèle. Les prélèvements préliminaires ont soulevé des questions après qu'ils eurent indiqué que la production cellulaire n'était non pas moindre mais, semblable à celle des trois sites situés légèrement plus au sud, pourtant séparés par plus de 300 kilomètres.

L'épinette noire étant une espèce très importante pour l'économie québécoise, il est primordial de comprendre la relation entre sa productivité et son environnement, surtout dans un contexte de changement climatique. L'utilisation d'un gradient latitudinal a permis d'observer une même espèce dans des milieux sensiblement semblables, mais dont la température et la photopériode varient. De plus, les sites permettent l'observation de la xylogénèse de l'épinette noire en forêt fermée dans toute son aire de distribution latitudinale au Québec, ce qui est une première selon nos connaissances.

Le premier article avait comme sujet l'observation de la production cellulaire avec l'aide de micro-carottes durant une saison de croissance à l'intérieur de cinq sites. L'objectif étant de déterminer l'influence de la température sur la xylogénèse et ces phases. Nous avons posé l'hypothèse qu'avec l'augmentation de la température, le début de la croissance serait plus hâtif et la fin plus tardive menant ainsi à une durée allongée de la xylogénèse. La production cellulaire s'élèverait avec l'augmentation de la température. Il était attendu que les dates observés de la phénologie serait proportionnellement retardé ou devancé selon les changements de température.

Le deuxième article réutilise les données du premier pour établir des modèles dans le but de déterminer l'exacte relation entre les variables de la xylogénèse et la localisation du site. Ces modèles ont ensuite été extrapolés sur une carte du Québec couvrant l'aire couverte par l'étude permettant ainsi une visualisation directe des phases de la xylogénèse ainsi que de la production cellulaire qui pourrait être observée dans les différentes régions du Québec.

Article #1

LENGTHENING OF THE DURATION OF XYLOGENESIS ENGENDERS DISPROPORTIONATE INCREASES IN XYLEM PRODUCTION

Lengthening of the duration of xylogenesis engenders disproportionate increases in xylem production

Sergio Rossi*, Marie-Josée Girard*, Hubert Morin*

*Département des Sciences Fondamentales, Université du Québec à Chicoutimi, 555 Boulevard de l'Université, Chicoutimi (QC), G7H2B1, Canada

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Ma participation pour cet article consiste au prélèvement sur le terrain et à l'analyse en laboratoire des échantillons ainsi qu'à l'écriture d'une partie du texte.

Abstract

In cold climates, the expected global warming will lead to earlier cambial resumptions in spring, with a resultant lengthening of the growing season but unknown consequences on forest productivity. The phenological traits of cambium activity and xylem formation were analysed at a short time scale along a thermal gradient represented by an alti-latitudinal range from the 48th to 53rd parallels and covering the whole closed black-spruce [*Picea mariana* (Mill.) BSP] forest in Quebec, Canada. A hypothesis was tested that warmer temperatures influence cambium phenology, allowing longer duration and higher intensity of growth, and resulting in proportionally increased xylem production. From April to October 2012, cell division in cambium and post-cambial differentiation of xylem were observed on anatomical sections obtained from microcores collected weekly from the stem of fifty trees. The southern and warmer site was characterized by the highest radial growth, which corresponded to both the highest rates and longest durations of cell production. The differences in terms of xylem phenology and growth were marginal between the other sites. Xylem growth was positively correlated with rate and duration of cell production, with the latter explaining most variability in growth. Within the range analysed, the relationship between temperature and most phenological phases of xylogenesis was linear. On the contrary, temperature was related with cell production according to an exponential pattern. Periods of xylogenesis of 14 days longer (+13.1%) corresponded to a massive increase in cell production (33 cells, +109%). This disproportionate change occurred at a May-September average temperature of c.a. 14 °C and a snow-free period of 210-235 days. At the lower boundary of the distribution of black spruce, small environmental changes allowing marginal lengthening of the period of cell division could potentially lead to disproportionate increases in xylem cell production, with substantial consequences for the productivity of this boreal species.

Keywords: black spruce, boreal forest, climate warming, growth, *Picea mariana*, phenology, temperature, threshold

Introduction

In the past, the Earth has experienced a number of climate fluctuations. Glaciations and warmings succeeded one another and shaped the world as we know it today (IPCC, 2007). The 21st century is witnessing a new era of climate change and human activities seem to have accelerated the processes (Galloway, 2004; IPCC, 2007). Within the next 100 years, the temperature will increase by about 1.4 to 5.2 °C. According to IPCC (2007), warming could be more pronounced at northern latitudes, with dramatic consequences on the life forms of the boreal and arctic ecosystems. The boreal forest covers 25% of the planet's forest surface and constitutes a huge sink of carbon stored in form of biomass and organic matter, representing one of the most important ecosystems in the world (Burton *et al.*, 2010). It is therefore a priority to understand the effects of climate change on these forests to predict the potential future evolution and the ecological consequences over time.

Like most living organisms, trees follow recurrent phenological activities, such as flowering, fructification, growth resumption and cessation (Forrest & Miller-Rushing, 2010). One of these, xylogenesis, or wood production, occurs annually in trees of temperate and cold climates according to the cycles of summer and winter, producing a distinct pattern on the wood conferred by the tree rings. Plants are unable to escape the unfavourable periods of the year, so the meristems suspend their activity during winter by becoming dormant, a state that is maintained even if environmental requirements of temperature or day length are met. In late winter, a change occurs from dormancy to a new state, quiescence, when growth cannot take place unless the required environmentally-favourable conditions are present (Begum *et al.*, 2007). At high altitudes or latitudes, the resumption of cambial activity for wood formation is an event mostly driven by temperature, according to either a gradual influence or threshold effect (Rossi *et al.*, 2007; Seo *et al.*, 2008; Swidrak *et al.*, 2011). As a consequence, the warming provided by higher temperatures could allow the threshold or heat sum triggering growth resumption to be reached earlier in spring (Rossi *et al.*, 2011; Boulouf Lugo *et al.*, 2012). It is expected that the consequent longer growing season of cambium will result in increased growth, in terms of wood or forest productivity (Boisvenue & Running 2006; Lupi *et al.*, 2010). However, forecasting the effects of warming on tree growth remains problematic because of the complexity of the abiotic (climate) and biotic (xylogenesis) systems investigated.

The relationship between temperature and growth is the subject of a fervent debate on the mechanisms by which the former influences the latter (Körner, 1998). Given the harsh environmental conditions and the short thermally-favourable season, it is not surprising that temperature is one of the key ecological factors controlling growth in boreal ecosystems. However, although numerous hypotheses have been proposed and discussed (Stevens & Fox 1991; Körner, 1998; Sveinbjörnsson, 2000), neither the control mechanisms (e.g. gradual influences versus threshold effects) nor the physiological processes (e.g. carbon assimilation versus allocation) involved have yet been clearly and definitively demonstrated. The growth is the result of cell division during the activity of the meristems that occurs for a precise period of time. Thus, increased growth may be the result of a longer growing season, or a higher growth intensity, or both (Rathgeber *et al.*, 2011; Rossi *et al.*, 2014).

This paper aims to identify the components of growth influenced by temperature by analysing the phenological traits of cambium activity and xylogenesis in a typical boreal species. Five sites were selected along a thermal gradient represented by an alti-latitudinal range covering the whole closed black-spruce [*Picea mariana* (Mill.) BSP] forest in Quebec, Canada, which to our knowledge provided the widest and most detailed range of cambial phenology and growth currently available. A hypothesis was tested that warmer temperatures influence the cambium phenology, allowing longer durations and higher intensity of growth, and resulting in increased cell production.

Material and methods

Study area and tree selection

The study was conducted in the boreal forest of Quebec, Canada, where five permanent sites [Simoncouche (abbreviated as SIM), Bernatchez (BER), Mistassibi (MIS), Camp Daniel (DAN) and Mirage (MIR)] were selected in mature even-aged black spruce stands at different altitudes and latitudes (Table 1). In each site, ten dominant or co-dominant 120-140-year-old trees with upright stems were chosen. Trees with polycormic stems, partially dead crowns, reaction wood or evident damage due to parasites were avoided. The height of the selected trees ranged between 13.1 and 18.3 m and decreased at increasing latitudes. No trend was observed for tree diameter along latitude or altitude (Table 1).

Xylem sampling and preparation

Tree-ring formation was studied from April to October 2012. 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 Trephor (Rossi *et al.*, 2006a). Trephor is chisel-shaped tool for a fast recovery of 2 mm diameter microcores. Its cutting tube is hammered into the wood, and the wood sample is separated from the xylem by rotating and extracting the tool like a corkscrew. The very small wounds inflicted by the thin piercing tubes of the tool and the consequently narrow areas of traumatized tissues around the sampling points allowed repeated samplings by microcore extraction (Forster *et al.*, 2000). Samples usually contained the recently formed tree rings and the developing annual layer with the cambial zone and adjacent phloem. Samples were always taken at least 5 cm apart to avoid getting resin ducts on adjacent cores.

The microcores were placed in Eppendorf microtubes with an ethanol solution (10% in water) and stored at 5 °C to avoid tissue deterioration. Microcores were dehydrated with successive 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. The sections were 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.

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 4-5 closely-spaced cells. At the onset of cambial activity, the cambial zone began to widen rapidly (within a week) 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 the zones of enlarging and cell wall thickening of tracheids. Because of the arrangement of cellulose microfibrils, the developing secondary walls shone when observed under polarized light. Instead, no glistening was observed in enlargement zones where the cells were still composed of primary wall (Abe *et al.*, 1997). The progress of cell wall lignification was detected with cresyl violet acetate reacting with the lignin (Rossi *et al.*, 2006b). Lignification was shown by a colour change from violet to blue. The colour 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 horizontal row of cells was observed in 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. Cambium phenology was computed in day of the year (DOY) corresponding to the dates of (1) first enlarging cell, (2) first wall-thickening cell, (3) first mature cell, (4) ending of cell enlargement, and (5) ending of cell wall lignification. The duration of xylogenesis was assessed as the number of days occurring between the onset of cell enlargement and the ending of cell wall lignification. The duration of cell production was assessed according to the phase of cell enlargement rather than on cell division in the cambial zone because (i) cambium can be active without necessarily increasing its cell number, (ii) cambium produces indistinct xylem and phloem cells, which are identical before undergoing differentiation; (iii) onset and ending of cell division and enlargement occur at approximately the same time, generally within one week (Gričar *et al.*, 2009; Rathgeber *et al.*, 2011).

Weather stations

At each site, a standard weather station was installed in a forest gap to measure air temperature, precipitation and snow depth. Snow depth was measured with an acoustic distance sensor that quantifies the elapsed time between emission and return of an ultrasonic pulse and automatically corrects for variations of the speed of sound during the year using the measurements of air temperature. Data were collected every 15 minutes and recorded as averages every hour by means of CR10X dataloggers (Campbell Scientific Corporation). Daily mean values were later calculated with the time series obtained from the 24 measurements per day. Annual statistics were calculated from October 2011 to September 2012, the period with available data for all weather stations.

Statistical analyses

The increase in the total number of xylem cells during the year was modelled for each tree with a Gompertz function [NLIN procedure in SAS 9.2 (SAS Institute Inc., Cary, NC)] defined as

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

where y is weekly cumulative sum of cells, t the time computed in DOY, A the upper asymptote, β the x-axis placement parameter and κ the rate of change of the shape. The resulting coefficients allowed the final radial number of cells produced by cambium to be estimated, represented by the upper asymptote, and the rate of cell production, calculated as $\kappa A/\mathrm{e}$ (Rathgeber *et al.*, 2011).

A multiple regression was performed to assess the relationships between duration and rate of cell production and the radial number of cells. The distribution of the dependent variable diverged significantly from normality and data were transformed using the common logarithm. The specific contribution of the two independent variables was determined by sensitivity analysis. This analysis consisted of computing the impact of the relative variations of the input variables (on the basis of their standard deviations) on the results of the model (Cariboni *et al.*, 2007).

Cambium phenology and cell production were regressed on the weather variables measured in the sites using simple regressions. The linearity of the relationships were verified on the resulting plots of studentized residuals.

Results

Climatic characteristics of the sites

The sites have a typical boreal climate with very cold winters and cool summers. The mean temperature in the study year varied between -1.6 and 4.1 °C, with the highest values recorded in SIM (Table 2). All sites had a winter with temperatures close to or below zero for a long period, attaining a minimum of between -29.8 and -47.1 °C. Summers were generally short with absolute maximum temperatures reaching 32.1 °C in MIS. The temperature changed according to latitude and altitude, with the sites located at the higher altitudes being the coldest in winter and the least warm in summer (Table 2). During May-September, the mean temperature varied between 11.1 and 14.6 °C, and 411.5-675.9 mm of rain were recorded, with MIS being the rainiest site. In winter, snow depth reached 130 cm in DAN. In spring, abrupt increases in soil temperature were observed after the complete snowmelt, with the organic layer showing May-September temperatures of 7.6-10.1 °C. In summer, the temperature of the two soil layers in SIM was similar because of the shallow depth of the organic matter.

Dynamics of xylem formation

The sites showed similar dynamics of xylem formation. In spring and autumn, when no cell production occurred, the dormant cambium was constituted of 4-5 cells. In spring, the number of cells in the cambial zone increased to 6-10, showing the onset of cell division. The first enlarging cells, corresponding to the onset of xylem differentiation, were observed from mid-May in SIM and MIS but only later at the higher latitudes and altitudes (Fig. 2a). Timings of the first wall-thickening and mature cells followed a similar trend to that of enlargement (Fig. 2c,e).

The ending of cell enlargement occurred between mid-July and mid-August (DOY 191-228) in all sites except SIM, where it was observed later, between mid-August and the beginning of September (DOY 220-249) (Fig. 2g). The last cells in cell wall lignification, which corresponded to the ending of xylem differentiation, were observed between mid-August and mid-October, with MIR and DAN being the earliest sites to complete differentiation (Fig. 2i). Duration of cell production was similar between sites except for SIM, where it was markedly longer (Fig. 2b). The period required for completing cell wall thickening and that for the whole process of xylogenesis showed durations that decreased with latitude (Fig. 2d,f).

Overall, trees produced between 0.2 and 1.1 cells day^{-1}, resulting in an average of 22.4 xylem cells along a radial file (Fig. 2h). The rate of cell production differed between and within sites, particularly in SIM and BER, where the highest variations were observed. SIM was characterized by the highest cell productions, which corresponded to both the highest production rates and the longest durations of differentiation, while the differences between the other sites were marginal (Fig. 2j).

Duration vs. rate of cell production

Multiple regression adequately fitted the data and produced a reliable model (F=51.48, $p<0.0001$) with a high variance accounted for, corresponding to an R^2 of 0.70. Intercept and duration of cell production were highly significant ($p<0.0001$), while the estimated coefficient for the rate of cell production was not significant ($p=0.26$) (Table 3). The analysis was performed with log-transformed data, thus the relation was clearly non-linear, as also revealed by the resulting prediction of the model (Fig. 3). The radial number of cells produced at the end of the season was positively correlated with duration and rate of cell production. However, the greatest increases were observed with cell productions lasting more than 70 days according to an exponential pattern. The rate of cell production only marginally affected the radial number of cells and essentially at the longer durations of cell production. These observations were confirmed by the sensitivity analysis of the model, which estimated the effects of the duration of cell production and rate of cell production at 86 and 14%, respectively. Thus, the radial number of cells was substantially more sensitive to the former variable and only marginally sensitive to the rate of cell production.

Phenology vs. temperature

Phenology and cell production were compared with the May-September temperatures measured in the sites, which represented the thermal gradient better than the alti-latitudinal coordinates. Dates of onset of cell differentiation (first enlarging [Fig. 4a-c] and first wall-thickening [Fig. 4d-f] cell) and first mature cell (Fig. 4g-i) became gradually earlier at increasing air temperatures. This indicated the phenological traits occurred earlier in the warmer sites according to a relationship that appeared approximately linear. A similar pattern, but with opposite slope, was observed for the end of lignification, which was completed later in the warmer sites (Fig. 4m-o). The linearity of the relationships were confirmed by the linear regressions and subsequent diagnostic tests (Fig. S1). For these variables, R^2 represented satisfying values of between 0.42 and 0.60. The studentized residuals showed scatter of points homoscedastic and with a normal distribution of errors, indicating that the regressions fitted adequately the datasets. The ending of cell enlargement differed from the other phenological traits, with four sites showing comparable values within the range between DOY 195 and 215, and one site completing enlargement between DOY 220 and 245 (Fig. 4j-l). This pattern markedly non-linear was detected by the low R^2 comprised between 0.10 and 0.45. Moreover, the regression models systematically underestimated cell enlargement at low and high temperatures, as exhibited by the residual plots, indicating a quadratic of exponential relationship (Fig. S1).

The durations of cell-wall thickening (Fig. 5e-h) and xylogenesis (Fig. 5i-l) gradually increased with May-September temperatures and the snow-free period according to linear patterns. The diagnostic tests of regressions confirmed the linearity of trends (Fig. S2). A different pattern was observed for the periods of cell production (Fig. 5a-d), which were similar at an average temperature lower than 14 °C (Fig. 5a). SIM, the warmer site and with a longer snow-free period, revealed markedly longer durations of cell production and larger amounts of cells in the xylem (Fig. 5q-t), always associated with a higher variability among trees. The lack in linearity was detected by both R^2 and residual plots for the variables duration and rate of cell production and radial number of cells (Fig. S2).

Discussion

Linear and nonlinear growth patterns

This study analysed the occurrence of cambium activity and xylogenesis to assess their changes along a wide thermal gradient. Sites showed similar dynamics of wood formation but with different timings of the phenological phases. As expected, warmer sites showed earlier onset and later ending of growth, resulting in longer durations and higher intensity of cell production. However, the wide gradient analysed revealed a number of non-linear trends, with relationships between phenology, cell production and air temperature following markedly exponential patterns. Linear models are the most widely known and applied methods to quantify changes in nature, and have been demonstrated to be a valid representation of several processes, or at least parts of them (Sparks *et al.*, 2009). However, linear patterns are a special case. Commonly, biological systems follow more complex, nonlinear, or non-monotonic (reversed) relationships (Burkett *et al.*, 2005; Porter & Semenov, 2005). According to Sparks *et al.* (2000), the relationships may be, or appear to be, approximately linear within a central, bounded interval of variation, but with marked deviations from linearity at the extreme margins of the range, as repeatedly observed in the warmer site.

Wood production is generally considered as a single growth process. On the contrary, the formation of xylem cells is the result of different biochemical processes underlying the sequential stages of cell division and maturation that are represented by the observed phenological traits (Prislan *et al.*, 2009). It is well known that a reduction in the period and amount of xylem production along the thermal gradient is associated with both later resumptions of growth in spring and earlier conclusions of xylem differentiation in autumn (Rossi *et al.*, 2007; Moser *et al.*, 2010). This study revealed at a fine temporal detail that air temperature differently affected duration and amount of growth: the phenology of xylem, in terms of beginning and ending of cell differentiation, gradually varied according to linear, or approximately-linear, patterns. In contrast, the duration of cell production and number of xylem cells in the annual tree-ring had an exponential pattern. Comparing the two warmer sites, SIM and MIS, an earlier growth resumption leading to a period of xylogenesis of 14 days longer (+13.1%) corresponded to a massive increase in cell production (33 cells, +109%). This disproportionate change occurred at a May-September average temperature of between 13.5 and 14.5 °C and a snow-free period of between 210-235 days.

Growth patterns and temperature

Heating experiments carried out in early spring induced localized reactivation of the cambium (Oribe *et al.*, 2001; Gričar *et al.*, 2006), demonstrating that cambial cells are highly responsive to temperature. The local application of high temperatures in stems of *Picea abies* affects the rate of cell division in cambium at the beginning of the growing season, although no effect was observed in the second part of the season, indicating that the influence of temperature on cambial activity varies during the year and is stronger in early spring (Gričar *et al.*, 2007). Also, heated trees showed higher amounts of tracheids in the xylem and earlier beginning of cell differentiation than control trees. Similar results have been observed at the treeline in southern Italy, where onset and duration of cell production and differentiation of *Pinus leucodermis* were drastically affected by the hot spring that Europe has experienced in 2003 (Deslauriers *et al.*, 2008).

Living organisms experience limiting factors that generate shifts and discontinuities, or contain thresholds beyond which responses change abruptly (Stenseth & Mysterud, 2002; Körner, 2003). Disproportionate responses of biological systems can be triggered by the coexistence or interaction of factors that result in cumulative effects (Burkett *et al.*, 2005; Körner & Basler, 2010). On one hand, the earlier cambial resumption in warmer sites allows more time for cambial cells to divide and produce new tracheids (Rossi *et al.*, 2012). On the other, in cold environments the higher temperatures speed up cell production once dormancy has broken, leading to higher rates of cell division being achieved. The relationship between cell division and temperature is non-linear. Körner (2003) reported that cell doubling time was quite constant at temperatures of 10–25 °C, but increased exponentially when the temperature fell from 10 to 5 °C, and approached infinity at 1– 2 °C. We observed that both factors, duration and intensity of growth, played a role in xylem cell production of black spruce, although the importance of the latter (rate of cell production) was marginal in respect to the former (duration). These results agreed with those reported by Lupi *et al.* (2010) for the same species, but were in contrast with other observations (Rathgeber *et al.*, 2011; Cuny *et al.*, 2012), which referred to different species and environmental conditions. To date, the scarcity of information on the timings and mechanisms explaining the variability in cell production prevents the question being resolved and a definitive conclusion being reached on this matter.

Xylogenesis has previously been investigated along the altitudinal range of distribution of European larch in the Alps (Moser *et al.*, 2010). Compared to the treeline (2150 m a.s.l.), the growing season of the stem was observed to be gradually delayed at intermediate altitudes (1660-1900 m a.s.l.). However, at the lowest level of species distribution (1350 m a.s.l.), this pattern changed abruptly, with an earlier ending of growth and shorter period of xylogenesis that resulted in a marked non-monotonic trend. Such a trend in the period of growth with altitude corresponded to similar reversed trends in cell production, probably associated to water stress at the lower altitudes due to the warmer summer temperatures and lower amount of precipitation (Moser *et al.*, 2010). Although black spruce occurs in mixed or open stands over a very wide area between the 40th and 60th parallels in North-eastern North-America, the sites considered in this work regarded only the latitudinal distribution of the closed boreal forest (Viereck & Johnson, 1990). At these latitudes, a significant amount of the precipitation falls as rain in the summer, allowing a constant water availability during the growing season even at the lower latitudes (Boulouf Lugo *et al.*, 2012). The low temperatures associated to the characteristics of the parent material lead to the formation of cold and only-partially permeable soils, which easily maintain the water over time. Thus, no effect of precipitation has been observed on the xylem features of black spruce trees in the studied area, and the occurrence of drought stresses significantly affecting xylem growth appears to be unlikely (Krause *et al.*, 2010, Belien *et al.*, 2012).

The range of localization of the study sites exceeded 5° in latitude and entailed a substantial, although not exceptional, gradient in day length. At the latitudes at which the monitoring was carried out, the reactivation of cambial activity corresponded to an approximate duration of daylight of between 15 to 17 h, in SIM and MIR, respectively. Because of the long-lasting winter and cold spring, the short period of cell production of boreal species begins when photoperiod is close to its culmination and ends at daylights of 14-16 h, thus exploiting the time window when photoperiod is longer. Previous studies have demonstrated that trees concentrate xylem cell division in the first part of the growing season, synchronizing cambial activity with day length, so that the rate of wood production culminates at the end of June, when photoperiod is longest (Heinrichs *et al.*, 2007). After that date, in July and early August, cell divisions have been observed to slow down (Rossi *et al.*, 2006b). By avoiding high cell productions during summer, trees guarantee enough time before winter for newly formed tracheids to complete their differentiation, particularly latewood cell wall formation and lignification. Day length is definitely recognized as key factor for bud phenology, but, at the time of writing, no effect has been demonstrated for the reactivation or conclusion of xylem growth. Accordingly, it could be concluded that the variation in phenology observed in this study is mainly due to the effect of the thermal gradient between the sites.

Growth patterns and climate change

In the last decades, long-term observations of primary meristems or reconstructions of xylem phenology have pointed out the occurrence of shifts in phenological events. Generally, the trends converge towards earlier growth resumptions and longer growing seasons, which have been ascribed to changes in the environment (Menzel *et al.*, 2006; Boulouf Lugo *et al.*, 2012, Anderson *et al.*, 2013). If the predictions of global warming are confirmed and the temperature continues to rise at the same rate, it is likely that further changes in plant phenology can be expected. Warmer temperatures in spring lead trees to achieve the thermal thresholds required for growth resumption earlier and allow a longer period for primary and secondary growth. In black spruce, longer durations of xylogenesis are estimated at higher temperatures, with increases of 8–11 days °C^{-1} and a lengthening of 25% with an increase of 3 °C in mean annual temperature (Rossi *et al.*, 2011). Körner & Basler (2010) affirmed that it is inappropriate to linearly extrapolate a few days of phenological advancement during warm years into a proportional lengthening of the growing season in climate warming scenarios. However, within the range reported in this paper, the relationship between temperature and most phenological phases of xylogenesis was clearly linear. On the contrary, temperature was related with cell production according to an exponential pattern. Accordingly, we observed that the changes in duration and amount of xylem growth are not proportional. Our results suggested that at the lower boundary of the distribution of the closed black-spruce forest, even slight warming allowing marginal lengthening of the period of cell division could potentially lead to disproportionate increases in xylem cell production. A higher xylem cell production results in a wider tree ring and greater growth in diameter of the stem. Moreover, warmer conditions allow trees to grow taller, as also observed by the height of the selected trees in this study. The cumulative effect of taller growth associated with larger tree rings could lead to substantial consequences for the productivity of these boreal stands.

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Table 1. Location of the five sites in the boreal forest of Quebec, Canada, listed at increasing latitude.

ID	Site	Latitude	Longitude	Altitude (m a.s.l.)
SIM	Simoncouche	48°13’	71°15’	338
BER	Bernatchez	48°51’	70°20’	611
MIS	Mistassibi	49°43’	71°56’	342
DAN	Camp Daniel	50°41’	72°11’	487
MIR	Mirage	53°47’	72°52’	384

Table 2. Weather at the five study sites in the boreal forest of Quebec, Canada. Annual statistics were calculated from October 2011 to September 2012.

	Annual temperature (°C)			May-September temperature (°C)		
ID	Mean air	Absolute maximum	Absolute minimum	Air	Organic layer	Mineral layer
SIM	4.1	31.0	-29.8	14.6	9.6	9.5
BER	1.8	29.6	-36.5	12.6	8.8	6.3
MIS	2.0	32.1	-36.9	13.6	10.1	8.4
DAN	0.0	30.6	-42.7	12.1	8.4	7.3
MIR	-1.6	30.0	-47.1	11.1	7.6	7.1

Table 3. Estimates of the regression analysis performed between two independent variables and $\log_{10}$ cell production.

Variable	Estimate	Standard error	t-value	P
Intercept	0.63	0.06	9.42	<0.0001
Duration of cell production (days)	$9.73\ 10^{-3}$	$1.38\ 10^{-3}$	7.06	<0.0001
Rate of cell production (cells day^{-1})	0.11	0.10	1.13	0.26

Caption list

Figure 1: Location of the five study sites in the boreal forest of Quebec, Canada.

Figure 2: Cambium phenology and cell production recorded in 2012 at the five study sites in the boreal forest of Quebec, Canada. Sites are listed at increasing latitude. Boxes represent upper and lower quartiles, whiskers achieve the 10th and 90th percentiles, and the median is drawn as horizontal solid line.

Figure 3: Variation of the radial number of cells estimated according to the duration and rate of cell production.

Figure 4: Cambium phenology vs. the May–September temperatures recorded in 2012 at the five study sites in the boreal forest of Quebec, Canada. Boxes represent upper and lower quartiles, whiskers achieve the 10th and 90th percentiles, and the median is drawn as horizontal solid line.

Figure 5: Durations of cell production and differentiation vs. the May–September temperatures and the snow-free period recorded in 2012 at the five study sites in the boreal forest of Quebec, Canada. Boxes represent upper and lower quartiles, whiskers achieve the 10th and 90th percentiles, and the median is drawn as horizontal solid line.

Figure 1.

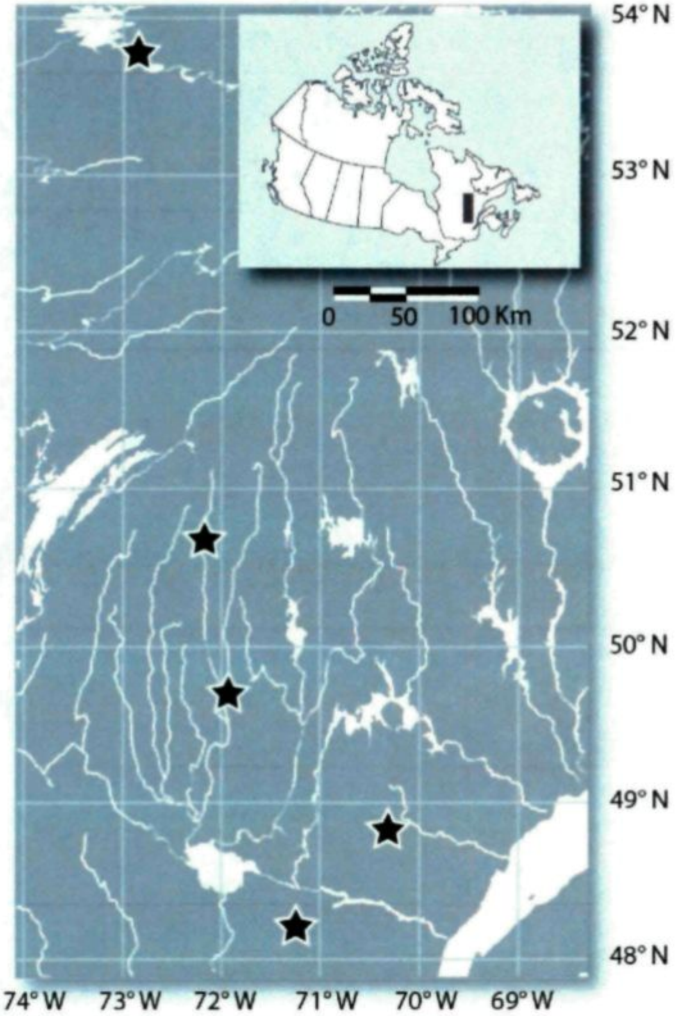
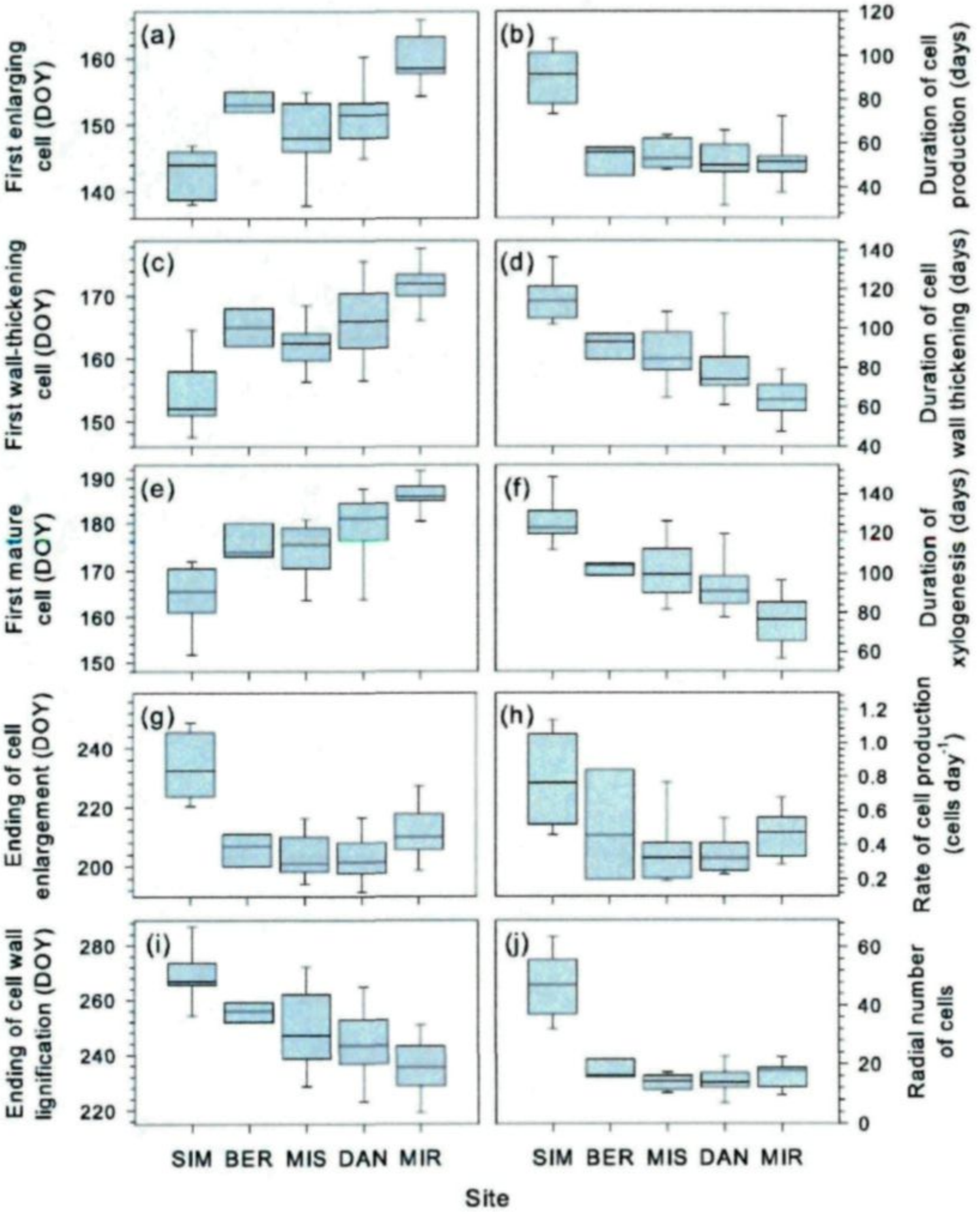


Figure 2.


(a)
(b)
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(j)
First enlarging cell (DOY)
140
150
160
Duration of cell production (days)
40
60
80
100
120
First wall-thickening cell (DOY)
150
160
170
Duration of cell wall thickening (days)
40
60
80
100
120
140
First mature cell (DOY)
150
160
170
180
190
Duration of xylogenesis (days)
60
80
100
120
140
Ending of cell enlargement (DOY)
200
220
240
Rate of cell production (cells day^{-1})
0.2
0.4
0.6
0.8
1.0
1.2
Ending of cell wall lignification (DOY)
220
240
260
280
Radial number of cells
0
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40
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SIM
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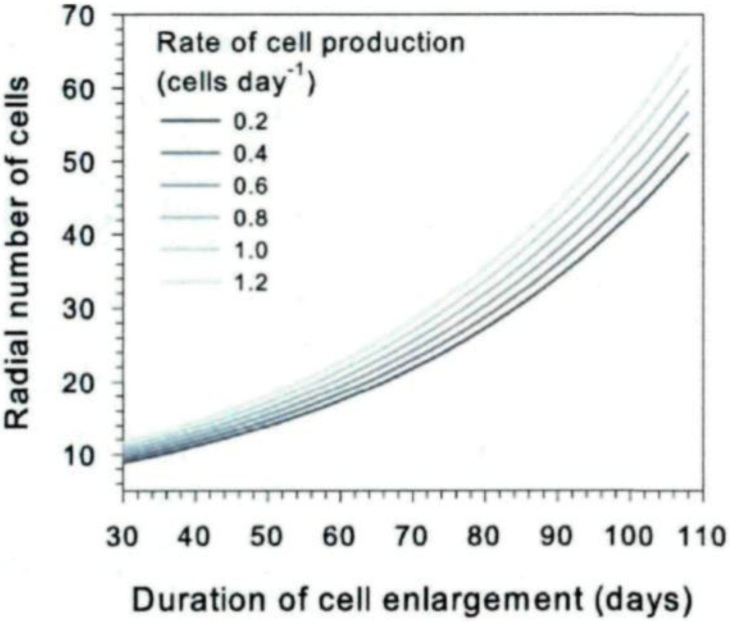
MIR
Site

Figure 3.


Rate of cell production
(cells day^{-1})
0.2
0.4
0.6
0.8
1.0
1.2
Radial number of cells
10
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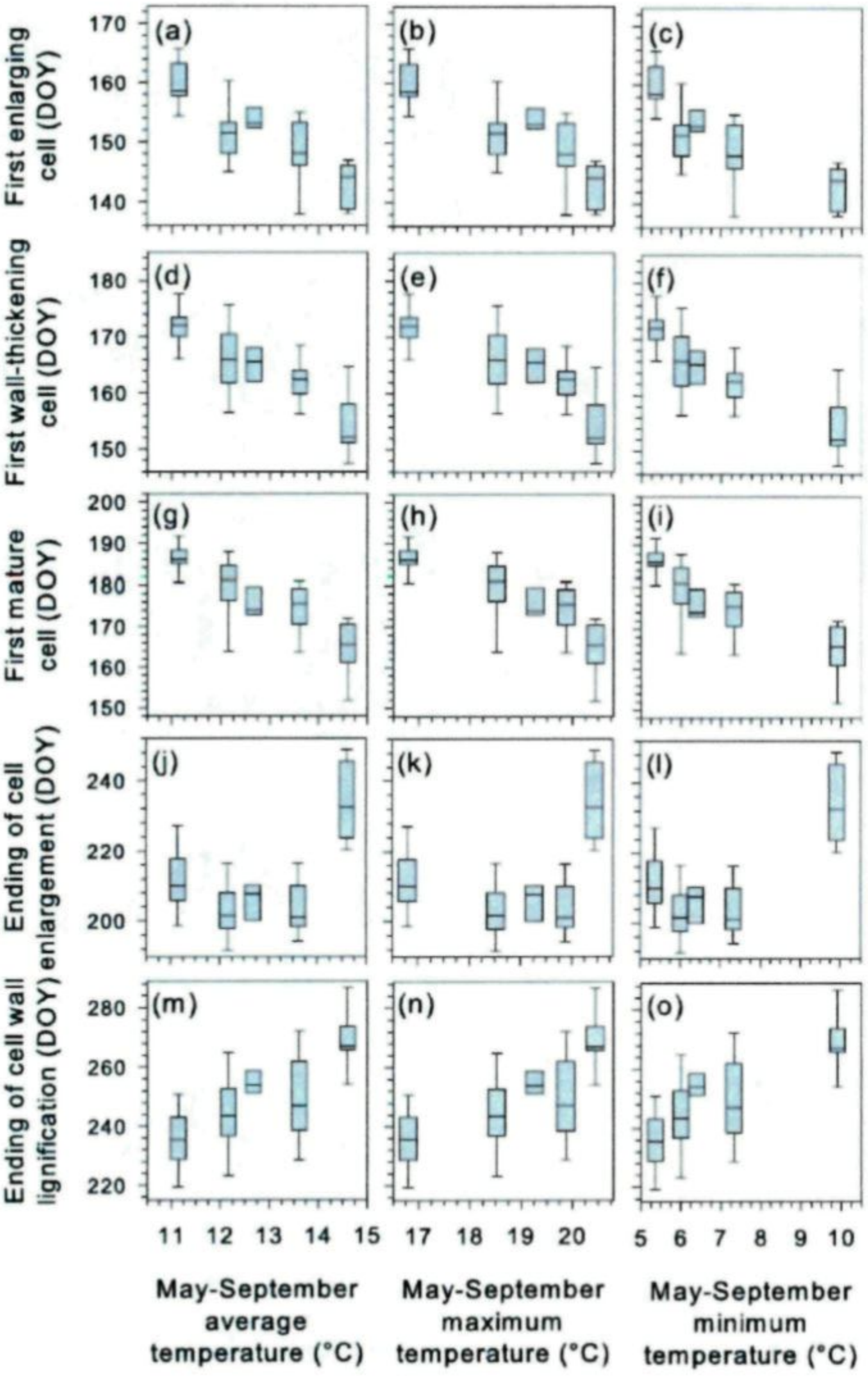
80
90
100
110
Duration of cell enlargement (days)

Figure 4.


First enlarging cell (DOY)
First wall-thickening cell (DOY)
First mature cell (DOY)
Ending of cell enlargement (DOY)
Ending of cell wall lignification (DOY)
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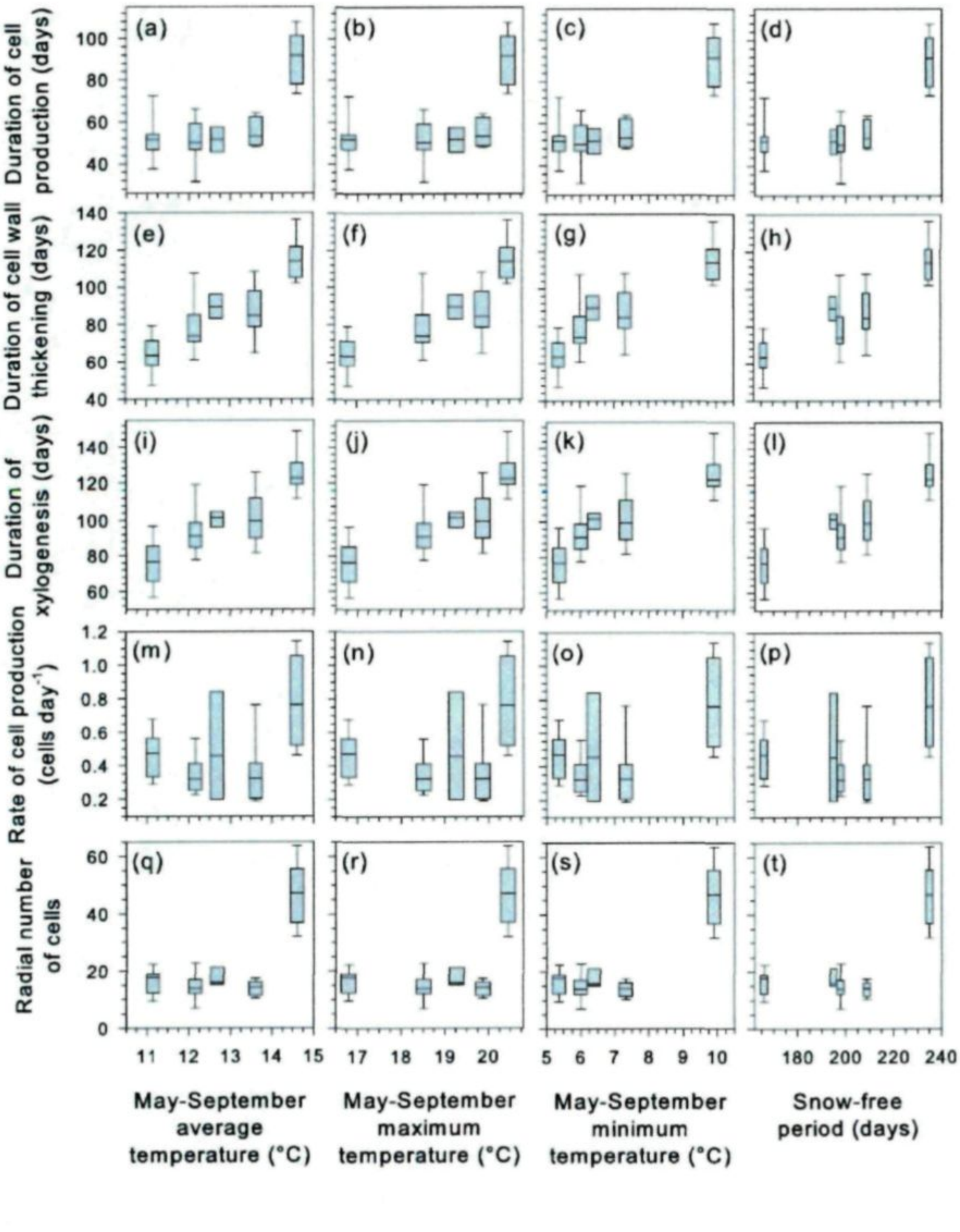
May-September average temperature (°C)
May-September maximum temperature (°C)
May-September minimum temperature (°C)

Figure 5.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(p)
(q)
(r)
(s)
(t)
Duration of cell production (days)
Duration of cell wall thickening (days)
Duration of xylogenesis (days)
Rate of cell production (cells day^{-1})
Radial number of cells
40
60
80
100
140
120
100
80
60
40
140
120
100
80
60
1.2
1.0
0.8
0.6
0.4
0.2
60
40
20
0
11 12 13 14 15
17 18 19 20
5 6 7 8 9 10
180 200 220 240
May-September average temperature (°C)
May-September maximum temperature (°C)
May-September minimum temperature (°C)
Snow-free period (days)

Supporting information

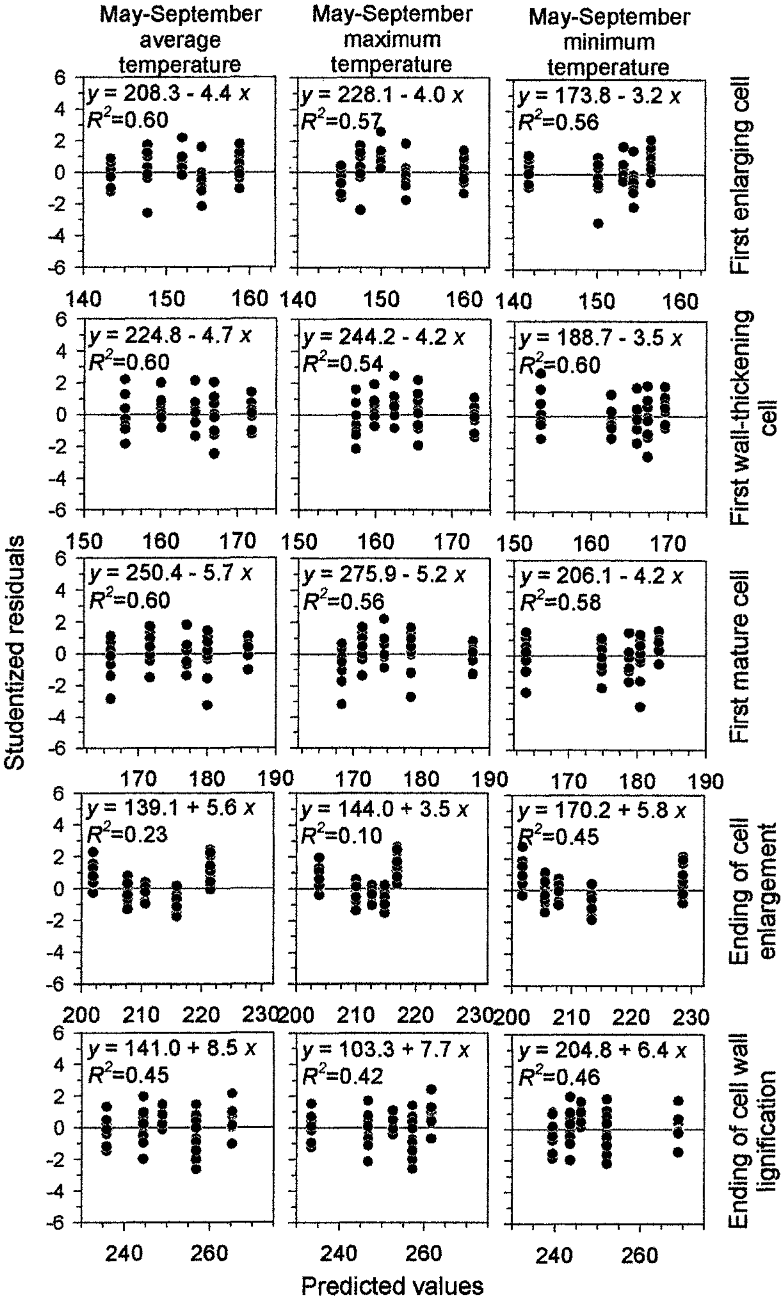

Figure S1. Studentized residuals of the regression models performed between cambium phenology and the May-September temperatures recorded in 2012 at the five study sites in the boreal forest of Quebec, Canada. Coefficients and R^2 of the regressions are reported in each plot.

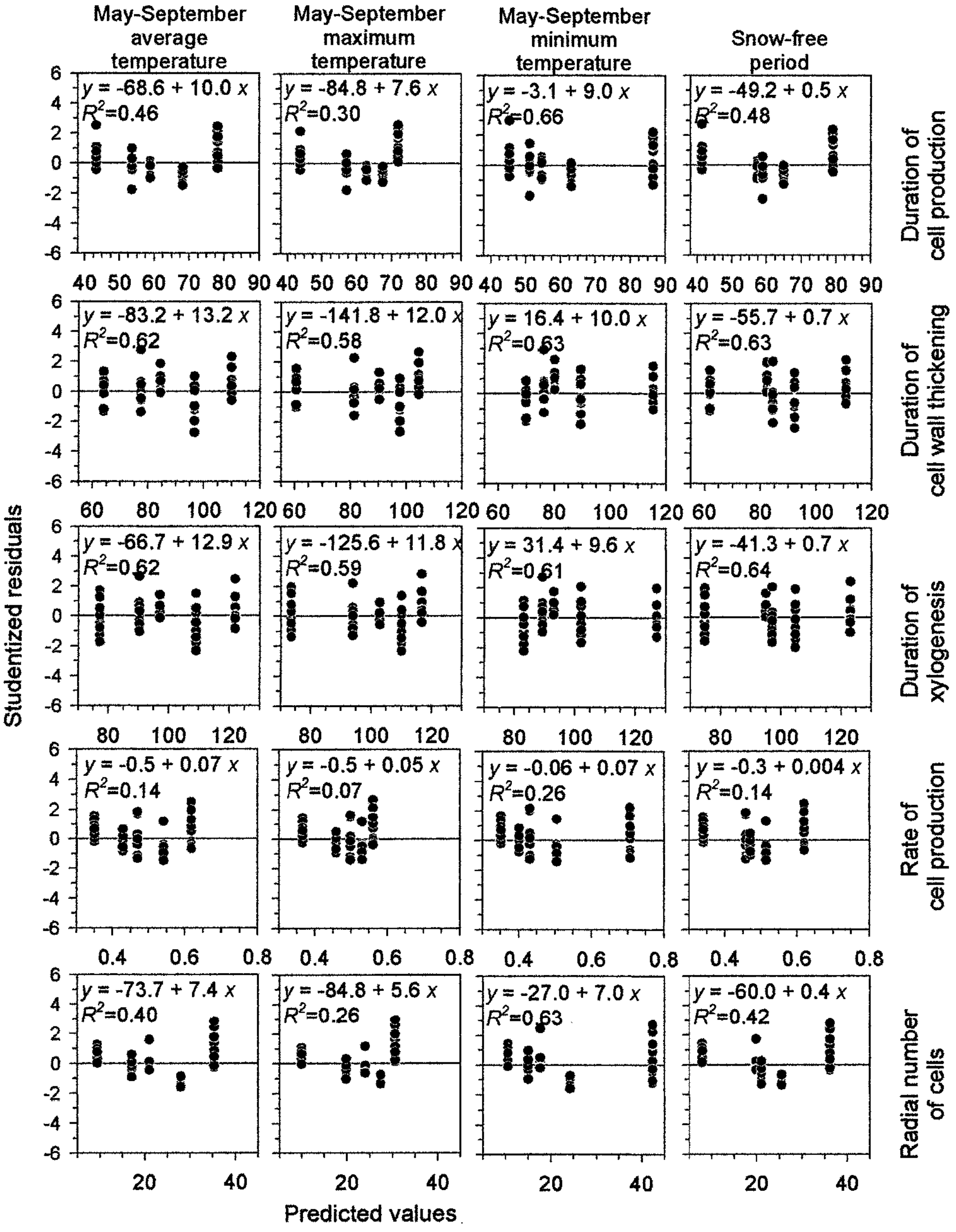

Figure S2. Studentized residuals of the regression models performed between durations of cell production and differentiation and the May-September temperatures and the snow-free period recorded in 2012 at the five study sites in the boreal forest of Quebec, Canada. Coefficients and R^2 of the regressions are reported in each plot.

ARTICLE #2

MODERN PORTRAYAL OF A BOREAL RELATIONSHIP: THE BLACK SPRUCE AND ITS PHENOLOGY

Modern portrayal of a boreal relation: the black spruce and its phenology

Marie-Josée Girard*, Sergio Rossi*, Hubert Morin*

*Département des Sciences Fondamentales, Université du Québec à Chicoutimi, 555 Boulevard de l'Université, Chicoutimi (QC), G7H2B1, Canada

(Soumis pour publication)

Ma participation pour cet article consiste au prélèvement sur le terrain et à l'analyse en laboratoire des échantillons. J'ai aussi fait les des analyses statistiques et j'ai réalisé la majorité de l'écriture.

Abstract

Models are a mathematic description of a phenomenon. They are useful to simplify and describe natural relationships and can be used to determine the link between the tree development and the surrounding environment. The aim of this research was to represent the relation between xylogenesis and it's alti-latitudinal location. The resulting equations were then extrapolated into a map covering the entire region studied by permanent plots. Microcores were taken weekly from April to October 2012 for 50 black spruce [*Picea mariana* (Mill.) BSP] distributed into five sites. Stations were located along a latitudinal gradient extending from the 48th to the 53rd parallel north and covering the entire distribution of the closed black spruce forest. Cellular analysis of each microcores has enabled to distinguish the dates of beginning and ending and then the duration in days for cell enlargement, cell wall thickening and xylogenesis and the total annual cells production. Latitude was the factor with the greatest impact while the altitude had minor or no effect, especially for nonlinear relations. Most results were linear such as the beginning of enlargement, beginning, ending and duration of lignification and the whole duration of xylogenesis. With the increasing latitude, dates were proportionally delayed for the beginning and earlier for the ending, thus creating a shorter duration. The temperature was suggested as the main factor driving these patterns. Duration and ending of enlargement and the complete cells production were nonlinear, with a regression similar to a negative exponential curve. This type of relationship seemed to indicate the presence of a limiting factor around the 49th parallel. This pattern was more complex and probably resulting from a combination of different factors.

Keywords: black spruce, boreal forest, growth, *Picea mariana*, phenology, threshold, gradient, linear model, xylogenesis, non-linear model

Introduction

Due to their immobility, plants are inexorably dependent on the immediate environment in which they are enrooted. Therefore, these organisms have developed a multitude of plastic adaptations to satisfy their metabolic and physiological needs and to ensure the survival of their offspring, even under changing, and sometimes hostile, conditions. Accordingly, the ecological, and consequently geographical, ranges of the species are closely related to their ability to adapt to different biotic and abiotic conditions, and constrained by the limits beyond which plants cannot growth and/or reproduce (Sexton *et al.*, 2009). For species exhibiting a wide distribution, the individuals of the population can experience environmental factors extending across huge ranges of variation. How do the organisms respond to these variations?

The impact of environment, mainly weather, on the seasonal dynamics of plants is studied through phenology (Forrest & Miller-Rushing, 2010), which investigates the variations of periodic or recurrent events over time in plants and animals. Long-term observations on trees revealed shifts in the timings of budding, flowering and fructification, probably due to the ongoing global warning (Menzel *et al.*, 2009). A reconstruction of cambial resumption in spring over the last 60 years estimated a similar trend for the dynamics of wood production (Boulouf-Lugo *et al.*, 2012). Wood production, or xylogenesis, is closely related to temperature, mainly in ecosystems of higher altitudes and latitudes, where it follows an annual cycle (Rossi *et al.*, 2007; Seo *et al.*, 2008; Swidrak *et al.*, 2011). During the thermally unfavourable season, trees are dormant and the metabolic activities are kept to a minimum in order to protect the tissues. The mild temperatures in spring allow cambium to reactivate, and new xylem cells to be produced (Begum *et al.*, 2007). These cells undergo a differentiation identified with the phenological phases of enlargement and cell-wall thickening, after which they reach their functional maturity. Xylogenesis is generally completed at the end of summer, before the temperature attains a critical threshold for cell maturation (Rossi *et al.*, 2008). Thus, in the last fifteen years, the phenological observations that were previously performed on primary and reproductive meristems (buds and flowers) are now applied on secondary meristems (cambium, xylem and phloem), providing noteworthy information on how and when trees produce the woody tissues.

The phenology represents an excellent example of sensitive response of the organisms to their environment and then is of great help to observe the climate change (Körner *et al.*, 2010). In general, different types of relationships have been observed between biological variables and abiotic factors. Linear relationship is the most commonly observed (Sparks *et al.*, 2009). However, in nature, relationships are rarely strictly linear, because of the intrinsic limits of adaptation of the organisms to their environment (Burkett *et al.*, 2005; Porter and Semenov, 2005). More frequently, the relationships appear non-linear, or show thresholds. The understanding of these trends can be of great help to assess ecological responses, mechanisms of functioning and reactions of the living organisms under environmental changes.

The aim of this study was to analyse the patterns of variation of cambium phenology and xylem cell production across the entire distribution the black spruce as closed forest. To do this, black spruce [*Picea mariana* (Mill.) BSP] was selected as representative species because of its wide geographical range of distribution across the boreal forest of Quebec, Canada. Five sites were chosen on an alti-latitudinal gradient covering more than 5° of latitude and representing the whole distribution of the closed forest of this species.

Material and methods

Study area and tree selection

The study was conducted in the boreal forest of Quebec, Canada (Fig. 1). The region has a gently rolling topography with hills reaching 500-700 m a.s.l. on thick and undifferentiated glacial till deposits. Five permanent sites [Simoncouche (abbreviated as SIM), Bernatchez (BER), Mistassibi (MIS), Camp Daniel (DAN) and Mirage (MIR)] were selected at different altitudes and latitudes in mature even-aged black spruce stands (Table 1) (Rossi *et al.*, 2011). The sites were isolated and without evidence of human impact, which means that stands could be considered to have developed under the influence of natural disturbance. In each site, ten dominant 120-140-year-old healthy trees with upright stems were chosen from a pre-selected group of trees with similar growth rates in terms of tree-ring width (Dufour and Morin, 2007; Lupi *et al.*, 2012).

Xylem sampling and preparation

Xylem formation was studied from April to October 2012. Wood microcores were collected weekly following a spiral trajectory on the stem at 30 cm below breast height using Trephor (Rossi *et al.*, 2006a). The very small wounds inflicted by the thin piercing tubes of the tools and the consequently narrow areas of traumatized tissues around the sampling points allowed repeated samplings by microcore extraction suitably representing tree growth (Forster *et al.*, 2000, Lupi *et al.*, 2014). Samples usually contained the previous 4 or 5 tree rings and the developing annual layer with the cambial zone and adjacent phloem. Samples were always taken at least 5 cm apart to avoid getting resin ducts on adjacent cores. The microcores were placed in Eppendorf microtubes with an ethanol solution (10% in water) and stored at 5 °C to avoid tissue deterioration. Microcores were oriented by marking the transverse side with a pencil under a stereomicroscope at magnifications of 10-20×, dehydrated with successive immersions in ethanol and D-limonene and embedded in paraffin (Rossi *et al.*, 2006c). Transverse sections of 7 μm thickness were cut from the samples with a rotary microtome. The sections were 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.

Microscopic observations

For 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 containing cells with large tangential sizes. 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 4-5 closely-spaced cells. At the onset of cambial activity, the cambial zone began to widen rapidly (within a week) as the number of cells increased, revealing that cell division had started. The new cell produced will enlarge twice the radial diameter with the augmentation of their vacuole and the primary wall will become thinner. Observations under polarized light will show no glistening for the enlargement zones where the cells were still composed of just primary wall (Funada *et al.*, 1997). Because of the arrangement of cellulose microfibrils, the developing secondary walls during the lignification shone when observed under polarized light because of the cresyl violet coloration reaction with lignin (Rossi *et al.*, 2006b).

Lignification was shown by a colour change from violet to blue. The colour change over the whole cell wall revealed the end of lignification and the tracheid reaching maturity (Gričar *et al.*, 2005).

The number of cells in the 3 rows was averaged for each tree and used to assess onset and ending of each phase of differentiation. In spring, when at least one cell was observed in the enlargement zone, 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 completed. Cambium phenology was represented by the dates corresponding to (i) the first enlarging cell, (ii) the first wall-thickening cell, (iii) the first mature cell, (iv) the ending of cell enlargement, and (v) the ending of cell wall lignification. The duration of xylogenesis was assessed as the number of days occurring between the onset of cell enlargement and the ending of cell wall lignification. The traits of cambium phenology, computed in days of the year (DOY), as well as the number of radial cells produced annually by the cambium were calculated for each tree.

Model definition and application

Linear and non -linear models were fit with the dates of the onset and beginning of each phenological phase and cell production according to the geographical location of the sites. Several models were tested before selecting the one that produced the best fitting. When linear models were not appropriate, a non-linear model was applied. The best findings were obtained by using longitude and altitude as independent factors according to the two following linear and non-linear equations:

$$y = \theta 1 \text{ latitude} + \theta 2 \text{ altitude} + \theta 3$$

$$y = \theta 1 \exp^{-\theta 2 \text{ latitude}} + \theta 3 + \theta 4 \text{ altitude}$$

where y represents xylem phenology (in DOY or days) or the final number of cell, and θ_1-θ_4 correspond to the coefficients of the equations. The statistical procedure for non-linear models regressed the residuals on the partial derivatives with respect to the parameters of the functions until the estimates converged. Several possible starting values were specified for each parameter, so that the procedure evaluated each combination of initial values using the interactions. Goodness-of-fit of each regression involved the proportion of variation accounted for (R^2), standard errors of the parameters, and the distribution of residuals. All fittings were performed using JMP 10.0 (SAS Institute Inc., Cary, NC).

The parameters calculated by the regressions were used to estimate the timings of xylem growth and cell production across the region interested by the sampling and comprised between 48 and 54 °N, and between 70 and 73 °W. The estimates were performed using the Canadian Digital Elevation Data generated on a 100-m-interval mosaic raster.

Results

Dynamics of xylem formation

In early spring, trees were still dormant, and cell production was not active (Fig. 2). At this time, the cambium was composed of 4-5 cells. In late spring, the cambium began its activity, and the amount of cambial cells increased to 6-10 cells, indicating the beginning of xylem cell production. The beginning of cell enlargement occurred in mid-May in SIM, and later in the other sites, according to the augmentation of the latitude or altitude (Fig 3). The first enlarging cell defined the beginning of xylem cell production, since the cells produced by the cambium can also differentiate in phloem tissue. The duration of enlargement lasted approximately 100 days in SIM, and 60 days in the other sites, and finished between late July and late August (Fig 2).

The beginning of the cell wall thickening exhibited a pattern among sites similar to cell enlargement, but with a delay of 10 days (Figs 2 and 3). The ending of cell differentiation ranged from late August to late September, depending on the latitude and altitude of the sites. The duration of the phases of cell enlargement and xylogenesis were longer in the sites located at lower latitudes and altitudes (SIM and MIS) (Fig 2). Trees in SIM produced an average of 45 cells. The other sites showed similar amounts of cell productions, with a mean of 15 xylem cells detected along the tree ring (Figs. 2 and 3).

Model definition

For all models, the regressions produced R^2 ranging between 0.55 and 0.66 and satisfying distributions of residuals (Fig. 4 and table 2). F-values were statistically significant and <0.0001. These results confirmed that the models suitably represented the data points. A residual variability within each site was observed, which was related to the between-trees variation (Fig. 4).

When using latitude and altitude as independent factors, five of the eight tested variables were suitably represented by linear models. The beginning of cell enlargement and cell wall thickening produced regressions with positive slopes, indicating that later beginnings were estimated at increasing latitude. Similarly, sites located at higher altitudes exhibited later beginning of growth. Ending and duration of cell wall thickening and duration of xylogenesis showed regressions with negative slopes. As a result, sites of higher latitudes and altitudes exhibited earlier ending of growth and shorter growing seasons.

Ending and duration of cell enlargement and the radial number of cells had a clear non-linear pattern (Fig. 4). For these variables, all curves showed an abrupt change between 48 and 49 °N, and attained a plateau at higher latitudes. Over 49 °N, the cell production was similar and the phase of cell enlargement was shorter. It was mainly affected by latitude, while the effect of altitude was small or negligible (Fig. 4 and Table 2).

Model application

The application of the models showed how xylem phenology and cell production changed across the region according with altitude and latitude (Fig 5). The effect of the latitude was clearly visible on all variables, with longer growing seasons and higher cell productions estimated in the Southern part of the region.

The altitude exhibited a stronger influence on the phenological phases represented by linear models, while non-linear models were only slightly or not affected by altitude. This was particularly evident for the ending of cell enlargement and cell production, where the changes with altitude were indiscernible on the maps (Fig. 5). For the other phenological phases, the variation was related to the orography. An earlier beginning and later ending of growth was observed on the plains around Lac Saint-Jean (on the bottom part of the study area) and along the Saguenay river. The shortest growing seasons were estimated on hills and mountains, as a consequence of the altitude.

Discussion

The aim of this investigation was to modelize the influence of localization on the cell production and differentiation of xylem. Cambium phenology and xylem cell production were assessed throughout the growing season along a latitudinal gradient from 48th to 53rd parallel, a wide geographical range of the distribution of black spruce. It is a complementary of the research of Rossi *et al.,* (2013) but, it goes further in the discoveries of the equations coefficients linking the different vectors and their extrapolation into a simulation of xylem phenology on an alti-latitudinal map of the region cover by the study.

Patterns of change in phenology and growth

Within the range analysed, linear patterns, were the most common relationship observed, with latitude and altitude leading to a proportional response in phenological phases. The dynamics of xylem growth were similar between sites, but occurred with delayed timings according to their location. As expected, in southern sites or at lower altitudes, xylogenesis began earlier and ended later, thus resulting in a longer growing season. Salmela (2014) also found a linear relation between latitude and growth duration. Previous studies performed in natural environments (Rossi *et al.*, 2007, 2008; Moser *et al.*, 2010) or using artificial warming (Gričar *et al.*, 2006; Oribe and Kubo, 1997) have demonstrated the key role of air temperature on cambial resumption, mainly in springtime. The relation was also linear for the end of the xylogenesis which is in conflict with the results observed in the Swiss Alps by Moser *et al.* (2010), where altitude showed little impact on the ending of lignification and enlargement. These results are probably due to the lower impact of altitude in comparison with latitude in the boreal zone

(Henttonen *et al.*, 2009). It has been suggested that late-occurring events, as the autumnal ending of growth, could be more related with photoperiod than temperature (Körner, 2003; Rossi *et al.*, 2008, Jackson, 2009). However, the way that environmental conditions influence the growth cessation is still partially known and subject to intense debate.

In nature, relationships are rarely linear, as living beings have physiological limits to their plasticity and adaptability. The wide latitudinal range covered by this study clearly demonstrated the non-linear responses of black spruce in terms of xylem cell production, which showed a pattern similar to a negative exponential curve. The regions under the 49th parallel had a final cell production 3 fold higher than the production estimated at higher latitudes. A rise in altitude leads to a cooling of the air which should affect phenology. However, altitude had no significant effect which is probably attributed to the low difference in height, which did not exceed 259m between the lowest site and the higher. In comparison, Moser *et al.* (2010) has 250m between two neighboring sites. In this research, the lower cell production was at the highest altitude, and all the other sites along the altitudinal gradient were having approximately the same cells production suggesting a common factor limiting production in north and at high location. In boreal and cold ecosystems, xylem cell production is well known to be closely related to environmental factors such as temperature, and photoperiod (Vaartaja, 1954; Johnsen *et al.*, 2005). Although precipitation has been observed to affect tree growth in black spruce of the boreal forest (Huang *et al.*, 2010), at intra-annual scale and in the sites analyzed in this work, this factor may be considered marginal (Belien *et al.*, 2012; Krause *et al.*, 2010).

Wood growth is often studied as a whole, but in this work the phases of cell differentiation were carefully identified and considered separately for a deeper understanding of the process of xylem formation (Rossi *et al.*, 2012). The derivatives, the cells produced by the cambium, must first expand by increasing the volume of their vacuole by water absorption. This process is considered energetically less demanding in comparison with cell wall maturation, which requires building and utilisation of structural carbohydrates, and is the main sink of carbon in trees (Cuny *et al.*, 2013). The end of enlargement shows a similar exponential pattern comparatively with cell production. On the other hand, cell wall thickening had a linear profile resembling that of phenological traits already known to be linked to the environment. Cuny (in progress) also found that these two phases were not controlled by the same factor. Enlargement was not influenced by the environmental condition, but rather by endogenous factors. Since it has a similar model as cell production, it is possible that these two processes are control by the same factor. Regarding lignification, our results indicate the dependence with the environment as shown by the linear relation.

Model application

The application of the linear and nonlinear models obtained from the permanent plot allowed to simulate xylem phenology over a wide geographical region. There is a readily visible impact of altitude in linear data in contrast to the non-linear data. The stands close to the lake Saint-Jean and along the rivers in the valley have longer growing seasons. On the contrary, the period of growth was shorter on mountains and at high altitudes. Rossi *et al.*(2011) had established a map of the beginning, ending and duration DOY of the xylem growth for a part of the Quebec. Maps are looking similar and exhibit similar pattern to our linear model except with minimal air temperature. The extrapolation of the model of this study was limited by particularities of the field that are independent with the altitude and latitude. For the purpose of the visualisation, it was supposed that all the area was composed of black spruce forest but some part were composed of another environment type, for example, wet area, wood cut, cities and etc... . Also, the map doesn't represent the detail of the geography, and some particularities were not accessible with the data, such as exposure, effects of wind or large mass of water. This map should be considered as a simplified representation of the phenology that could be observed inside a wide geographical range for one species.

Temperature vs photoperiod

This study didn't allows the identification of the climatic factors influencing the establishment of the models. However, the alti-latitudinal gradient of this study represent a variation of two main environmental factors already known to have an impact on xylogenesis: temperature and day length. Temperatures are colder and daily illumination longer with the augmentation of latitude. It can be then assumed that each site where having proportional temperature and photoperiod link to its location. Amount of daily sunlight could also be an important vector of the cell production (Rossi *et al.*, 2006b; Heinrichs *et al.*, 2007) and since it didn't change with the altitude, it could explain the unconnected relation of altitude and non-linear model. Interaction could also been observed in the results such as photosynthesis who's dependent of temperature and photoperiod, as observed by Körner (2003). He pinpoints the fact that net photosynthesis exhibits a bell shape when put in relation with the temperature (Lin *et al.*, 2012), but is related to dark respiration by means of an exponential pattern (Tjoelker *et al.*,1999). It can be suppose that temperature and photoperiod are controlling the cells production with a day by day process where interaction between the amount of sunlight and the temperature guide the cell activity.

Making prediction

Existing models are often used for understanding the past, but few are known about predicting future events. Jochner (2013) used an urban gradient of birch to simulate climate change and pollution over space and time. Established models with space data allowed to emit good spatial prediction but were not accurate for predictions in time. In a context of climate change where temperature, but not photoperiod, is expected to increase (IPCC, 2007), linear relationships are suspect to react in a proportional way. Some studies already observe and advancement in the phenology (Menzel and Fabian, 1999; Menzel *et al.*, 2006). But, non-linear reaction, such as cell production, seems more complex to predict considering that they are connected in too many and different way with the environment. Several factor are implicate in the xylogenesis in the course of a year and more data in time and in space should be collected before making false assumption (Mäkelä, 2013).

Conclusion

At our knowledge, the latitudinal gradient of more than 600 km used in this study is the largest monitoring available for the phenology of cambium, and represents most of the latitudinal range of the closed boreal forest in Quebec, Canada. Sampling a wide range of thermal conditions allowed to observe the differences in timings and amount of growth throughout the season revealing distinctive relationships between trees and their environment. The more common pattern was linear, where the phenological timings were proportional to latitude and altitude of the site. Negative exponential patterns were observed for cell enlargement and cell production, which could be related to a cumulative effect of several environmental factors on cambial activity. Further studies are required for a better understanding of the environmental drivers causing such patterns in cell production. The linear and exponential relationships observed in this work have several important implications for modelling or estimating tree growth under climate change scenario and how the ongoing warming will affect the productivity in forests of the boreal ecosystems. Care should be taken in defining the correct relationship to ensure reliable expectations of the growth models.

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Table 1. Location of the five study sites in the Quebec boreal forest

ID	Site	Latitude (°N)	Longitude (°W)	Altitude (m a.s.l.)
SIM	Simoncouche	48° 12’	71° 14’	382
BER	Bernatchez	48° 51’	70° 20’	597
MIS	Mistassibi	49° 43’	71° 56’	338
DAN	Camp Daniel	50° 41’	72° 11’	481
MIR	Mirage	53° 47’	72° 52’	396

Table 2. Coefficients and goodness of fit of the linear and non-linear models linking xylem phenology and cell production to latitude and altitude

	Coefficient						
	θ1	θ2	θ3	θ4	R^2	F	p
Beginning of cell enlargement	4.54	2.58	0.03	---	0.62	36.30	<0.0001
Ending of cell enlargement	89596.56	6.69	219.84	-0.02	0.61	-	-
Duration of cell enlargement	22667.31	5.28	75.10	-0.03	0.72	-	-
Duration of xylogenesis	525.22	-7.79	-0.06	---	0.66	44.50	<0.0001
Beginning of cell wall thickening	3.45	2.88	0.03	---	0.55	28.49	<0.0001
Ending of cell wall thickening	529.76	-5.21	-0.08	---	0.54	25.91	<0.0001
Duration of cell wall thickening	526.32	-8.09	-0.05	---	0.66	43.41	<0.0001
Radial number of cells	2273.95	3.45	16.71	$3,00 \times 10^{-3}$	0.77	-	-

Figure 1.

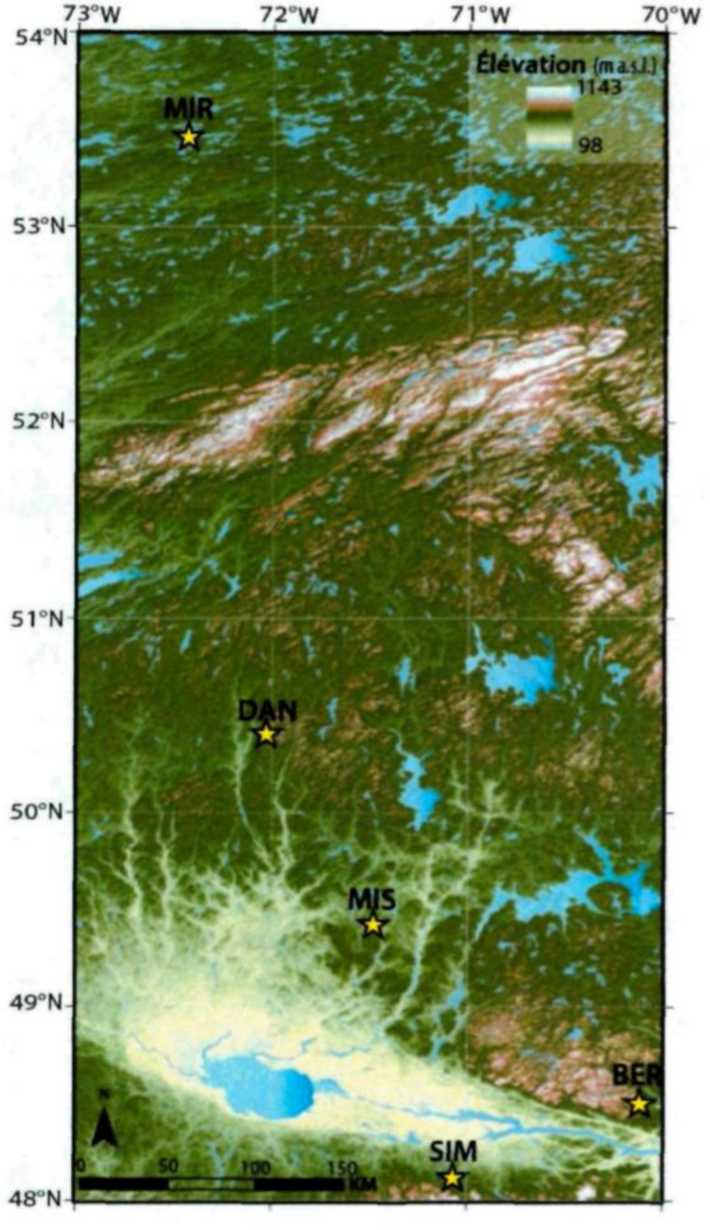
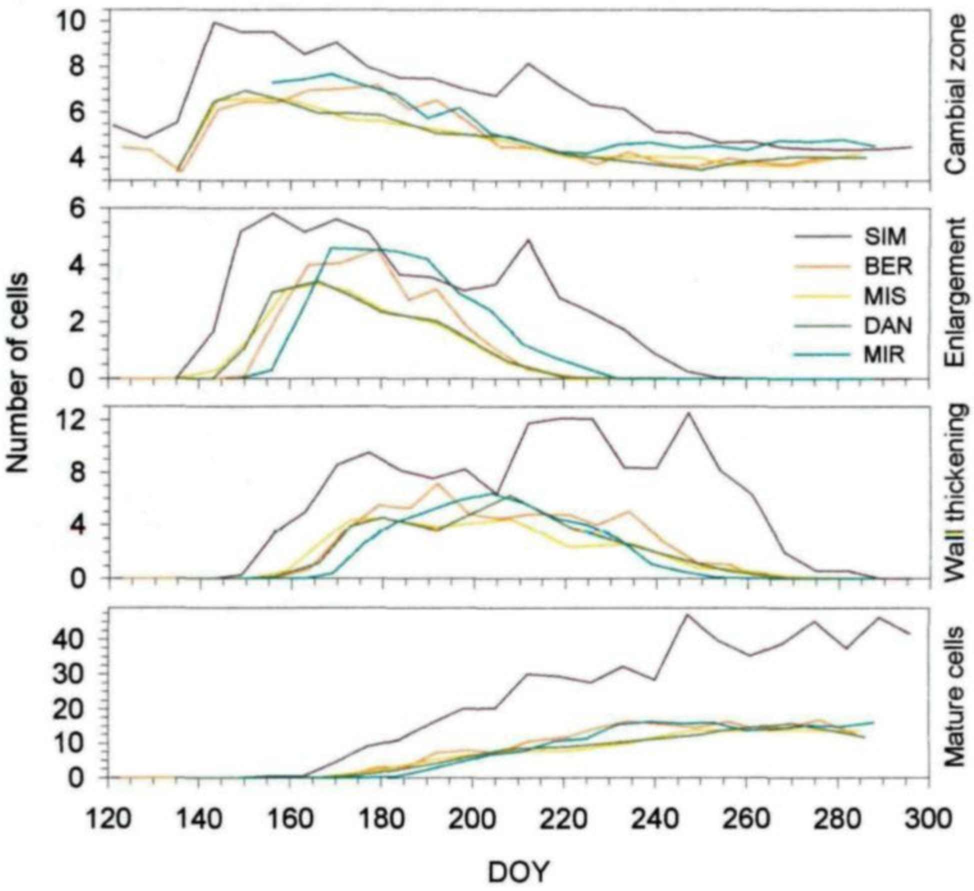
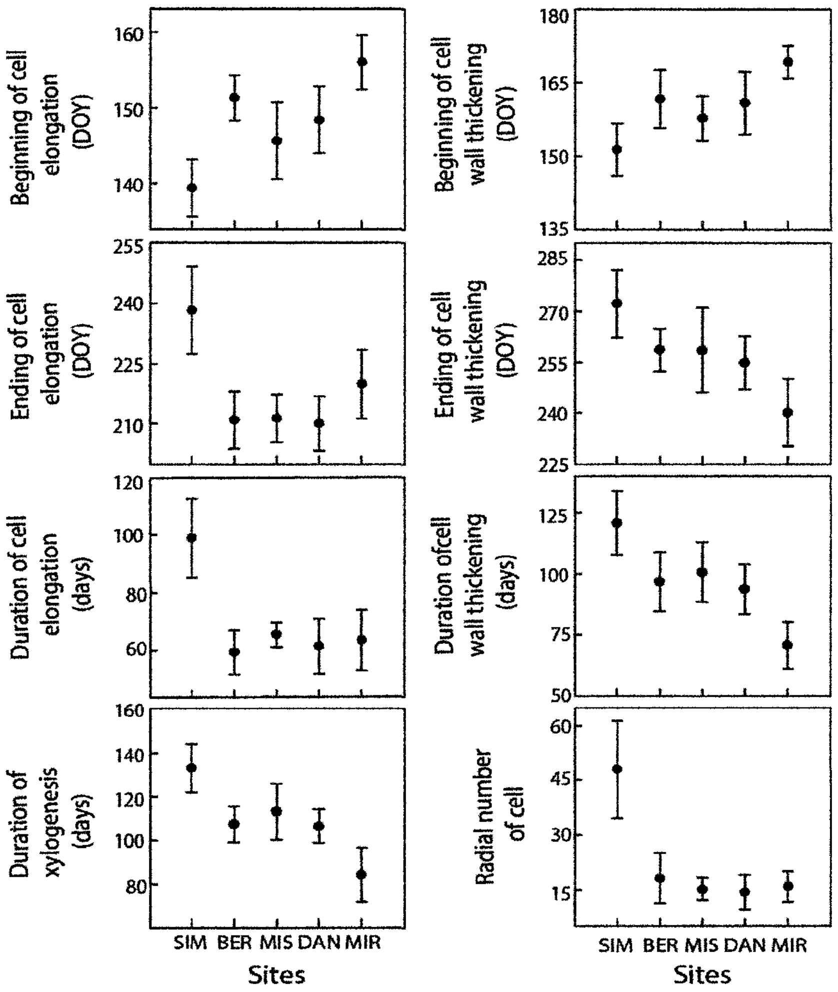

73°W
72°W
71°W
70°W
54°N
53°N
52°N
51°N
50°N
49°N
48°N
Élévation (m.a.s.l.)
1143
98
MIR
DAN
MIS
BER
SIM
N
0
50
100
150
KM

Figure 2.



10
8
6
4
Cambial zone
6
4
2
0
SIM
BER
MIS
DAN
MIR
Enlargement
12
8
4
0
Wall thickening
40
30
20
10
0
Mature cells
Number of cells
120
140
160
180
200
220
240
260
280
300
DOY

Figure 3.



Beginning of cell elongation (DOY)
160
150
140
Ending of cell elongation (DOY)
255
240
225
210
Duration of cell elongation (days)
120
100
80
60
Duration of xylogenesis (days)
160
140
120
100
80
Beginning of cell wall thickening (DOY)
180
165
150
135
Ending of cell wall thickening (DOY)
285
270
255
240
225
Duration ofcell wall thickening (days)
125
100
75
50
Radial number of cell
60
45
30
15
SIM BER MIS DAN MIR
Sites
SIM BER MIS DAN MIR
Sites

Figure 4.

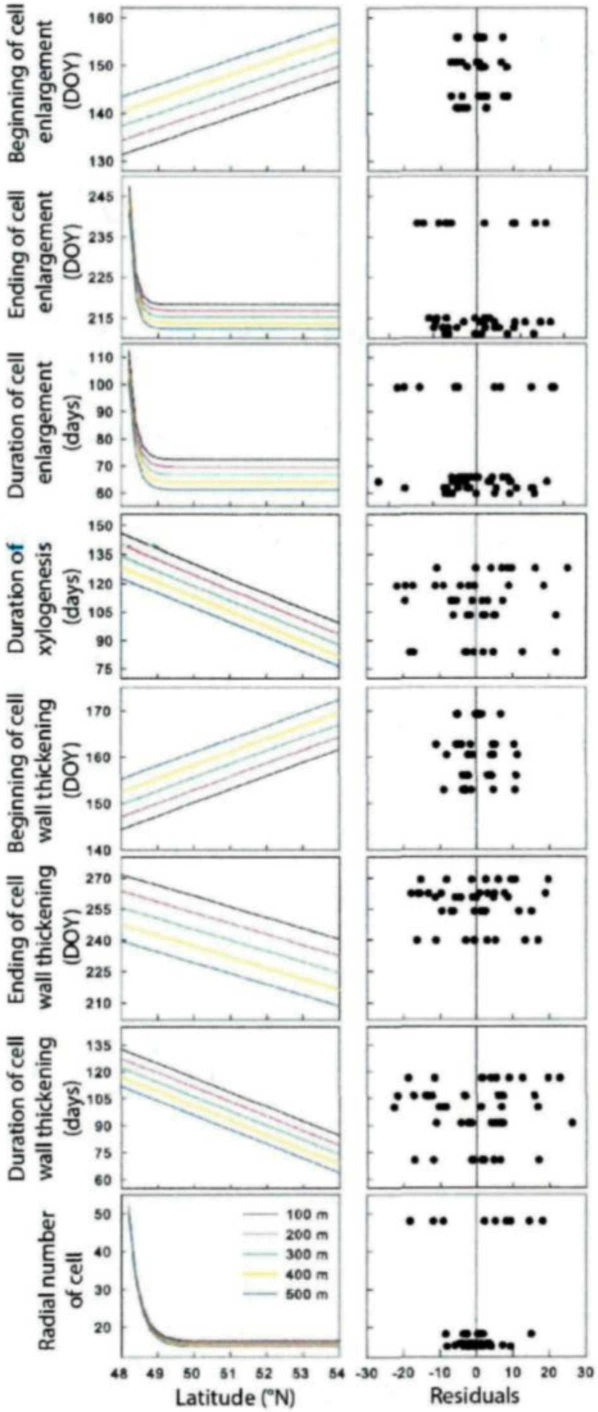
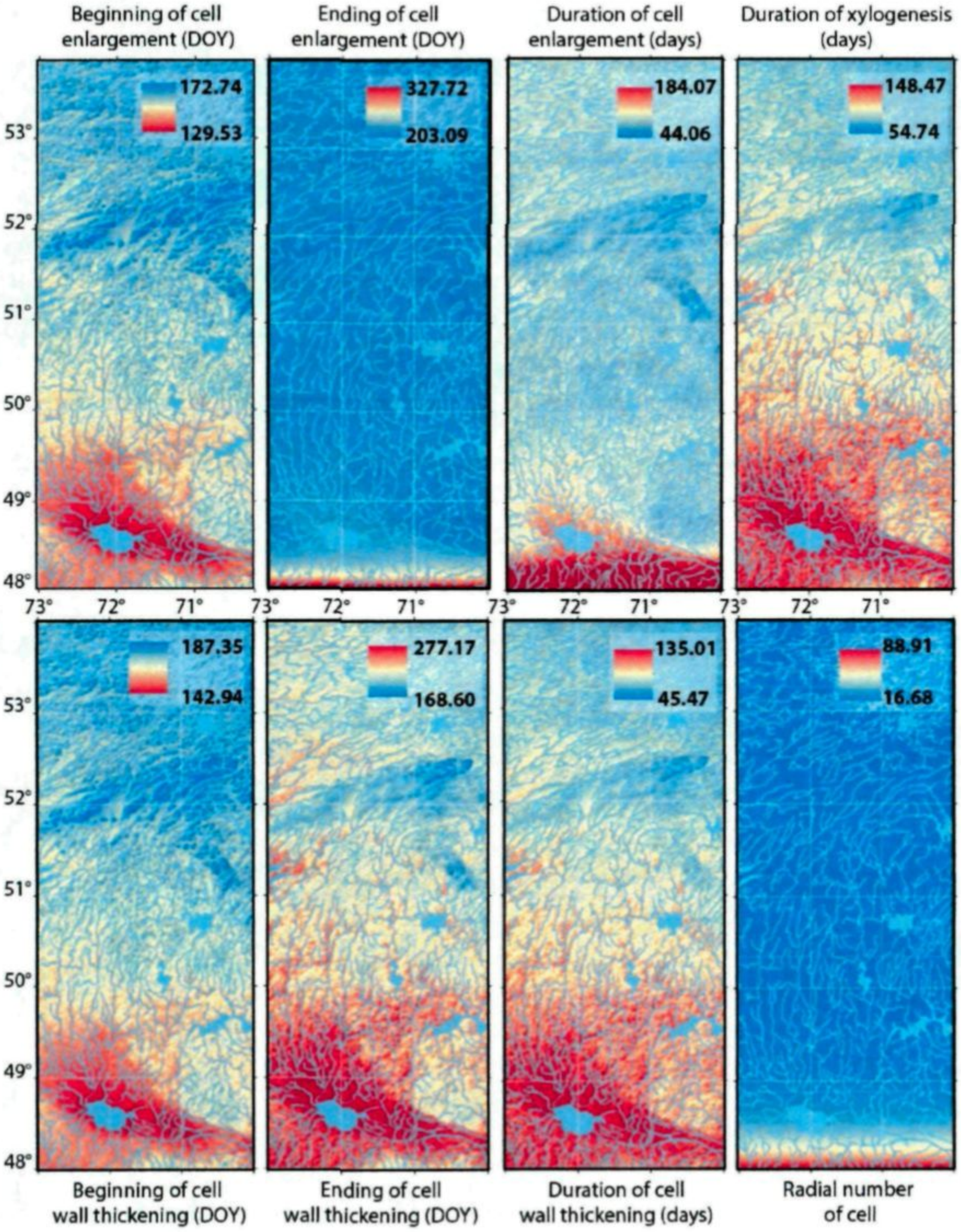

Beginning of cell enlargement (DOY)
130
140
150
160
Ending of cell enlargement (DOY)
215
225
235
245
Duration of cell enlargement (days)
60
70
80
90
100
110
Duration of xylogenesis (days)
75
90
105
120
135
150
Beginning of cell wall thickening (DOY)
140
150
160
170
Ending of cell wall thickening (DOY)
210
225
240
255
270
Duration of cell wall thickening (days)
60
75
90
105
120
135
Radial number of cell
20
30
40
50
100 m
200 m
300 m
400 m
500 m
48
49
50
51
52
53
54
Latitude (°N)
-30
-20
-10
0
10
20
30
Residuals

Figure 5.



Beginning of cell enlargement (DOY)
Ending of cell enlargement (DOY)
Duration of cell enlargement (days)
Duration of xylogenesis (days)
172.74
129.53
327.72
203.09
184.07
44.06
148.47
54.74
53°
52°
51°
50°
49°
48°
73°
72°
71°
187.35
142.94
277.17
168.60
135.01
45.47
88.91
16.68
Beginning of cell wall thickening (DOY)
Ending of cell wall thickening (DOY)
Duration of cell wall thickening (days)
Radial number of cell

Caption list

Figure 1: Elevation map of the study area including the five study sites

Figure 2: Average number of cells in cambium and differentiation observed during the growing season. DOY represents the day of the year.

Figure 3: Xylem phenology and cell production recorded in the five study sites during 2012. Data are reported as mean and standard deviation

Figure 4: Linear and non-linear models representing the changes in xylem phenology and cell production according to latitude and altitude. The distribution of the residuals of each model is also reported.

Figure 5: Application of the models predicting xylem phenology and cell production on the study area covering the whole latitudinal distribution of the closed forest of black spruce in Quebec, Canada

CONCLUSION GÉNÉRALE

Les modèles étaient linéaires pour la plupart, avec des réponses proportionnelles aux changements alti-latitudinaux. Ces relations étaient supposées être principalement reliées à la température et à la latitude. En effet, de nombreuses études font déjà état de la relation entre la xylogénèse et la température, notamment lors de recherche sur les changements climatiques. Le coefficient élevé de la latitude dans les équations indiquaient sa forte influence alors que l'altitude n'en avait pratiquement aucune. Par opposition, la production cellulaire ainsi que la durée et la fin de l'élargissement avaient une réponse non linéaire selon la température et la localisation. Les modèles non linéaires, similaires à des courbes exponentielles négatives, étaient d'explication plus complexe et pourraient découler d'une interaction entre la photopériode et la température. Un changement brusque dans ces modèles était observé aux alentours du 49e parallèle, ou d'une température de 14°C. Au nord de ce parallèle, les sites possèdent un nombre de cellules finales similaires alors que le site au sud de cette limite a une production trois fois plus élevée. Des études supplémentaires seraient nécessaires pour un approfondissement des connaissances sur les modèles non linéaires et des facteurs qui les contrôlent.

Un apport important de cette recherche a été l'utilisation de l'épinette noire dans toute son aire de répartition en tant que forêt fermée. Au nord et au sud de nos sites en limite de la distribution, l'épinette noire est encore disponible, mais devient clairsemé probablement dû à un mélange de compétitions et de facteur climatique. L'existence de sites à la limite nord et sud de la distribution a été très importante, car elle a permis d'établir un modèle exponentiel pour la production cellulaire, modèle qui aurait été linéaire en leur absence. Il serait important, lors de futures études sur la phénologie, d'effectuer des prises de données couvrant le plus possible l'entière distribution de l'espèce pour obtenir des modèles représentant les réalités de la nature.

Dans une perspective de changement climatique, il est certain que les arbres de la forêt boréale seront influencés par l'arrivée de ces nouvelles conditions. Cette étude a permis d'établir une linéarité entre plusieurs phases phénologiques et la température. Une augmentation de celle-ci pourrait donc amener un réveil hâtif de l'activité cellulaire au printemps et un rallongement de la saison de croissance. La production cellulaire est cependant plus difficile à prévoir. Il est à prendre en compte qu'une seule année a été échantillonnée rendant incertaine l'extrapolation dans le temps de nos résultats. Des années supplémentaires de micro-carottages sur ces sites seront nécessaires avant de pouvoir émettre des prédictions fiables.