Tracheid production phenology of *Picea mariana* and its relationship with climatic fluctuations and bud development using multivariate analysis

BORIS DUFOUR¹,² and HUBERT MORIN¹

¹ Département des sciences fondamentales, Université du Québec à Chicoutimi, Chicoutimi, Canada
² Corresponding author (boris_dufour@uqac.ca)

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Summary Research on cambium phenology in trees and its limiting factors in natural conditions is still at an early stage of development, restricting our capacity to precisely evaluate the effect of growing season length and climate fluctuations on tracheid production. The first objective of this paper was to describe cambial tracheid production phenology of black spruce (*Picea mariana* (Mills.) BSP). Repeated tree ring sampling was performed from 2002 to 2006 on four sites (48°13.78′ N, 71°15.18′ W; 48°51.92′ N, 70°20.57′ W; 49°43.92′ N, 71°56.88′ W; and 50°41.78′ N, 72°11.03′ W) representative of closed black spruce forest in Quebec, Canada. The timing of cambial initiation and cambial cessation in black spruce differs from year to year, the first occurring on 4 June on average, whereas the second occurs on 15 August. During a single year, these events do not vary significantly in space within the study area. The duration of cambial tracheid production does not vary significantly in either time or space. The second objective of this study was to identify the climatic factors that explain variations in initiation and cessation. Air temperature and humidity, soil temperature and water content, rain precipitations, snow cover as well as photosynthetically active radiation were monitored at each studied site. These were then used to create sets of candidate regressors to explain timing of phenological events. Timing of cambial initiation is primarily dependent on mean temperature between mid-March and initiation itself. Vapor pressure during this period is also important but in a negative way. A significant effect of the previous year’s August soil and air temperature conditions suggests a link with spring bud activity resumption, an interpretation that is supported by an analysis significantly linking measured timing of bud break to cambial initiation. Cessation of cambial tracheid production is influenced by factors linked to photosynthesis during the period from mid-July to cessation. Those related to water status, namely saturation vapor pressure, soil water content and vapor pressure are particularly influential, but light intensity and soil temperature also have an effect. Also, because mid-July corresponds to the timing of bud set and because the previous late summer’s soil temperature has a significant effect, a clear link is established with apical cessation.

Keywords: boreal forest, cambium, climate changes, eco-physiology, multiple regression, repeated tree ring sampling.

Introduction

Black spruce (*Picea mariana* (Mills.) BSP) is a rather small and slow-growing but widely distributed and commercially valuable conifer tree of North America. In Quebec, it dominates in the major part of the commercial boreal forest, hence playing an important ecological and economic role. Climate change will likely alter the timing and length of the black spruce tracheid production season, so consequences on growth can be expected and thus on the competitive capacity (Murray et al. 1994, Kramer et al. 2000) and also on timber yield and quality. It is therefore relevant not only to know the timing of tracheid production over the years and across the territory but also to determine which factors are limiting and how. Timing could subsequently be included in growth analysis to evaluate its significance, which could help to estimate the consequences of climate change on growth or to improve yield predictions.

Despite the fact that substantial work has been done on bud phenology, there are still few ecophysiological studies aiming to explain or predict cambial growth initiation and cessation. The precise link between bud and cambial phenology and their synchronism at the beginning and end of the growing season still remain unclear. While some authors believe in a rather tight control of cambium by terminal buds (Heide 1974a, 1974b, Kozlowski and Pallardy 1997), others seem to agree with a more or less pronounced independence of these meristems (Schmitt et al. 2004, Rossi et al. 2009). If cambial initiation can be locally and independently stimulated in spruce species (Orike et al. 2003, Gričar et al. 2006), probably due to cambial overwintering auxins (Egierszdorff 1981, Little and Wareing 1981), it seems that only buds...
can provide a sufficient amount of hormones to support a sustained dividing cambium (Barnett and Miller 1994, Kozlowski and Pallardy 1997).

If the cambium is controlled by buds, it could be hypothesized that cambium initiation is influenced not only by spring conditions but also by the previous year’s late summer conditions since this can influence bud dormancy intensity (Chuine and Cour 1999). Concerning growth cessation, the eco-physiological response depends on the growing mode of the plant material. In trees whose growth season finishes with a free-growing phase, photoperiod is commonly considered to be the triggering factor for cessation of terminal (Wareing 1950a, Pollard and Ying 1979) as well as cambial activity (Heide 1974a, O’Reilly and Owens 1989). In this case, for trees growing in their native photoperiod, cessation timing should be influenced only by conditions prevailing during the delay between the triggering signal and the response (Coursolle et al. 1998, Johnsen and Skroppa 2000). But when a tree exhibits an exclusively determined shoot-growing mode, like mature black spruce (Logan and Pollard 1975), an influence of the previous year’s late summer conditions should be suspected. This is due to development of the terminal shoot primordium in the bud, which occurs after active shoot elongation and then influences the following year’s cessation as this latter cannot happen before a complete predetermined shoot elongation (Wareing 1950b, Heide 1974b, Lanner 1993). Better environmental conditions during black spruce bud maturation, like warm temperature, high soil moisture and good nutrition result in a more developed primordial shoot (Pollard and Logan 1979, Colombø 1986), leading to longer apical development (Cannell et al. 1976) and possibly longer cambial activity.

Research on climate influence as a cause of tree phenological fluctuations in natural conditions is still relatively new (Larcher 2003). So far, studies have involved a few different climatic variables or just air temperature alone. However, this kind of assessment does not represent a very realistic evaluation since trees grow in complex ecological contexts that expose them to many fluctuating factors simultaneously (Grossnickle 2000, Larcher 2003). Moreover, climatic variables are prone to intercorrelation (Briffa 1999, Fritts 2001). This means that correlating a growth parameter with a single climatic factor leads to a coefficient that results not only from the single effect of the concerned climatic variable but also from a part of what is due to its collinear links with the other variables (Neter et al. 1990, Draper and Smith 1998, Briffa 1999, MacNally 2000). These considerations are particularly true when studying trees growing in climate conditions far from any adaptive extreme. In these situations, univariate links are difficult to establish and/or account for a small part of growth variations (Fritts 2001, Pensel et al. 2006). It can therefore be supposed that measuring many climatic parameters and analyzing their influence in a multivariate statistical context would be more appropriate.

The first objective of this paper is to characterize the timing of tracheid production initiation and cessation for mature black spruce growing in mesic stand conditions. The second objective is to identify climatic factors explaining the timing of these events and how they relate to known bud phenology influences. We hypothesize that these climatic factors are: soil temperature, water availability, air temperature, air humidity and light intensity. The influence of these factors on spruce growth or other physiological responses has been demonstrated experimentally, as reported in reviews (Lamhamedi and Bernier 1994, Grossnickle 2000), and this is considered sufficient justification to hypothesize an influence on phenology. Also, these factors taken together represent the whole operational environment of the tree (Fritts 2001), which is an important requirement motivated by full modeling, the chosen statistical approach (Burnham and Anderson 2002). Furthermore, conditions occurring immediately before each phenological event (initiation or cessation) are considered as well as those of the period of bud maturation or dormancy development in the previous year to account for the possible influence of apical activity.

Materials and methods

Study area and sampling plots

Efforts were made to choose sites representative of the mesic conditions prevailing in the middle of Quebec’s continuous boreal forest vegetation subzone. Four permanent plots disposed along a latitudinal transect have been sampled in order to account for the climatic variability within this subzone. From south to north, two are situated in the balsam fir–white birch bioclimatic domain: Simoncouché (Sim: 48°13.78’ N; 71°15.18’ W) and Bernatchez (Ber: 48°51.92’ N; 70°20.57’ W). The other two are situated in the spruce–moss bioclimatic domain: Mistassibi (Mis: 49°43.92’ N; 71°56.88’ W) and Daniel (Dan: 50°41.78’ N; 72°11.03’ W). Each plot is installed on even-aged, mature, closed and pure black spruce stands. The trees, established 120–140 years ago, are growing on gentle slopes (8–17%) and moderately (three plots) to imperfectly drained glacial tills (one plot: Dan). Site index (mean height at 50 years of age), mean current dominant tree height and mean diameter at breast height are: 13.6 m, 16.0 m and 20.4 cm at Sim; 6.9 m, 17.6 m and 21.1 cm at Ber; 11.4 m, 18.6 m and 21.4 cm at Mis; 8.7 m, 16.8 m and 20.0 cm at Dan, respectively.

Assessment of in-progress cell production

All the main steps of the methods used are summarized in this section, but emphasis is placed on the differences between these and the more detailed methods given in Dufour and Morin (2007).

From 2002 to 2006, five dominant trees at each site were sampled repeatedly throughout the growing season to monitor tree ring formation on a weekly or bi-weekly frequency from mid-May until the initiation of the active cell production period and thereafter on a weekly (Sim 2002–2006; Ber, Mis
and Dan 2002–2003) or fortnightly basis (Ber, Mis and Dan 2004–2006) until mid-October. At each sampling, a single micro-core was taken from the stem of each tree using a bone marrow sampling needle (DBMNI-1501 inter-V medical), extracting cores about 1 mm in diameter and up to 20 mm long (Deslauriers et al. 2003). Coring points were arranged along a counter-clockwise rising spiral centered at breast height (1.3 m). Spacing between points was at least 3 cm horizontally and 2 cm vertically, which has been observed to be enough to avoid resin duct formation due to prior sampling trauma.

Micro-cores were processed using standard histological methods involving dehydration in alcohol, paraffin embedding and cutting with a rotary microtome. Staining was done with 0.15% cresyl violet acetate filtered solution. Observations were made while the sections were gently stretched under a polarized light-equipped transmission microscope to unfold the tissues compressed by coring, making cell types easy to distinguish (for pictures, see Thibeault-Martel et al. 2008). Cells (tracheids) were then counted in each of the following developmental zones: cambial zone, radially enlarging and enlarged (includes the ones that have undergone lignification and the mature ones).

The counts for enlarging cells and already enlarged cells were standardized to account for variability due to different sampling positions around the stem (Deslauriers et al. 2003, Rossi et al. 2003). Each count was therefore multiplied by the ratio of the mean ring width of the previous ring of all samples taken on the tree in the same year, divided by the ring width of the previous ring specific to the counted sample.

Mean values from each site and year have been computed by averaging counts from the five trees. The two standardized counts for enlarging and enlarged cells were summed to create datasets of the number of tracheids produced. We fitted a linear and a quadratic model to the summed dataset and kept only the solution showing the best fit. The fit was restricted to the points where tracheid numbers increase (excluding zero and asymptotic observations), so the fit is specifically focused on the period of tracheid production. The first observation of this period is the first when a non-zero number of cells is observed in enlargement, and the last one is the last before the number of cambial cells returns to its base value (4 ± 1 cells).

The timing of the observation points alone can serve directly as estimates of the phenological timing (Thibeault-Martel et al. 2008), but precision and reliability depend on the observation interval. In this study, observations were sometimes 2 weeks apart, and this was judged as being too long to use observation timing directly. Instead, we used extrapolations from the functions fitted as described above. Two pieces of timing information have been extracted from them. First, the day on which the function achieves a value of one cell gives the day of tracheid production initiation (variable TPinit). Second, the day on which the function achieves the total number of cells, determined as the mean value of the asymptotic observations excluded from the fit, is the day of tracheid production cessation (variable TPcess). However, the use of this procedure for TPcess is conditional on an extrapolation lying between the two observation points encompassing the period when cell production ceased. In five out of 20 cases, TPcess evaluation fell outside the time period defined by the observation points, so for these cases, the timing of the closest of these corresponding observations has been used.

Assessment of bud break

For 3 years at Sim and 2 years at Dan, bud development in spring was monitored on five trees per site, which were different from those sampled for cambium phenology. On each tree, a twig was harvested using a clipper fixed at the tip of a 9-m pole for a total sampling height of 10 m, corresponding roughly to one-third to one-half of the crown. Terminal branchlet buds were then observed, and bud break was determined as the time when needles were distinguishably free of bud scales (Colombo 1986). Sampling was repeated once or twice weekly. Bud break was revealed to be a fast and clear development stage. Consequently, two patterns have been observed. The first is when two successive samplings showed respectively 0 and 100% broken, within as well as between trees, so the middle day between the sampling dates was determined as the day of bud break. In the second pattern, only one intermediate sampling showed some level of partition between unbroken and broken buds on the sampled twigs either within or between trees. When that was observed, day of bud break was directly determined by the date of this partitioned sample.

Meteorological monitoring and climatic variability

As mentioned earlier, sites were chosen to be representative of the mesic closed black spruce forest, but the spatial layout was also planned to assess a representative climatic variability within the study area. To assess this variability, a weather station was installed in a gap (♂ 20 m) on each site. Each system was controlled by a CR10X datalogger (Campbell Scientific®) to which weather sensors were connected (one per parameter) that monitored the following parameters:

- Air temperature, 2 m above ground in the gap
- Relative humidity, 2 m above ground in the gap
- Incident photosynthetically active radiation (PAR) at 8 m high in the gap
- Air temperature, 2 m above ground, under cover
- Humus temperature (∼5–15 cm below surface)
- Soil upper mineral layer temperature (∼25–35 cm below surface)
- Soil water content (time domain reflectometry; TDR), upper 30 cm layer
- Precipitation
- Snowpack depth

Measurements were taken every 5 min; hourly total (precipitation only), minima/maxima (both air temperature) and means (all measurements except precipitation) were recorded. From this hourly record, daily total, minima/maxima and
means were computed. Compilations made from this daily defined database are described in the next sections.

In all sites during the studied period, the warmest month of the year was July, while the coldest was January (Figure 1). Yearly mean temperature was, from south to north: 1.8, −0.1, 0.7 and −1.3 °C. Monthly mean temperature was typically above zero from May to October in all sites but also in April at Sim, the warmest site. During this period, monthly total rain precipitation is, in general, gradually more abundant from south to north. Total precipitations from May to October ranged from 511 mm (Sim) to 655 mm (Dan). The thickest snow accumulations are found in the coldest (Dan) and the highest (Ber) sites and peak at 90 (Sim) to 120 (Ber) cm in March. Snowpack usually melts almost completely in April at Sim and in May at the other sites.

Modeling method

Since each site has its own weather station, observations can be considered as independent among sites, so all the analyses have been done pooling observations from years and sites.

The statistical approach used is a full model, which means inclusion of all the regressors (factors) that are mechanically expected to influence the response. These full models are known to be the most effective to decipher the real effect of a factor in a multivariate context, i.e., regression coefficients are not biased (MacNally 2000, Burnham and Anderson 2002, Whittingham et al. 2006). Basically, the factors tested are in accordance with the hypothesis stated in the introduction: soil temperature, water availability, air temperature, air humidity and light intensity. However, the basic five-factor set is repeated for each time period considered, including time just preceding the phenological event and time of shoot primordia development during the preceding season. Once factors had been chosen, we proceeded to an optimization of the full model. Thus candidate variables, often more than one per factor, were computed (Table 1). All the possible models including every factor but changing the representing variable for each one were computed, and the solution with the lowest AIC was used.

Full models are not parsimonious, which means they consider all the variables put in the model to be important. But since we were interested in identifying the most important variable and because overfit was a concern, a parsimonious final solution was reached. To do this, the full model with \( k \) parameters is first compared with all possible models having one factor less. If any of these \( k-1 \) parameter models shows an AIC reduction compared with the \( k \) parameters model, then the model with the lowest AIC is selected. This procedure is repeated at least until the number of regressors represents no more than one-third of the number of observations and then until no further AIC decrease can be obtained.

Compilation of regressors and transformation of response variables

Climatic variables have been compiled as mean daily values for the period lasting between an arbitrarily fixed starting day,
common to all observations, and the day of the phenological event itself (i.e., \( TP_{init} \) or \( TP_{cess} \)), which is specific to each observation. However, many possibilities for the starting day have been compared, and selection of the best was made by choosing the one leading to the best model in terms of statistical fit (adjusted \( R^2 \)). The resulting best starting day defines what we called the period of climate influence (of a given phenological stage). Other candidate variables, belonging to the previous year’s conditions (Table 1), are also included in each iteration fitting process but are constant (i.e., not the object of differential period compilation) among the iterations.

Neither \( TP_{init} \) nor \( TP_{cess} \) has been used directly as the response variable in their respective analyses. The reason for this is that building a model with regressors, compiled according to observation-specific periods to explain the variable that also makes these periods fluctuate, is a tautology. If it does not inhibit the possibility of judging the most important factors, it clearly prevents any prediction of the model because knowing the right period for compilation would necessitate having a priori knowledge of what has to be predicted (i.e., phenological timing). Since we preferred to also have a predictive utility for our model, response variables have been transformed as follows. Throughout the period of climate influence, which ends with the phenological stage occurrence, the underlying process can be considered to evolve from 0 to 100%. Then, dividing 100 by the duration (in days) gives the mean daily advancement percentage of that process. This transformation of \( TP_{init} \) and \( TP_{cess} \) results in the response variables ‘initiation daily advancement percentage’ (\( I_{adv\%} \)) and ‘cessation daily advancement percentage’ (\( C_{adv\%} \)), respectively. Having a model that predicts a daily phenological advancement percentage by daily meteorological conditions allows the timing of the phenological event that comes at the end to be predicted since it happens when the summed daily advancement reaches 100%. This procedure is only valid with strictly linear models. Our models meet this requirement since we model with multiple linear regression and apply no non-linear transformation to regressors or response.

**Comparing the influence of each important factor**

As mentioned by Quinn and Keough (2002), the partial regression coefficients are differently scaled so they do not allow straightforward comparison of the effect of each factor, nor does their standardized version (standard beta) since these are biased by collinearity. To efficiently assess the rel-

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Table 1. Check list of candidate regressors tested for their influence on various phenological response variables of different nature and period of influence. Regressors are all means computed from daily data and covering periods specific to each model. Response variables abbreviation: BB, bud break; \( I_{adv\%} \), daily percentage of advancement to tracheid production initiation; \( C_{adv\%} \), daily percentage of advancement to cessation of tracheid production.

<table>
<thead>
<tr>
<th>Factor type</th>
<th>Analogous variable (units)</th>
<th>Response variable and climate influence period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Abbreviation</td>
<td>BB current year</td>
</tr>
<tr>
<td>Air humidity</td>
<td>Vapor pressure (kPa)</td>
<td>VP</td>
</tr>
<tr>
<td>Light</td>
<td>Photosynthetically active radiation (( \mu \text{mol} \text{ s}^{-1} \text{ m}^{-2} ))</td>
<td>PAR</td>
</tr>
<tr>
<td>Air temperature</td>
<td>Saturated vapor pressure (kPa)</td>
<td>SatP</td>
</tr>
<tr>
<td></td>
<td>Temperature ( (°C) )</td>
<td>T</td>
</tr>
<tr>
<td></td>
<td>Maximum temperature ( (°C) )</td>
<td>( T_{max} )</td>
</tr>
<tr>
<td></td>
<td>Minimum temperature ( (°C) )</td>
<td>( T_{min} )</td>
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<td>Degree-days over 0 °C</td>
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<td></td>
<td>Degree-days over 6 °C</td>
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<td>Undercover temperature ( (°C) )</td>
<td>( T_{uc} )</td>
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<td></td>
<td>Maximum undercover temperature ( (°C) )</td>
<td>( T_{uc,max} )</td>
</tr>
<tr>
<td></td>
<td>Minimum undercover temperature ( (°C) )</td>
<td>( T_{uc,min} )</td>
</tr>
<tr>
<td>Soil temperature</td>
<td>Humus temperature ( (°C) )</td>
<td>( T_{hu} )</td>
</tr>
<tr>
<td></td>
<td>Mineral soil temperature ( (°C) )</td>
<td>( T_{mn} )</td>
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<tr>
<td>Water availability</td>
<td>Soil water content (%)</td>
<td>SWC</td>
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<tr>
<td></td>
<td>Precipitations (mm)</td>
<td>( P )</td>
</tr>
<tr>
<td>Snow cover</td>
<td>Snowpack depth (m)</td>
<td>( S_{depth} )</td>
</tr>
<tr>
<td>Timing of initiation</td>
<td>Initiation of tracheid production (day of year)</td>
<td>( TP_{init} )</td>
</tr>
</tbody>
</table>
Results

Timing of tracheid production and bud break

Modeling the observed in-progress cell production allowed an evaluation of the times when cell production started and ceased. The fitted functions, showing $R^2$ values ranging from 0.91 to 0.99 with a mean of 0.97, have been used to extrapolate cambium phenology events (Figure 2A). These have also all been averaged by sites and by years (Figure 2B and C).

The mean dates for $T_{P_{\text{init}}}$ and $T_{P_{\text{cess}}}$ were day 153 (2 June) and day 227 (15 August) respectively, and the mean length of cell production was 74 days. During the assessed years, variability of $T_{P_{\text{init}}}$ was clearly higher between years than between sites as revealed by standard deviations (Figure 2B); the year-to-year variations at a single site are generally higher than the site-to-site variations during a single year. Indeed,
variations among years led to a higher standard error and a significant Kruskal–Wallis test \( (P = 0.0097) \) but a non-significant one for variations among sites \( (P = 0.3594) \). Overall variations of cessation were weakly significant among years \( (P = 0.059) \) and not significant among sites \( (P = 0.8231) \).

The pattern for \( TP_{cess} \) is similar to \( TP_{init} \) at Ber, Mis and Dan together, an early initiation was followed by an early cessation \( (\text{Figure 2A, } P = 0.01, r = 0.64) \), but Sim diverges from that rule since it shows a non-significant, negative tendency. Nevertheless, cell production duration \( (\text{Figure 2C}) \) was constant among sites \( (P = 0.1995) \) and among years \( (P = 0.3244) \).

The timing of bud break has been assessed for five cases. We fitted a multiple regression model \( (\text{Table 2}) \) to explain bud break variations by \( TP_{init} \) and mean undercover temperature \( (T_{uc}) \) for periods specifically adjusted between \( TP_{init} \) and bud break. \( T_{uc} \) was selected as the best fitting variable from the same air temperature candidates as those used for tracheid production initiation \( (\text{Table 1}) \). The result is a weakly significant test \( (\alpha = 0.1, \text{ Table 2}) \) with both regressors being significant since the model including both of them has the lowest AIC.

**Influence of climatic variations on timing of tracheid production initiation**

Iterations of the modeling process for \( I_{adv\%} \) have been made with a 7-day lag of the starting day, so there were 12 repetitions using starting days ranging from day 55 to day 132. \( \text{Table 1} \) lists the current year variables, which have been compiled according to the different periods of time, along with variables for the previous late summer, always represented by August conditions.

The best starting date was determined by choosing the one that gave the best fit between the specific selected regressors and \( I_{adv\%} \) using adjusted \( R^2 \) as criterion \( (\text{Figure 3}) \). Day 76 (17 March) was revealed to be the best estimation of the starting day. The corresponding multivariate regression is detailed in \( \text{Table 3} \).

Residual plots were checked, but no obvious tendency appeared, and Shapiro–Wilk normalcy test was non-significant \( (P = 0.9594) \); thus, residuals are normal and show no obvious heteroscedasticity. No autocorrelation remains once climate effect is removed so the assumption of independence of errors is met \( (\text{Draper and Smith 1998, Legendre and Legendre 1998, Quinn and Keough 2002}) \).

The relative importance of each isolated effect is expressed by the percentage of independent effect \( (IE_{\%}) \) given from hierarchical partitioning, so the parameters are classified in descending order of importance in \( \text{Table 3} \). By far the most influential factor is \( T \), being responsible for almost half of \( I_{adv\%} \) explained variations, while vapor pressure \( (VP) \) accounts for about a quarter. Higher mean daily temperature in spring promotes growth initiation, whereas increasing vapor pressure has a retarding effect. The previous year’s late summer conditions account for the last quarter of the explained variance as growth initiation is delayed by higher undercover minimum temperature in August but promoted by warm humus temperature.

The daily probability of reaching a temperature above 0 °C \( (\text{Figure 4}) \) indicates that day 76 occurs at the beginning of a period when the probability of reaching non-freezing temperatures shows a regular increase from values close to 0 towards values around 1.

**Influence of climatic variations on timing of tracheid production cessation**

Factor types and their respective analog variables tested for their influence on tracheid production cessation are displayed in \( \text{Table 1} \). Only the end of the period during which the cessation process operates has been measured \( (\text{corresponding to } TP_{cess}) \). We therefore proceeded once more with progressive duration modeling, varying the starting date. First, we put the starting date back lagging 4 days, in steps ranging from day 202 back to day 174. The lag was then lengthened to 7 days
for the period extending back to day 118, this second series extending before the summer solstice (Figure 5) to investigate for a possible influence of photoperiod on the triggering of TP_cess.

The period showing the best fit begins on day 194, considered as a ±3-day evaluation (consequent to the lag of 4 days) of the signal triggering the growth cessation process. The lag between day 172 (summer solstice) and day 194 corresponds, from the southernmost to the northernmost site, to 21–23 min diminution of the photoperiod (Lammi 2008).

The best model is shown in Table 4. Residual plots showed no heteroscedasticity. Spatial autocorrelation of residuals is not significant ($r = -0.06, P = 0.8318$), indicating no real spatial autocorrelation once climate is taken into account.

Mean daily saturation vapor pressure, a function of temperature, is the most influential factor and has an accelerating effect on the cessation process. Some other significant factors have a retarding effect, reducing the daily advancement of the cessation process: previous late summer’s mineral temperature and current year soil water content, PAR and vapor pressure. Humus temperature is the least significant factor and

<table>
<thead>
<tr>
<th>Regressor</th>
<th>Mean value</th>
<th>Intersite standard deviation</th>
<th>Interannual standard deviation</th>
<th>Coefficient</th>
<th>Coefficient standard deviation</th>
<th>IE%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>3.0126</td>
<td>0.2738</td>
<td>47.7</td>
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<tr>
<td>$T$</td>
<td>1.3413</td>
<td>1.0662</td>
<td>1.3116</td>
<td>0.1281</td>
<td>0.0176</td>
<td>47.7</td>
</tr>
<tr>
<td>VP</td>
<td>0.5124</td>
<td>0.0360</td>
<td>0.0360</td>
<td>-3.8575</td>
<td>0.5799</td>
<td>25.2</td>
</tr>
<tr>
<td>$T_{\text{uc, min, aug}}$</td>
<td>8.3904</td>
<td>0.7878</td>
<td>0.8014</td>
<td>-0.0384</td>
<td>0.0172</td>
<td>19.9</td>
</tr>
<tr>
<td>$T_{\text{hu, aug}}$</td>
<td>11.5078</td>
<td>0.9135</td>
<td>0.3553</td>
<td>0.0522</td>
<td>0.0192</td>
<td>7.2</td>
</tr>
</tbody>
</table>

1 Regressors are basically observation-specific daily mean compiled from day-of-year 76 to tracheid production initiation. Subscript 'aug-1' indicates previous year August mean daily conditions. For units, see Table 1.

Figure 4. Daily probability for daily mean temperatures ($T$) over 0 °C to occur. These have been calculated by dividing the number of observed occurrences of $T > 0$ over all observed cases (20) on the four sites from 2002 to 2006. The arrow points to the starting day of the best fit period of climate influence as previously determined (day 76).

Figure 5. Adjusted $R^2$ for 16 iterations of the modeling process (see Materials and methods) for daily percentage of advancement to tracheid production cessation ($C_{\text{adv}}$) in function of climatic variables. In each iteration (bar), the length of the considered period for climate influence is changed by modifying its beginning day, in such a way for climate and response variables to be differentially compiled for each of these periods. All models are significant ($\alpha = 0.05$).
shows a stimulating effect on the process, hence resulting in earlier growth cessation.

Discussion

Modeling methods

The motivation for pooling observations from sites and years comes from our aim to draw conclusions that are valuable in time and within the whole study area. The factors included in the models are shared in terms of spatial and temporal dominance of their variability: some are more variable in time, others in space and some are quite even (Tables 3 and 4). Therefore, averaging either sites or years would probably have neglected important variability and resulted in overfitted models, i.e., a too-specific fit to be generalized in space or time (Ginzburg and Jensen 2004, Whittingham et al. 2006). Also, we made sure that the whole extent of variation belonging to the study context reduces the risk of coefficient biases because shortness of data is harmful to coefficient evaluation, and this is exacerbated by collinearity (Belsley 1991).

The starting day for climate influence has been evaluated by comparing many arbitrarily fixed days because we had no data assessing the beginning and its variability in time or space. That this variability is high or low does not inhibit the possibility of using the best compromise given by the best fit as the starting day for climate compilation. But having no variability information means we are unable to draw any conclusion about the constancy of the starting day.

Timing of tracheid production

The general rule concerning tracheid production phenology could be that initiation and cessation are synchronous over the covered territory but variable over years. Despite spatial statistical dependence of phenological responses, initiation and cessation have been significantly related to climate fluctuations, and nothing of this dependence remains in the residuals, so there is no real autocorrelation (Legendre and Legendre 1998).

Duration is not significantly different between sites or between years. Constant duration is thus due to a relative synchronism between initiation and cessation. The synchronism is probably not caused by a real dependence of cessation on initiation but rather by covariance of the factors influencing both events. Indeed, among the 24 possible correlations that could be computed from the four and six factors influencing initiation and cessation, respectively, 10 are significant ($P < 0.05$), including the one between the most influential factor for each, i.e., temperature from day 76 and saturation vapor pressure from day 194 ($P = 0.0136$, $r = 0.56$). Nevertheless, some extreme durations have been observed, as duration ranges from 2 to 3 months. Thus, large but occasional variations of duration may occur.

Tracheid production initiation: link with bud initiation and climate

For the hypothesis of bud-controlled cambium initiation (Reinsing and Owens 1994, Kozlowski and Pallardy 1997, Grossnickle 2000) to be proved, it would be best to directly observe if bud mitosis initiation is, at the very least, concomitant with or prior to cambium mitosis initiation. We did not evaluate bud mitosis initiation but bud break instead. It is consistent to consider that bud break happens 4 to 6 weeks after mitosis initiation (Owens and Simpson 1988, Westin et al. 1999), so that the fact that bud break happens after cambium initiation (Owens and Simpson 1988, O’Reilly and Owens 1989, Westin et al. 1999, Rossi et al. 2009) cannot disprove bud-controlled cambium hypothesis. On the contrary, this study demonstrates that cambium initiation can be significantly related to bud break, a direct but delayed consequence of bud mitosis initiation, as long as the modulation

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Table 4. Multiple regression ANOVA, climatic variables’ mean and standard deviations, model coefficients and their standard deviation, and percentage of independent effect (IE$_{fl}$) given by hierarchical partitioning of the best multiple regression found for daily percentage of advancement to tracheid production cessation (C$_{adv}$) vs daily climatic variables.

<table>
<thead>
<tr>
<th>Regressor</th>
<th>Mean value</th>
<th>Intersite standard deviation</th>
<th>Interannual standard deviation</th>
<th>Coefficient</th>
<th>Coefficient standard error</th>
<th>IE$_{fl}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.2492</td>
<td>1.3981</td>
<td>40.5977</td>
</tr>
<tr>
<td>SatP</td>
<td>1.8943</td>
<td>0.1228</td>
<td>0.0884</td>
<td>13.9474</td>
<td>1.6004</td>
<td>40.5977</td>
</tr>
<tr>
<td>$T_{\text{min,LS-1}}$</td>
<td>9.0865</td>
<td>0.8470</td>
<td>0.2757</td>
<td>-1.8227</td>
<td>0.1606</td>
<td>16.6046</td>
</tr>
<tr>
<td>SWC</td>
<td>53.4962</td>
<td>10.3770</td>
<td>7.3330</td>
<td>-4.3187</td>
<td>1.0424</td>
<td>16.4567</td>
</tr>
<tr>
<td>PAR</td>
<td>353.0513</td>
<td>43.5584</td>
<td>43.1139</td>
<td>-0.0469</td>
<td>0.0041</td>
<td>14.9993</td>
</tr>
<tr>
<td>VP</td>
<td>1.4000</td>
<td>0.0768</td>
<td>0.0675</td>
<td>-13.3251</td>
<td>1.9559</td>
<td>7.4295</td>
</tr>
<tr>
<td>$T_{\text{sat}}$</td>
<td>11.6258</td>
<td>0.8848</td>
<td>0.5482</td>
<td>0.6396</td>
<td>0.1485</td>
<td>3.9121</td>
</tr>
</tbody>
</table>

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1Regressors are basically observation-specific daily mean compiled from day-of-year 194 to tracheid production cessation. Subscript ‘LS-1’ indicates previous year late summer (August–September) mean daily conditions. For units, see Table 1.
The effect of air temperature in the days prior to bud break is taken into account.

The link between bud and cambium initiation is also supported by the inclusion of the two factors representing the previous year’s August conditions. The most influential of these, undercover minimum temperature, has a retarding effect on cambium initiation in the following spring. Other authors reported similar observations for spruce species bud break, and the explanation seems to be that during dormancy setting up, higher air temperature promotes a deeper rest (Heide 1974b, Granhus et al. 2009). The other previous August factor, soil temperature, has a weaker but opposite effect. High soil temperature is considered to enhance nutrition of the tree (Krause 1991, Domisch et al. 2002), and during bud development, this produces a more expanded bud structure (Pollard and Logan 1979, Colombo and Smith 1988). Bigger buds (Nienstaedt 1966, Colombo 1986) and high fertilization (Murray et al. 1994, Floistad and Kohmann 2004) have been associated with an earlier bud flush in the following spring.

Thus, cambium initiation is related to bud activity in spring, bud shaping in the previous year and bud dormancy setting up. Also, cambium reactivation without concomitant natural or artificial bud auxin supply has also been revealed to be either non-existent (Little et al. 1990, Barnett and Miller 1994) or only temporary and unproductive (Little and Bonga 1974, Oribe et al. 2003, Gricar et al. 2006). All these considerations taken together bring the conclusion that cambium initiation depends on shoot initiation.

Climate influence on tracheid production initiation in spring begins on day 76. Whether this reflects a varying triggering in time and space or a constant one is a relevant question. The concomitance with the rise of the daily probability of reaching a temperature above 0 °C (Figure 4) supports the hypothesis of a varying triggering since temperature is clearly, on an annual basis, a variable factor. The consequence would be that, for a better initiation prediction, the time when daily mean temperature reaches 0 °C in spring should be checked instead of systematically computing from a constant day.

So far, cambial initiation has been notably related to a heat-sum approach, using a single base temperature, +5 °C (Schmitt et al. 2004, Seo et al. 2008), in a similar fashion to heat sum applied to bud phenology (Hänninen 1990, Chuine and Cour 1999, Kozłowski and Pallardy 2002). In this study, we also assumed a heat-sum dependency, but many base temperatures have been compared, all in a full model approach, the best suited one to avoid coefficient bias and evaluate the real effect of factors (MacNally 2000, Burnham and Anderson 2002, Whittingham et al. 2006). However, despite the possibility of fitting a significant model including a base temperature heat sum with our data, none has a fit as good as the one including daily mean temperature, i.e., using no base temperature.

We suggest that the retarding effect of high air humidity on cambial division initiation could be linked to water conductance dynamics, a hypothesis that is also supported by the non-base-temperature result. Using a base temperature implicitly means that the modeled process is uninfluenced by temperatures below this base temperature, whereas a process modeled without base temperature experiences backward evolution in subzero temperatures. The latter is consistent with the conductance dynamics because conifers experience increasing xylem cavitation following freeze–thaw cycles (Sparks et al. 2001, Pittermann and Sperry 2006, Mayr and Charra-Vaskou 2007). Although the mechanism of recovery from cavitation is unknown, it is likely driven by the same factors that create the flowing force through the xylem, i.e., high transpiration demand from the leaf, to which low vapor pressure is closely linked (Nobel 2005). Additional support for this hypothesis are that recovery occurs when the shoot is very dry (Sperry and Robson 2001) and that stomata opening in spring occurs beyond photosynthetic requirements (Day et al. 1990).

Snow cover has been tested but not considered significant, despite the work of Kirdyanov et al. (2003) that reports a positive effect of snowmelt timing on cambial initiation. Snow depth is significantly and negatively correlated with two of the explicative factors for initiation ($T$ and $T_{\text{bud, aug}}$, $R^2 = 0.67$), and our method allows for a confrontation of snow cover vs these collinear factors. The result is an initiation of radial growth that is much more explained by spring air temperature and the previous year’s August humus temperature than by spring snow depth alone, proving that the latter is not necessarily good as a cause.

**Influence of climatic variations on timing of tracheid production cessation**

Although we do not have any monitoring of bud set, we can draw indirect conclusions about the bud influence on tracheid production cessation. Bud set can be triggered either by photoperiod in free-growing trees (Wareing 1950a, Heide 1974a, Pollard and Ying 1979, O’Reilly and Owens 1989) or by complete primordia elongation in determined growth trees (Wareing 1950b, Heide 1974b, Lanner 1993). Mature black spruce is a determined growth tree (Logan and Pollard 1975), and this study reveals that its cambium activity cessation is retarded by high late summer soil temperature in the previous year. Since no preformed, overwintering cambial structure has been observed in our material or reported in the literature and also because late summer corresponds to the primordial shoot development period (Colombo and Teng 1992), an obvious link with bud is established. The retarding effect likely works as follows: low soil temperature limits nitrogen mineralization from accumulated organic matter (Krause 1991, Domisch et al. 2002), and the resulting lower nutrition reduces the number of leaf primordia in the bud (Pollard and Logan 1979, Colombo and Smith 1988), which then reduces the time of apical elongation during the following growing season (Cannell et al. 1976), a tendency followed by cambium as well (Heide 1974b, this study).

The determination of day 194 as the starting day for current year climatic influence also supports the hypothesis for
Cambial phenology to be controlled by bud development, although it first appears to support a photoperiodic triggering since it occurs after summer solstice. In determined growth species like spruces, the first year is the only year in which apical growth cessation is doubtless triggered by photoperiod, since there is no possible determined growth (Heide 1974b, Cannell et al. 1976). Because first-year cessation effectively happens in mid-September in natural photoperiod at our latitude (Pollard and Ying 1979, Colombo et al. 1989, Coursolle et al. 1998) and considering that the delay between a triggering signal and cessation of apical elongation is shorter than a month (Colombo et al. 1989, Bigras and D’Aoust 1992, Coursolle et al. 1998), the photoperiodic triggering signal cannot happen before mid-August. This implies a difference of at least a month from day 194, so the signal occurring on the latter is unlikely to be photoperiodic by nature. Consequently, a different kind of triggering should be considered. As mid-July corresponds to bud set timing, as observed in mature black spruce (O’Reilly and Parker 1982), predetermined shoot elongation completion is a more convincing hypothesis to describe the nature of a cambial-cessation-triggering signal.

Cambial division cessation response is delayed in time from the triggering signal initiating the cessation process. During the lag, climatic variations modify the timing of cessation. Air temperature is the most important factor to explain cambial cessation variations: the warmer the late summer, the earlier the occurrence of cessation. Such a hastening effect of temperature is already known for shoot elongation completion (Heide 1974a, Johnsen and Skrøppa 2000). The selection of saturation vapor pressure as air temperature analog, instead of any other, reveals that temperature effect is at the very least partly linked to tree water loss. The significance and signs of soil water content and vapor pressure support this idea in a consistent way. Most reviews count water stress among the factors that can trigger shoot dormancy in trees (Kozlowski and Pallardy 2002, Arora et al. 2003). Considering also the retardant effect of higher light intensity, a consistent convergence toward photosynthesis appears. The obvious conclusion is therefore that photosynthesis promoters, i.e., those in favor of a good water status and good illumination, extend the tracheid production season in the bole.

**Predictive utility**

Although it was not the most important objective of this study, we built the models to make them usable for predictive purpose. As the response variable predicted by the model is a daily percentage of process advancement, the day of phenological stage occurrence is the one when summed daily predictions from the starting day reach 100%. It is important to note, however, that this procedure is made possible only because we assumed a linear relationship between climate and phenological responses. From the predicted day of tracheid production initiation, it is also possible to predict the day of bud break using the presented model for it. Making predictions has many useful applications like simulating effects of climate changes on tracheid production duration, but it would also be interesting to compare prediction for various other cases with real measurements of responses to validate the models or evaluate their scopes more precisely.

**Black spruce tracheid production season vs climate changes**

Possible effect of climate changes on tracheid production duration can be inferred from the models, even though no simulation has been made so far. Quebec boreal forest is presently experiencing a climatic warming that could reach 2 °C in spring and summer for the period 1990–2020 (Bourque and Simonet 2007). Our model suggests that this leads to earlier tracheid production initiation since air temperature in spring has a prevailing influence on this phenomenon. Summer temperature influence on tracheid production cessation is also important, but its direct effect for an earlier cessation could be attenuated by the concomitant warming of soil, which has a second-order opposite effect. Then, both phenological stages happen earlier with higher temperature, but overall duration of tracheid production should increase because initiation is more hastened than cessation.

**Conclusion**

Mature black spruce growing in the heart of its native range and in moderate stand conditions has a tracheid production phenology that is fairly variable in space but more variable in time and results in a rather constant duration of production. Its average initiation takes place in early June, whereas its cessation happens in mid-August.

Tracheid production phenology in the studied context is subjected to complex links with climate that can be well understood by taking into account its relationship with shoot activity. Timing of initiation and cessation is related to conditions during shoot primordium formation (bud maturation) and dormancy setting up, both occurring during the preceding year. Cambium initiation is also controlled by factors influencing bud break and, possibly, xylem conductance dynamics from mid-March until its occurrence. Cessation is hastened by high air and soil temperatures occurring between mid-July and cessation itself, but during the same period it is retarded by photosynthesis promoters, namely light intensity, soil water content and air humidity. These results have major implications for the evaluation of the precise effects of climate change on black spruce phenology. The resulting models are also very interesting tools for cambial and apical phenology predictions.

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References


